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Ethical, legal and social implications of genetically modified organism in the shadow of advanced genetic tools

Jasmin Šutković [1], Lejla Mahmutovic[1], Emina Huseinbegovic[1], Muhamed Adilovic[1], Fatima Sinanovic[1] Faruk Berat Akcesme[2]
[1]Faculty of Engineering and Natural Sciences, International University of Sarajevo, Bosnia
[2]Sağlık Bilimleri University, Faculty of Medicine, Turkey

ABSTRACT

In order to define the term GMO, different scientific definitions and legal explanations are available. In the regulation process of GM foods, the US and EU legal frameworks are based on the methodologies themselves. Currently, for the production of GMOs, several genome editing tools are available. Along with different site-directed nucleases (ZFN, TALENs, etc.), RNAi and CRISPR/Cas9 have proven to be the very effective tools for genome editing. According to the current EU legislative, introduced in 2018, CRISPR/Cas9 and RNAi techniques are regulated as methods that produce GMOs, because the methodology of the process itself resembles the traditional breeding methods. In the past few years, a large number of scientific publications have confirmed that CRISPR/Cas9 and RNAi technology produce GMOs, supporting and suggesting that the legislation policies in the EU and especially in the USA have to be elaborated. Besides, a huge public pressure makes it difficult to develop and implement new methodologies for GMO production. For this reason, ELSI society is responsible to investigate and question whether the new genetic engineering techniques produce GMO food that is safe for human consumption.

Keywords: Genome edition tools, GMO, Ethical, Legal and Social Implications (ELSI)

Corresponding Author:

Jasmin Šutković
Genetics and Bioengineering
Faculty of Natural Sciences and Engineering
International University of Sarajevo
Hrasnicka cesta 15
71210 Sarajevo
E-mail: jsutkovic@ius.edu.ba

1. Introduction

1.1. Genetically Modified Organisms (GMOs)

The first organisms with desired traits were produced thousands of years ago via traditional breeding methods involving mating species many times [1]. In order to produce a crop with the desired phenotype, this method took many years. The farmers from these times were not aware that they were manipulating the plant`s genetic material [2]. Such traditional breeding methods could be considered as the first gene manipulation that came along with the time of natural adaptation and mating species. Genetically Modified Organisms (GMOs) can be defined in various ways. In the EU directive, GMO is defined as an organism, except for human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination [3]. In the annex of GMO EU directive, each technique that is used for genetic modification was described as following: *(1)* "recombinant nucleic acid techniques involving the formation of new

combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation";(2) "techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation"; *(3)* "cell fusion (including protoplast fusion) or hybridization techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally" [3].

In many countries, legislations regarding GMOs have been established to guarantee the traceability of food/feed products on the market and to protect the consumer freedom of choice [4]. Flavrsavr tomato was the first commercial GMO product produced by Calgene Inc. in 1987. Based on Calgene Inc. requests, FDA approved the usage of commercial Flavrsavr tomato in 1994 for the US market [5]. However, the commercialization of biotech or genetically modified (GM) crops started in 1996. Since this date a 94-fold increase (from 1.7 million hectares in 1996 to 160 million hectares in 2011) was observed, making biotech crops the fastest adopted crop technology in the history of modern agriculture [6]. Some other examples of genetically modified (GM) foods include herbicide-resistant corn and soybeans - modified to tolerate the herbicide glyphosate found in Roundup, virus-resistant papaya - modified to be able to withstand the ringspot virus, and golden rice that produces beta-carotene - an antioxidant that the body can turn into vitamin A [7].

The use of these GMOs in the nation's food supply has received increasing media attention due to growing concerns regarding their safety. Various studies have called to question the safety of these foods. Chemical herbicides are substances engineered specifically for the purpose of killing or damaging undesirable plants [8], and their use has become popular worldwide. However, crops which result from genetic modifications, and are resistant to the chemicals, are classified as safe with no long-term studies available to provide an evidence base. Similarly, according to the WHO, GM foods found on the international market have passed safety assessments and do not pose risks for humans [9]. Also, no effects on human health were reported as a result of the consumption of such foods by the general population in the countries where they have been approved. There are concerns regarding the biosafety, ethics, and issues related to the release of GMOs in the environment. Many countries and NGOs have opposed the release of GMOs due to these reasons [10]. When considering all GMO related issues, the most important ones are about the safety of consumers and potential health risks. This review will summarize the current understandings of the three main issues regarding the use of genetic engineering in human food consumption.

1.2 Genome editing tools considered to produce GMO

Besides the traditional breeding methods, several genome editing technologies are available, all using the cell's endogenous repair system when specific genomic regions are altered. All of these techniques begin with the introduction of a targeted DNA double-stranded break at a predetermined locus using a sequence-specific nuclease. These techniques involve the use of zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and meganucleases[11].

