GENETICALLY EDITED ORGANISMS – CRISPR/Cas9 AS A NEW TOOL IN FOOD PRODUCTION

Tatjana Marinkovic[1], PhD; Dragan Marinkovic[2], PhD; Veljko Samardzic[3], MSc
1. College of Applied Sciences Uzice, Serbia, tatjana.marinkovic@hotmail.com
2. University of Belgrade, Faculty for Special Education and Rehabilitation, Belgrade, Serbia, dragan.marinkovic@hotmail.com
3. Higher Educational Institution for Applied Studies of Entrepreneurship, Belgrade, Serbia, vsamardzic75@gmail.com

Abstract: *Few topics in science raised so much controversy as that of genetically modified organisms, or GMOs. Engineered by technics of recombinant DNA, GMOs include genes from other organisms to produce a particular trait, such as disease or pesticide resistance. However, while GMO technology is still considered to be "new" and not fully understood, recent discovery of gene-editing tool CRISPR/Cas9, with ability to cut out genes and splice in new ones, even more, to do it in vivo, gives a promise for a completely new approach to the production of crop with desirable genetic traits. There is a distinction between 'genetically modified organisms' (GMOs) generated through the transgenic introduction of foreign DNA sequences and 'genome-edited crops' (GECs) generated through precise editing of an organism's native genome. In this paper, we will give an overview of CRISPR/Cas9 applications, as well as of benefits and major issues regarding the use of genetically edited organisms in food production and medicine.*

Keywords: *Genetically edited organisms, CRISPR/Cas9, food, genome modification, food safety*

1. INTRODUCTION

Since the beginning of civilization, man had selection approach in the use of plants and animals for their own needs and with the first cross breeding between organism man has created the first engineering by crosses of superior plants with other compatible plants to achieve more productive or pathogen-resistant plants, for example. When it was realized that genes are determining qualitative or quantitative traits desired by breeders, the intention arose to mutate these genes specifically. Thus, as an addition to traditional plant selective breeding, mutagenesis using chemical compounds or irradiation application, followed by screening of mutation populations for the desirable traits was used over the last 60 years. It is important to notice that traditional plant breeding techniques, including conventional mutagenesis, translocation breeding and intergeneric crosses, are intrinsically very non-specific. As it happens, a large genome part instead of a single gene is transferred by crossing, or thousands of nucleotides are mutated instead of the desired single one [1].

The results achieved in 1973 with using restriction enzymes, bacterial nuclease, which accurately recognize and cut the DNA chain, whereupon such fragments can be reconnected i.e. recombined, gave a completely new approach in modification of living organisms. Since their first introduction in 1970's, genetically modified organisms (GMO) are drawing a large amount of attention by both scientists and general public. Creation of living organisms with desired features by technology of recombinant DNA appeared to be of major significance and brought a benefit in wide areas of human activities, including science, production of wide variety of molecules for the medical and economical purposes. When it comes to the food production, however, many debating and controversy occurred. GM plants were readily accepted by one part of society, mostly by USA and South America seed producers, while even more than 30 years after first commercially available plant, European countries are still resisting to the wide application of GM plants in food production [2], [3]. Despite the promise that GM crops hold for global food security, their use is affected by largely unsubstantiated health and environmental safety concerns. Government regulatory frameworks that aim to safeguard human and environmental biosafety have led to significant cost barriers to the rapid widespread adoption of new GM traits [4].

However, while GMO technology is still considered to be "new" and not fully understood, recent discovery of gene-editing tool CRISPR/Cas9, with ability to cut out genes and splice in new ones, even more, to do it in vivo, gives a promise for a completely new approach to the production of crop with desirable genetic traits. There is a distinction between 'genetically modified organisms' (GMOs) generated through the transgenic introduction of foreign DNA sequences and 'genome-edited crops' (GECs) generated through precise editing of an organism's native genome.
Several new plant breeding techniques (NPBTs) have been developed during the last decade, and make it possible to precisely perform genome modifications in plants. Clearly, new, advanced technique for the alteration of genome raised a question whether the resulting plants and their products are covered by GMO legislation.
Here, we will give and overview of new plant-breeding technique that includes genome editing and discuss how it fits into current GMO legislation.

2. GENOME EDITING TOOLS

Genome-editing tools provide advanced biotechnological techniques that enable the precise and efficient targeted modification of an organism's genome [5]. Genome editing recently come under the spotlight through the development of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas systems which provide simplicity and ease of targeted gene editing [6]. CRISPR/Cas system use typical sequence-specific nucleases (SSNs) that can be induced to recognize specific DNA sequences and to generate double-stranded breaks (DSBs). This is the mechanisms that provide bacteria and archaea with adaptive immunity against viruses and plasmids through CRISPR/Cas-dependent silencing of invading nucleic acids [6]. The plant's endogenous repair systems fix the DSBs either by non-homologous end joining, which can lead to the insertion or deletion of nucleotides thereby causing gene knockouts, or by homologous recombination (HR), which can cause gene replacements and insertions [7]