1.2.1 Zinc-finger nucleases (ZFNs)

The zinc-finger domain is one of the most common types of DNA-binding motifs found in eukaryotes [12]. This fact enabled the ZFNs to effectively induce double-strand breaks within the cellular DNA region, affecting the DNA repair processes that lead to both targeted mutagenesis and targeted gene replacement at remarkably high frequencies [13]. The first success reported on plant genome engineering with ZFNs was in *Arabidopsis thaliana* and later on in *Nicotiana tabacum* [14], [15]. In 2009, the IPK1 gene that encodes the key enzyme for the phytic acid biosynthesis in maize was disrupted with the use of ZFNs, resulting in induced

herbicide tolerance [16]. Through the use of ZFNs, scientists have successfully introduced a point mutation in the Acetolactate Synthase (ALS) gene in tobacco, which is the target of sulfonylurea (SU) and imidazolinone (IMI) herbicides [17]. A study performed on Soybean (Glycine max) in 2011 demonstrated that ZFNs-based mutagenesis provides an efficient method for making mutations in duplicate genes that are otherwise difficult to study due to redundancy. In this study, the ZFNs induced mutations in DCL4a and DCL4b genes which are involved in seed development and plant growth [18]. Long-term use of these herbicides has led to the formation of herbicide-resistant weeds, which have a site-specific mutation mainly on the ALS gene [19]. However, ZFNs-based technology has some disadvantages, such as the complexity and high cost of protein domains construction for each particular genome locus. In addition, there is a possibility of inaccurate cleavage of target DNA due to single nucleotide substitutions or inappropriate interaction between domains [20].

1.2.2 Transcription activator-like effectors nucleases (TALENs)

TALENS operate on a similar principle as ZFNs. TALENs are proteins of bacterial origin, having the function of restriction enzymes [21]. Transcription activator-like proteins can recognize a unique DNA sequence, consisting of 17,5 tandem repeats on average, composed of 33-34 amino acid repeating motifs, followed by a spacer region and lastly by FOK1 restriction endonucleases [22]. There are several publicly available platforms for constructing TALENs or dTALEs. Such platforms include Golden Gate assembly methods for the seamless construction of TALE repeat arrays (11) and ligation-based systems (8). One of the main advantages of TALENs is that all engineered TALENs have multiple functionalities, making them the tool of choice for many genome engineering applications. Likewise, TALENs allow a researcher to target a DNA locus on average every 10 bp, which is more frequent than with use of ZFNs [18].

The TALENs technology has enabled the creation of several successful genetic modifications on the *Arabidopsis thaliana genome,* such as mutations in *ADH1, TT4, MAPKKK1, DSK2B, NATA2, and GLL22 genes* [23]. A year later, using the TALENs technology, the Soybean (*Glycine max*) lines that are low in polyunsaturated fats were generated. The modification was done by introducing mutations in two fatty acid desaturase 2 genes (*FAD2-1A* and *FAD2-1B*), which convert the monounsaturated fat, oleic acid, to the polyunsaturated fat, linoleic acid in the seed [24]. Another research performed on Barley (*Hordeum vulgare*) in 2013 showed that TALENs-induced double-stranded breaks have led to the introduction of short deletions at the target site, thus making the plant antibiotic-resistant [25].

In hexaploidy wheat bread, the S-gene was successfully knocked out using the TALENs and CRISPR/Cas9 system, resulting in the formation of fungi-resistant wheat [26]. In 2015, Voytas and coworkers designed TALENs constructs to mutate *Vascular Invertase genes*, known to convert sucrose to glucose and fructose in a potato tuber. This study has shown that at high temperatures and after cold storage the genetically modified potatoes produced fewer brown pigments and acrylamides compared to wild-type potatoes [27].

1.2.3 Meganucleases

Meganucleases have been used for more than 15 years to induce gene targeting, and are divided into five families based on sequence and structure motifs: LAGLIDADG, GIY-YIG, HNH, His-Cys box and PD-(D/E) XK [28]. The LAGLIDADG proteins are mostly used due to their availability (found in many kingdoms). LAGLIDADG proteins function as RNA maturases that are involved in facilitating the splicing of their intron, and as highly specific endonucleases capable of recognizing and cleaving the exon-exon junction sequences [29]. In 2009, Gao and his team showed that re-designed homing endonucleases are a useful engineering tool for the introduction of targeted mutations in a living organism, specifically a maize plant *Zea mays* [30].

In addition, this targeted mutagenesis procedure yielded small deletions and insertions at the Ems26 target site of the maize genome, thus changing the cytochrome P450-like gene (MS26) of the Male maize plant, and resulting in plant fertility [31]. Furthermore, through the biolistic manipulation of two herbicide tolerance genes (EPSPS and HPPD) in cotton by meganucleases, an herbicide-tolerant and insect-resistant cotton was created [32].

1.3 Tools for non-GMO production

1.3.1 RNA interference (RNAi)

RNAi is a technique that can selectively inhibit the function of specific genes at the posttranscriptional level. Double-stranded RNA molecules, like small interfering RNAs (siRNA) or microRNAs (miRNA) are considered as executors of RNAi in eukaryotic cells. Upon the cleavage by DICER, ≈22 nucleotides long dsRNA molecules bind the Argonaute protein, where the guide RNA is selected and remains bound to the Argonaute. Assuming the presence of several accessory proteins, this complex is known as RNA-induced silencing complex (RISC). In this manner, guide RNA strand leads RISC to the target mRNA, where the translation of a specific mRNA will be inhibited, or the Argonaute will catalyze the cleavage of target mRNA [33]. In that way, expression of specific genes can be silenced or reduced. Several minor crops were developed through the RNAi-mediated methods, inducing the virus resistance. An example of such engineering is the papaya plant resistant to the Ringspot Virus, grown in Hawaii, and the plum resistant to the Pox Virus. RNAi suppresses and inhibits the desired gene expression, as shown in Arabidopsis, Tobacco, Tomato, Cotton, and other organisms [34, 35, 36, 37]. In 2007, in tobacco and tomato, the expression of the Mshigene was controlled via RNAi [38]. Furthermore, a study from 2016 showed the RNAi-based methods succeeded to downregulate the gene expression of two genes in a small green aphid (*Myzuspersicae*) [39].

Since RNAi is based on sequence complementarity between siRNAs and mRNA, it can lead to potential silencing of genes other than targeted ones. Off-target effects, or popularly known as side effects, can occur in the GM plant itself or organisms exposed to it [40]. One of the conclusions from the European Food Safety Authority (EFSA) workshop held in 2014 was that the bioinformatics analyses could be an important factor in the risk assessment of RNAi-based GM plants [40]. However, information obtained from bioinformatics analysis cannot be reliably used on its own for predicting the presence of RNAi activity at present [41], due to the lack of genomic data and insufficient knowledge of mechanisms governing mRNA–siRNA interactions [42].

1.3.2 CRISPR/Cas9

CRISPR/Cas9 system is the unique mechanism providing organism protection against foreign DNA [12] via RNA-guided DNA cleavage. In the CRISPR/Cas9 system, short segments of foreign DNA termed ‘spacers’ are integrated within the CRISPR genomic loci, transcribed and processed into short CRISPR RNA (crRNA) [12, 20]. These crRNAs anneal to transactivating crRNAs (tracrRNAs), direct sequence-specific cleavage, and silencing of pathogenic DNA by Cas9 proteins. A study from 2015 has shown that target recognition by the Cas9 protein requires a conserved dinucleotide-containing PAM sequence upstream of the crRNA- binding region [20,43,44]. In this way, the CRISPR/Cas9 system can be retargeted to cleave virtually any DNA sequence by redesigning the crRNA. Importantly, the CRISPR/Cas9 system has been shown as directly portable to human cells by co-delivery of plasmids expressing the Cas9 endonuclease and the necessary crRNA components [43], [44]. According to the previous studies, CRISPR/Cas9-mediated genome editing has been successfully demonstrated in zebrafish and bacterial cells [12, 20]. In addition, the type II CRISPR/Cas9 system is currently the most widely used, owing to its high efficiency and simplicity, with alternatives such as

the CRISPR-Cpf1 system that offers numerous future perspectives [45]. Actually, the CRISPR-Cpf1 system has been already used as a genome editing tool in bacteria, plants, mammalian cells, *Drosophila melanogaster*, frogs, zebrafish, etc. [46]. Using CRISPR/Cas9, the genome modification in the following plants was achieved: *Arabidopsis thaliana*, *Nicotiana benthamiana*, *Tobacco*, *Sorghum*, *Oryza sativa*, *Triticumaestivum*, Sweet orange, and *Zea mays* [47]. The most common implications of the CRISPR/Cas9 technique assume research in the field of human genetic disorders, cancer treatment, and targeted therapies [48, 49].

The Netherlands Commission on Genetic Modification (COGEM) report on CRISPR/Cas was that CRISPR-Cas is a novel genome editing technique that is rapidly adopted in science and applications of this technique fall under the legislation on GMOs, within the current EU legal framework. On the other hand, CRISPR-Cas can be used for different purposes, and some of them should qualify to be exempted from the regulations. New techniques such as CRISPR-Cas and other new breeding techniques (oligonucleotide-directed mutagenesis, cis-genesis, RNA-dependent DNA methylation, and others) are showing that the current EU GMO legislation is due for revision and the COGEM suggested that Dutch Government and the European Commission should consider revising the EU legislation on GMOs, especially to strengthen the transparency of the risk assessment process [50]. Making a decision on subjecting the applications and products of CRISPR-Cas9 to the GMO regulations can impact the innovation within the EU and the European company's economic position.

The RNAi and CRISPR/Cas9 technology, as new plant breeding techniques (NPBTs), are faster than traditional breeding methods. Modification of plants with the use of NPBTs results in plants that are identical to the ones that are classically bred, indicating that these techniques should be evaluated according to the final product rather than the creation process itself. Concluding, Government regulations for GM foods vary, with relaxed policies in the U.S. focusing on the final food product, to strict rules in the EU that consider the genetic engineering process used to make the food [51, 52]. The NPBTs are under revisions by advisory and regulatory authorities concerning the GMO legislation in the EU and USA, in order to develop an appropriate science-based risk assessment procedure, within existing plant protection products legislation [52].

2. Ethical, legal and social implications and GMO

The most important aspect of ELSI is to consider possible side effects GMOs could have both for humans as well as for the environment [53]. If we interfere with natural processes without adequate preparation and research, the natural homeostasis may be disturbed while editing plant genes, which has been functioning for thousands of years as such, The natural homeostasis might be disrupted even if we are not working with delicate things such as the DNA [54]. Disrupting this balance can create a lot of negative consequences for the environment and living organisms present. One such example is the transfer of specific species, so-called invasive species, into a new habitat, which in turn disrupts the natural balance [55,56, 57]. While GMOs have generally been labeled as safe and there are benefits tied to them [58], some examples of GMOs side effects tell us that we have to put the in-depth study of possible side effects for modifications to organisms' DNA as a requirement before releasing them out of the laboratory. This is, unfortunately, being hindered by the practice of today's companies that prevent the scientists from performing further tests on their products [59], which is another reason why the legal framework has to support such activities for the benefits of everyone.