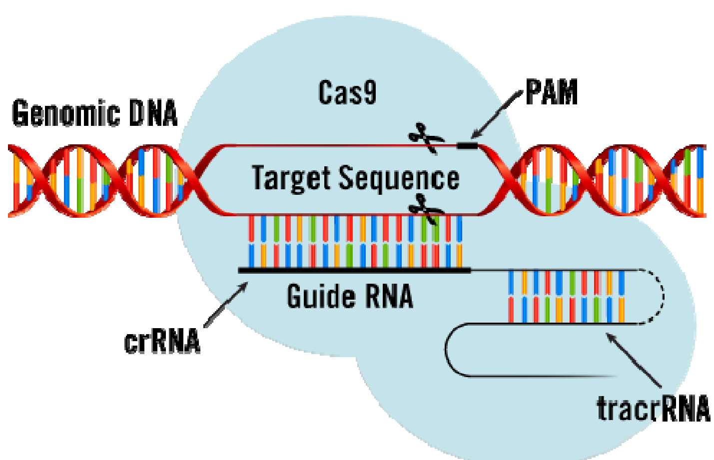

Figure 1. CRISPR/Cas system: Cas9 binds and accurately cuts genomic DNA via a guide RNA, enabling for useful genome engineering (adopted from [8])

Beside CRISPR/cas system, several more site-specific or site-directed nuclease system are described: meganucleases (MNs), zincfinger nucleases (ZFNs), transcription activatorlike effector nucleases (TALENs) [9].

Altering genomes through the use of genome-editing technology does not have a hazard that may arise from GMPs is uncontrolled integration of recombinant DNA into the genome. Random integration of transgene may result in interrupted or de-regulated genes of the host plant, and this may lead to the formation of unpredictable changes in host genome. The risks involved in are significantly lower than those associated with GM crops because most edits alter only a few nucleotides, producing changes that are quite similar to those found throughout naturally occurring populations [10].

It could be told that gene editing tools work like a biological find-and-replace function to cut out genes and splice in new ones. This technique is a highly promising for the development of potential treatments for genetic diseases. Recently, He Jiankui of Southern University of Science and Technology in Shenzhen, claimed he altered embryos for seven couples during fertility treatments, with one pregnancy resulting so far, with the goal was not to cure or prevent an inherited disease, but to try to bestow a trait that few people naturally have: an ability to resist possible future infection with HIV. This result raised a huge resistance in the scientific community, as this kind of gene editing is banned in most countries as the technology is still experimental and DNA changes can pass to future generations, potentially with unforeseen side-effects, as commented in Guardian journal.
"If true, this experiment is monstrous," said Julian Savulescu, a professor of practical ethics at the University of Oxford. "The embryos were healthy. No known diseases. Gene editing itself is experimental and is still associated with off-target mutations, capable of causing genetic problems early and later in life, including the development of cancer." [11]

However, when it comes to crops production, gene-editing tools seems more acceptable, when compared with GMOs, as the genetic changes produced by these methods are not different than ones naturally occurring. In contrast to "classical" GMOs that contain DNA from other organisms, which would not be found in nature, gene-editing leads to the outcome similar to the one obtained by the conventional breeding methods, just with significantly higher levels of efficiency and precision [5], [10].

3. GENE-EDITED CROPS VS. GMOS, LEGAL PERSPECTIVE

Since first description of transgenesis in 1970's, more than 20 years of research and regulation of genetically modified plants have passed since the first field trials in the EU took place. The Council of the European Communities adopted in 1990 Directive 90/219/EEC (for contained use of GMOs) and Directive 90/220/EEC (for deliberate release of GMOs) to protect human and animal health and the environment [1]. In 1996, commercial planting of GMPs started with approximately 1.7 million hectares worldwide, which had increased 100 fold by 2012. In contrast, the total area of cultivation of GMPs in Europe was a mere 129 000 hectares in 2012, due to strong societal and political opposition against agro-food biotechnology [12], [13]. All GMOs produced by transgenic procedures are regulated in the EU by Directive 2001/18/EC for cultivation and Regulation 1829/2003 for genetic modification (GM) food or feed [14].

In Directive 2001/18/EC, which replaced the older Directive 90/220/EEC, a GMO is defined as follows: 'Genetically modified organism (GMO) means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination'.

To repeat one more time, gene-editing technologies do not introduce foreign DNA into genome, it's just a modification of existing genes. Recently, in the journal Nature Genetics, Huang J. outlines the differences between gene-edited crops (GECs) and GMOs, and lays out some rough guidelines for how to regulate the GEC [15].
As clearly pointed, "A distinction must be established, particularly in the public sphere, between 'genetically modified organisms' (GMOs) generated through the transgenic introduction of foreign DNA sequences and 'genome-edited crops' (GECs) generated through precise editing of an organism's native genome."[15].
Importantly, the commentary's authors are proposing that we regulate the product, not the technology used to create it.

4. CONCLUSION

The number technologies for crop genetic improvement is raising every day. Elaborate transient transfer and expression techniques, as well as modern concepts such as synthetic genomics or reverse breeding, aided by sophisticated high-throughput analytical techniques, provides a set of superior tools to quickly and precisely alter the genomic sequences of plants. Using these techniques, potential adverse effects are even less likely than in conventional transgenic plants or plants resulting from conventional breeding

However, even if new technologies are reducing environmental and health risks of genetic manipulations, there is a strong opinion that gene-edited foods should be let off the hook completely — a numerous scientists believe that GECs should be subject to the same registration process as traditionally bred crop varieties, but they shouldn't be subjected to additional government oversight, which could be unnecessarily restrictive, they say.
As commented in Nature Genetics, "The potential benefits of GECs should not be impeded as a result of misinformation, so disclosure and education are the best ways to promote sound policies".

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