Even though GMOs are made in order to provide additional benefits to humans, GM foods are becoming an increasing public health risk due to the microbial and chemical contamination, food adulteration, additives, mislabeling, food allergens, etc. [60, 61]. Moreover, increased antibiotic resistance of bacteria also poses a threat since antibiotic resistance genes are commonly used in almost any genetic engineering experiments as selection method [62, 63]. Additionally, there are concerns from an economical perspective since GMO technology is expensive and as such is used by large companies widening the gap between them and local farmers [64]. In general, one of the main downsides of GMO is a counterargument for every benefit,

especially since there is also a lack of scientific data as an answer to these counterarguments. For example, increased pesticide resistance is a benefit of GMO. However, this results in increased pesticide usage in nature.

Furthermore, GMO production results in bigger and better BIOTECH companies, but these companies are gradually creating a monopoly in agriculture. For example, current research shows that the ten most developed GMO companies hold more than two-thirds of the global proprietary seed market [59].

While some ethical concerns related to GMO may not be the product of raw scientific data, they are still important since they reflect the opinions of consumers which are the most critical and the most numerous part of the population having an influence on and being affected by the GMOs [65]. It is important to protect consumers since the threat of hunger outweighs the threat of potential consequences of GMO, and people in need will choose to eat GMO in order to live, even though they might be in the long-term risk [65]. Despite the risks of GMO, people with lower economical income will not be able to afford more expensive "organic" food, while those with good economic status will be able to avoid GMO completely. Consequently, this raises many social issues. This data leads to additional legal issues that dysfunctional governments and economies are the ones that hinder both production and distribution of food making it inaccessible to the ones in need [66].

When considering GMO related issues, the most important ones are the safety of consumers and potential health risks. Thus, labeling of the GMO products which contain at least 0.9% of modified protein expression is mandatory [67]. However, there are additional issues related to the labeling itself [68, 69]. For example, a recent study performed at Western Kentucky University suggests that many products labeled as "organic" or "non-GMO" actually contain genetic modifications. Namely, 75% of soy and 83% of corn containing USDA Organic Certification contained evidence of genetic modification [69].

3. Discussion and conclusion

New Breeding Techniques are a group of molecular gene editing techniques that can introduce new traits in agricultural plants [70]. Most of these techniques are based on site-directed Nucleases (ZFNs, TALENs, meganucleases), RNAi, and CRISPR/Cas9 technology. However, CRISPR/Cas9 and RNAi among all named methods have shown sufficient efficiency in the production of GMOs and therefore are commercially available since 2015. The modified organisms produced by these two technologies are not termed as transgenic organisms, as it is traditionally known and accepted by the public, where no foreign gene was implanted into the host genome, but only a slight change was induced (what may occur in nature as well). These methods are operating with the genes by turning them on or off or completely removing them from the genome, in this way resulting in the desired phenotype. However, there is still a need for the development of the regulatory status of employment of these techniques in GMO production, particularly in regards to worldwide regulations on Genetically Modified Organisms [70]. Some plants developed using RNAi and CRISPR/Cas are already commercially available, and they were not legally regulated. For example, the genetically modified mushroom, which was the first CRISPR-edited organism that passed the regulation processes but its production was not regulated by the US Department of Agriculture (USDA) [71]. The agency has also previously determined that it will not regulate several crops developed using zinc-finger nucleases and TALENs. Calyxt, a plant biotechnology company in Minnesota, for example, used TALENs to edit a single gene in the parent plant and produce a variety of wheat with improved resistance against powdery mildew [72]. There is still a lack of clarity over how products of the new plant-breeding technologies will be regulated around the world. Even though the US FDA has stated that at least five products generated using genome editing are not regulated products in the US, some non-governmental organizations published an open letter to the European Commission with an urge to ensure that: "organisms produced by these new techniques will be regulated as genetically modified organisms under existing EU regulations" [3,73]. The US

Department of Agriculture has indicated that they will not consider the GE plants without any foreign DNA as GMOs.

In regards to RNAi technology, certain studies showed that this technology can be used to improve certain aspects of agriculture, such as improving disease resistance, insect and nematode resistance, and resistance against parasitic weeds. Furthermore, it can be used for improving drought tolerance and nutritional value, for metabolic engineering, improving forage digestibility, biomass and grain yield, improving biofuel production, etc. Risk assessment of the RNAi itself is rarely formally considered unless the dsRNA made by the GM plant is supposed to act as a pesticide [74]. The safety evaluation of GM plant varieties that are developed and made using RNAi technology and foods that are derived from them are discussed in the context of GE crop safety assessment within the Food and Drug Administration (FDA) Centers in manuscript on protein safety in GE crops [41]. Risks of consuming the siRNA GM crops are triggered by the indications that any nucleic acids that are present in consumable food can be digested and end up in the bloodstream and organs. Therefore, there is a risk that these dsRNAs can interact with human genes and have an effect on their expression, which can be even heritable. The process of assessment for GE plant varieties developed using RNAi is similar to the one for transgenic proteins, with the addition that safety assessment for RNAi possesses one unique element, which is that it is not intended for a new protein to be expressed from the RNAi sequence element. A large number of small, non-coding, regulatory RNAs are naturally expressed in plants and they can be consumed by humans and animals [41].

While making decisions about the legal assessment of GM varieties, one of the points to consider is whether the externally produced heritable genetic material is introduced into the organism. If this point is true, that makes the particular technique as the one which falls under the provisions of Article 2(2) and Annex I A part I of EU Directive 2001/18 [50]. If we take CrispCas9 as an example, within the EU directive in technical means, this genome editing tool generally cannot be regarded as a mutagenesis tool [50]. Furthermore, in the last few years the public awareness about GMOs has increased, leading to more social and public media debates, which end in the conclusion that organic food is much safer than GMO food [75, 76]. In the last few years, several scientific studies have shown that the GMOs are not only organisms with transgenic genes but also organisms that have their phenotypic characteristics altered due to simple changes in their genomes, by using RNAi and Crisp-Cas9 technology.

Due to the lack of clear and concise regulations in the US on the production of GMO, the GMOs edited with RNAi and Crisp-Cas9 technologies, are not classified as GMOs. Therefore, these genetically edited plants are not subjected to the same time-consuming, rigorous approval process that genetically modified crops are going through, and could reach the market much more quickly; without the need to label the GMO product [77].

There are indeed a lot of precaution measures implemented in order to make GMO safer. However, it is not treated equally in all parts of the world and laws differ from country to country[67,68, 69]. Appropriate view of GMOs will also aim to protect all participants economically including small companies and local farmers, not only large biotech companies. This is partially due to the fact that awareness will be raised among people; the general public will have a better knowledge of GMOs and a better understanding of the differences in food production. Thinking globally, GMO controversy also teaches us not to neglect other points that can be improved. Namely, by better food distribution, we can partially solve a world's hunger problem, without even relying on the GMO in the first place.

The main idea is that we should take a moderate stand, while there are many benefits from GMO, there are also potential risks and a lot of counter-arguments. These are the reasons why information regarding GMOs should be transparent and available to the public in order to give them the options to choose from.

In all of these regards, we support the proposal of new amendments to the current laws, especially in the US, so that the public gains full benefits from the latest advances in plant genetics and genomic methodologies for GMO production.

References

[1] E. Bellucci *et al.*, "Population Structure of Barley Landrace Populations and Gene-Flow with Modern Varieties," *PLoS ONE*, vol. 8, no. 12, p. e83891, Dec. 2013, doi: 10.1371/journal.pone.0083891.

[2] "History of Plant Breeding," in *Principles of Plant Genetics and Breeding*, Chichester, UK: John Wiley & Sons, Ltd, 2012, pp. 22–39.

[3] "Directive 2001/18/EC of the European Parliament 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 - Commission Declaration," Europian Union, Official Journal of the European Communities.

[4] M.-A. Fraiture, P. Herman, I. Taverniers, M. De Loose, D. Deforce, and N. H. Roosens, "Current and New Approaches in GMO Detection: Challenges and Solutions," *BioMed Res. Int.*, vol. 2015, pp. 1–22, 2015, doi: 10.1155/2015/392872.

[5] R. Meyer, "Nachweis gentechnologisch vernderter Pflanzen mittels der Polymerase Kettenreaktion (PCR) am Beispiel derFlavr Savr TM-Tomate," *Z. Fr Lebensm.-Unters. -Forsch.*, vol. 201, no. 6, pp. 583–586, Nov. 1995, doi: 10.1007/BF01201590.

[6] S. R. M. Broeders, S. C. J. De Keersmaecker, and N. H. C. Roosens, "How to Deal with the Upcoming Challenges in GMO Detection in Food and Feed," *J. Biomed. Biotechnol.*, vol. 2012, pp. 1–11, 2012, doi: 10.1155/2012/402418.

[7] X. K. Morin, "Genetically modified food from crops: progress, pawns, and possibilities," *Anal. Bioanal. Chem.*, vol. 392, no. 3, pp. 333–340, Oct. 2008, doi: 10.1007/s00216-008-2313-4.

[8] M. Manno, "Herbicides," in *Human Toxicology*, Elsevier, 1996, pp. 551–560.

[9] "Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance : scientific assessment." World Health Organization, 2004.

[10] B. M. Maghari and A. M. Ardekani, "Genetically modified foods and social concerns," *Avicenna J. Med. Biotechnol.*, vol. 3, no. 3, pp. 109–117, Jul. 2011.

[11] A. E. Ricroch and M.-C. Hénard-Damave, "Next biotech plants: new traits, crops, developers and technologies for addressing global challenges," *Crit. Rev. Biotechnol.*, pp. 1–16, Feb. 2015, doi: 10.3109/07388551.2015.1004521.

[12] A. A. Nemudryi, K. R. Valetdinova, S. P. Medvedev, and S. M. Zakian, "TALEN and CRISPR/Cas Genome Editing Systems: Tools of Discovery," *Acta Naturae*, vol. 6, no. 3, pp. 19–40, Jul. 2014.

[13] D. Carroll, "Genome Engineering With Zinc-Finger Nucleases," *Genetics*, vol. 188, no. 4, pp. 773–782, Aug. 2011, doi: 10.1534/genetics.111.131433.

[14] A. Lloyd, C. L. Plaisier, D. Carroll, and G. N. Drews, "Targeted mutagenesis using zinc-finger nucleases in Arabidopsis," *Proc. Natl. Acad. Sci.*, vol. 102, no. 6, pp. 2232–2237, Feb. 2005, doi: 10.1073/pnas.0409339102.

[15] D. A. Wright *et al.*, "Standardized reagents and protocols for engineering zinc finger nucleases by modular assembly," *Nat. Protoc.*, vol. 1, no. 3, pp. 1637–1652, Aug. 2006, doi: 10.1038/nprot.2006.259.

[16] V. K. Shukla *et al.*, "Precise genome modification in the crop species Zea mays using zinc-finger nucleases," *Nature*, vol. 459, no. 7245, pp. 437–441, May 2009, doi: 10.1038/nature07992.

[17] J. A. Townsend *et al.*, "High-frequency modification of plant genes using engineered zinc-finger nucleases," *Nature*, vol. 459, no. 7245, pp. 442–445, May 2009, doi: 10.1038/nature07845.

[18] S. J. Curtin *et al.*, "Targeted Mutagenesis of Duplicated Genes in Soybean with Zinc-Finger Nucleases," *Plant Physiol.*, vol. 156, no. 2, pp. 466–473, Jun. 2011, doi: 10.1104/pp.111.172981.

[19] S. B. Powles and Q. Yu, "Evolution in Action: Plants Resistant to Herbicides," *Annu. Rev. Plant Biol.*, vol. 61, no. 1, pp. 317–347, Jun. 2010, doi: 10.1146/annurev-arplant-042809-112119.

[20] T. Gaj, C. A. Gersbach, and C. F. Barbas, "ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering," *Trends Biotechnol.*, vol. 31, no. 7, pp. 397–405, Jul. 2013, doi: 10.1016/j.tibtech.2013.04.004.

[21] J. Boch and U. Bonas, "*Xanthomonas* AvrBs3 Family-Type III Effectors: Discovery and Function," *Annu. Rev. Phytopathol.*, vol. 48, no. 1, pp. 419–436, Jul. 2010, doi: 10.1146/annurev-phyto-080508-081936.

[22] J. D. Sander and J. K. Joung, "CRISPR-Cas systems for editing, regulating and targeting genomes," *Nat. Biotechnol.*, vol. 32, no. 4, pp. 347–355, Apr. 2014, doi: 10.1038/nbt.2842.

[23] M. Christian, Y. Qi, Y. Zhang, and D. F. Voytas, "Targeted Mutagenesis of *Arabidopsis thaliana* Using Engineered TAL Effector Nucleases," *G3amp58 GenesGenomesGenetics*, vol. 3, no. 10, pp. 1697–1705, Oct. 2013, doi: 10.1534/g3.113.007104.
[24] W. Haun *et al.*, "Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family," *Plant Biotechnol. J.*, vol. 12, no. 7, pp. 934–940, Sep. 2014, doi: 10.1111/pbi.12201.
[25] T. Wendt *et al.*, "TAL effector nucleases induce mutations at a pre-selected location in the genome of primary barley transformants," *Plant Mol. Biol.*, vol. 83, no. 3, pp. 279–285, Oct. 2013, doi: 10.1007/s11103-013-0078-4.
[26] Y. Wang *et al.*, "Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew," *Nat. Biotechnol.*, vol. 32, no. 9, pp. 947–951, Sep. 2014, doi: 10.1038/nbt.2969.
[27] B. M. Clasen *et al.*, "Improving cold storage and processing traits in potato through targeted gene knockout," *Plant Biotechnol. J.*, vol. 14, no. 1, pp. 169–176, Jan. 2016, doi: 10.1111/pbi.12370.
[28] J. P. Gogarten and E. Hilario, "Inteins, introns, and homing endonucleases: recent revelations about the life cycle of parasitic genetic elements," *BMC Evol. Biol.*, vol. 6, no. 1, p. 94, 2006, doi: 10.1186/1471-2148-6-94.
[29] B. L. Stoddard, "Homing Endonucleases: From Microbial Genetic Invaders to Reagents for Targeted DNA Modification," *Structure*, vol. 19, no. 1, pp. 7–15, Jan. 2011, doi: 10.1016/j.str.2010.12.003.
[30] H. Gao *et al.*, "Heritable targeted mutagenesis in maize using a designed endonuclease," *Plant J.*, vol. 61, no. 1, pp. 176–187, Jan. 2010, doi: 10.1111/j.1365-313X.2009.04041.x.
[31] V. Djukanovic *et al.*, "Male-sterile maize plants produced by targeted mutagenesis of the cytochrome P450-like gene (MS26) using a re-designed I-CreI homing endonuclease," *Plant J. Cell Mol. Biol.*, vol. 76, no. 5, pp. 888–899, Dec. 2013, doi: 10.1111/tpj.12335.
[32] K. D'Halluin *et al.*, "Targeted molecular trait stacking in cotton through targeted double-strand break induction," *Plant Biotechnol. J.*, vol. 11, no. 8, pp. 933–941, Oct. 2013, doi: 10.1111/pbi.12085.
[33] G. J. Hannon, "RNA interference," *Nature*, vol. 418, no. 6894, pp. 244–251, Jul. 2002, doi: 10.1038/418244a.
[34] D. Beris, G. Kapolas, P. Livanos, A. Roussis, D. Milioni, and K. Haralampidis, "RNAi-mediated silencing of the Arabidopsis thaliana ULCS1 gene, encoding a WDR protein, results in cell wall modification impairment and plant infertility," *Plant Sci.*, vol. 245, pp. 71–83, Apr. 2016, doi: 10.1016/j.plantsci.2016.01.008.
[35] W. G. Burke, E. Kaplanoglu, I. Kolotilin, R. Menassa, and C. Donly, "RNA Interference in the Tobacco Hornworm, Manduca sexta, Using Plastid-Encoded Long Double-Stranded RNA," *Front. Plant Sci.*, vol. 10, p. 313, Mar. 2019, doi: 10.3389/fpls.2019.00313.
[36] U. e Ammara, S. Mansoor, M. Saeed, I. Amin, R. W. Briddon, and A. M. Al-Sadi, "RNA interference-based resistance in transgenic tomato plants against Tomato yellow leaf curl virus-Oman (TYLCV-OM) and its associated betasatellite," *Virol. J.*, vol. 12, no. 1, p. 38, Dec. 2015, doi: 10.1186/s12985-015-0263-y.
[37] A. Ahmad *et al.*, "Solubility, solubility parameters and solution thermodynamics of thymoquinone in different mono solvents," *J. Mol. Liq.*, vol. 272, pp. 912–918, Dec. 2018, doi: 10.1016/j.molliq.2018.10.104.
[38] A. P. S. Sandhu, R. V. Abdelnoor, and S. A. Mackenzie, "Transgenic induction of mitochondrial rearrangements for cytoplasmic male sterility in crop plants," *Proc. Natl. Acad. Sci.*, vol. 104, no. 6, pp. 1766–1770, Feb. 2007, doi: 10.1073/pnas.0609344104.
[39] M. Mulot *et al.*, "Comparative Analysis of RNAi-Based Methods to Down-Regulate Expression of Two Genes Expressed at Different Levels in Myzus persicae," *Viruses*, vol. 8, no. 11, p. 316, Nov. 2016, doi: 10.3390/v8110316.
[40] M. Ramon *et al.*, "RNAi-based GM plants: food for thought for risk assessors," *Plant Biotechnol. J.*, vol. 12, no. 9, pp. 1271–1273, Dec. 2014, doi: 10.1111/pbi.12305.
[41] J. H. Sherman, T. Munyikwa, S. Y. Chan, J. S. Petrick, K. W. Witwer, and S. Choudhuri, "RNAi technologies in agricultural biotechnology: The Toxicology Forum 40th Annual Summer Meeting," *Regul. Toxicol. Pharmacol.*, vol. 73, no. 2, pp. 671–680, Nov. 2015, doi: 10.1016/j.yrtph.2015.09.001.
[42] J. M. Casacuberta, Y. Devos, P. du Jardin, M. Ramon, H. Vaucheret, and F. Nogué, "Biotechnological uses of RNAi in plants: risk assessment considerations," *Trends Biotechnol.*, vol. 33, no. 3, pp. 145–147, Mar. 2015, doi: 10.1016/j.tibtech.2014.12.003.

[43] E. M. Anderson *et al.*, "Systematic analysis of CRISPR–Cas9 mismatch tolerance reveals low levels of off-target activity," *J. Biotechnol.*, vol. 211, pp. 56–65, Oct. 2015, doi: 10.1016/j.jbiotec.2015.06.427.
[44] J. M. Smith, *Genetic roulette: the documented health risks of genetically engineered foods*. Fairfield, Iowa: Yes! Books, 2007.
[45] S. Huang, D. Weigel, R. N. Beachy, and J. Li, "A proposed regulatory framework for genome-edited crops," *Nat. Genet.*, vol. 48, no. 2, pp. 109–111, Feb. 2016, doi: 10.1038/ng.3484.
[46] F. Safari, K. Zare, M. Negahdaripour, M. Barekati-Mowahed, and Y. Ghasemi, "CRISPR Cpf1 proteins: structure, function and implications for genome editing," *Cell Biosci.*, vol. 9, no. 1, p. 36, Dec. 2019, doi: 10.1186/s13578-019-0298-7.
[47] N. J. Baltes and D. F. Voytas, "Enabling plant synthetic biology through genome engineering," *Trends Biotechnol.*, vol. 33, no. 2, pp. 120–131, Feb. 2015, doi: 10.1016/j.tibtech.2014.11.008.
[48] H. Li, Y. Yang, W. Hong, M. Huang, M. Wu, and X. Zhao, "Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects," *Signal Transduct. Target. Ther.*, vol. 5, no. 1, p. 1, Dec. 2020, doi: 10.1038/s41392-019-0089-y.
[49] S. Baliou *et al.*, "CRISPR therapeutic tools for complex genetic disorders and cancer (Review)," *Int. J. Oncol.*, Jun. 2018, doi: 10.3892/ijo.2018.4434.
[50] C. Bruetschy, "The EU regulatory framework on genetically modified organisms (GMOs)," *Transgenic Res.*, vol. 28, no. Suppl 2, pp. 169–174, 2019, doi: 10.1007/s11248-019-00149-y.
[51] K. Chen, Y. Wang, R. Zhang, H. Zhang, and C. Gao, "CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture," *Annu. Rev. Plant Biol.*, vol. 70, no. 1, pp. 667–697, Apr. 2019, doi: 10.1146/annurev-arplant-050718-100049.
[52] B. Mezzetti *et al.*, "RNAi: What is its position in agriculture?," *J. Pest Sci.*, May 2020, doi: 10.1007/s10340-020-01238-2.
[53] D. Macer, "Ethical, legal and social issues of genetically modifying insect vectors for public health," *Insect Biochem. Mol. Biol.*, vol. 35, no. 7, pp. 649–660, Jul. 2005, doi: 10.1016/j.ibmb.2005.02.010.
[54] M. Worall, "Homeostasis in nature: Nest building termites and intelligent buildings," *Intell. Build. Int.*, vol. 3, no. 2, pp. 87–95, Jul. 2011, doi: 10.1080/17508975.2011.582316.
[55] M. I. Westphal, M. Browne, K. MacKinnon, and I. Noble, "The link between international trade and the global distribution of invasive alien species," *Biol. Invasions*, vol. 10, no. 4, pp. 391–398, Apr. 2008, doi: 10.1007/s10530-007-9138-5.
[56] L. Timar and D. J. Phaneuf, "Modeling the human-induced spread of an aquatic invasive: The case of the zebra mussel," *Ecol. Econ.*, vol. 68, no. 12, pp. 3060–3071, Oct. 2009, doi: 10.1016/j.ecolecon.2009.07.011.
[57] D. Lassuy R. and P. Lewis N., "Invasive Species - Human Induced. Arctic Biodiversity Assessment," in *Arctic Biodiversity Assessment: Status and Trends in Arctic Biodiversity*, Conservation of Arctic Flora and Fauna, pp. 559–565.
[58] A. Bakshi, "Potential Adverse Health Effects of Genetically Modified Crops," *J. Toxicol. Environ. Health Part B*, vol. 6, no. 3, pp. 211–225, Jan. 2003, doi: 10.1080/10937400306469.
[59] S. Mueller, "Are Market GM Plants an Unrecognized Platform for Bioterrorism and Biocrime?," *Front. Bioeng. Biotechnol.*, vol. 7, p. 121, 2019, doi: 10.3389/fbioe.2019.00121.
[60] Z. Gizaw, "Public health risks related to food safety issues in the food market: a systematic literature review," *Environ. Health Prev. Med.*, vol. 24, no. 1, p. 68, Nov. 2019, doi: 10.1186/s12199-019-0825-5.
[61] A. M. Branum and S. L. Lukacs, "Food Allergy Among Children in the United States," *PEDIATRICS*, vol. 124, no. 6, pp. 1549–1555, Dec. 2009, doi: 10.1542/peds.2009-1210.
[62] T. Midtvedt, "Antibiotic resistance and genetically modified plants," *Microb. Ecol. Health Dis.*, vol. 25, no. 0, Sep. 2014, doi: 10.3402/mehd.v25.25918.
[63] M. Woegerbauer *et al.*, "Antibiotic resistance marker genes as environmental pollutants in GMO-pristine agricultural soils in Austria," *Environ. Pollut.*, vol. 206, pp. 342–351, Nov. 2015, doi: 10.1016/j.envpol.2015.07.028.
[64] T. Philips, "Genetically Modified Organisms (GMOs): Transgenic Crops and Recombinant DNA Technology," *Nat. Educ.*, vol. 1, no. 1, p. 213, 2008.
[65] B. E. Carter, C. C. Conn, and J. R. Wiles, "Concern About Hunger May Increase Receptivity to GMOs," *Trends Plant Sci.*, vol. 21, no. 7, pp. 539–541, 2016, doi: 10.1016/j.tplants.2016.05.003.
[66] A. Kumar, "A Comprehensive Assessment and Perception of Genetically Modified Foods," *J. Genet. Syndr. Gene Ther.*, vol. 2, no. 3, 2011, doi: 10.4172/2157-7412.1000110.

[67] R. Lee, "The Regulation of Genetically Modified Organisms: Comparative Approaches. Edited by LUC BODIGUEL and MICHAEL CARDWELL (eds)," *J. Environ. Law*, vol. 22, no. 3, pp. 511–514, Jan. 2010, doi: 10.1093/jel/eqq015.
[68] D. A. Yeh, M. I. Gómez, and H. M. Kaiser, "Signaling impacts of GMO labeling on fruit and vegetable demand," *PloS One*, vol. 14, no. 10, p. e0223910, 2019, doi: 10.1371/journal.pone.0223910.
[69] A. A. Moghissi, L. M. Jaeger, D. Shafei, and L. L. Bloom, "Regulatory science requirements of labeling of genetically modified food," *Crit. Rev. Biotechnol.*, vol. 38, no. 3, pp. 386–393, May 2018, doi: 10.1080/07388551.2017.1356804.
[70] A. I. Whelan and M. A. Lema, "Regulatory framework for gene editing and other new breeding techniques (NBTs) in Argentina," *GM Crops Food*, vol. 6, no. 4, pp. 253–265, Oct. 2015, doi: 10.1080/21645698.2015.1114698.
[71] E. Waltz, "Gene-edited CRISPR mushroom escapes US regulation," *Nature*, vol. 532, no. 7599, pp. 293–293, Apr. 2016, doi: 10.1038/nature.2016.19754.
[72] H. Ledford, "Gene-editing surges as US rethinks regulations," *Nature*, vol. 532, no. 7598, pp. 158–159, Apr. 2016, doi: 10.1038/532158a.
[73] H. D. Jones, "Are plants engineered with CRISPR technology genetically modified organisms?," *The Biochemist*, vol. 38, no. 3, pp. 14–17, Jun. 2016, doi: 10.1042/BIO03803014.
[74] J. A. Heinemann, S. Z. Agapito-Tenfen, and J. A. Carman, "A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments," *Environ. Int.*, vol. 55, pp. 43–55, May 2013, doi: 10.1016/j.envint.2013.02.010.
[75] A. Mie *et al.*, "Human health implications of organic food and organic agriculture: a comprehensive review," *Environ. Health Glob. Access Sci. Source*, vol. 16, no. 1, p. 111, 27 2017, doi: 10.1186/s12940-017-0315-4.
[76] S. Hurtado-Barroso, A. Tresserra-Rimbau, A. Vallverdú-Queralt, and R. M. Lamuela-Raventós, "Organic food and the impact on human health," *Crit. Rev. Food Sci. Nutr.*, vol. 59, no. 4, pp. 704–714, 2019, doi: 10.1080/10408398.2017.1394815.
[77] J. Lau, "Same Science, Different Policies: Regulating Genetically Modified Foods in the U.S. and Europe," *Science in the news Harward University*, Aug. 09, 2015.