Food and Agriculture Organization of the United Nations

Gene editing and food safety

Technical considerations and potential relevance to the work of Codex Alimentarius

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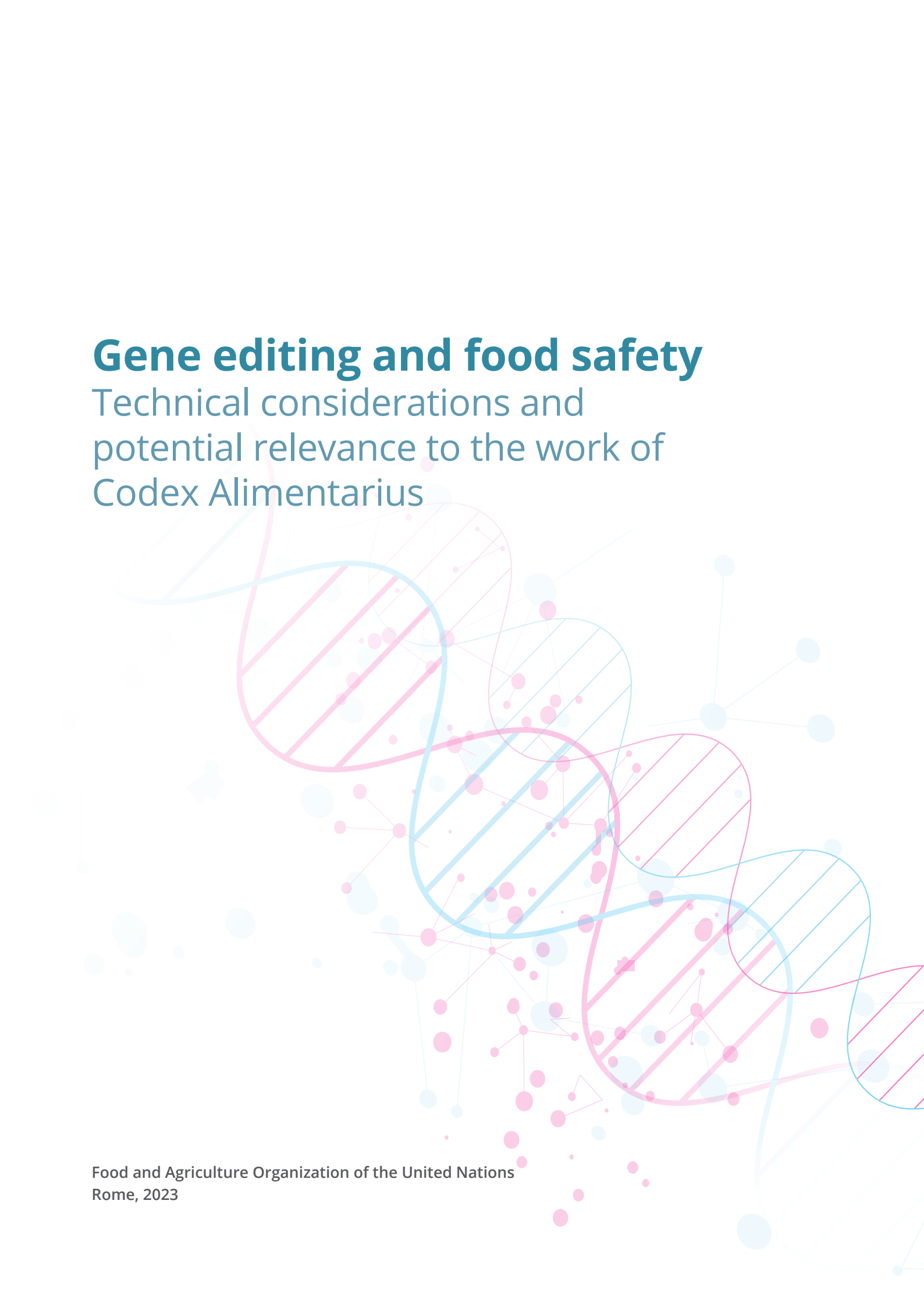

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Contents

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Abbreviations and acronyms

ALV	avian leukosis virus
CAC	Codex Alimentarius Commission
CBD	Convention on Biological Diversity
CRISPR	clustered regularly interspaced short palindromic repeats
CTNBio	National Technical Biosafety Commission of Brazil
DNA	Deoxyribonucleic acid
EPA (NZ)	Environmental Protection Authority (of New Zealand)
FDA	United States Food and Drug Administration
GABA	gamma-aminobutyric acid
GMO	genetically modified organism
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
HDL	high-density lipoprotein
IAEA	International Atomic Energy Agency
ICA	Agricultural Institute of Columbia
ODM	oligonucleotide-directed mutagenesis
LDL	low-density lipoprotein
LMIC	low- and middle-income countries
LMO	living modified organism
MAFF	Ministry of Agriculture, Forestry and Fisheries - Japan
MHLW	Ministry of Health, Labour and Welfare - Japan
MoE	Ministry of Environment - Japan
NBA	National Biosafety Authority - Kenya
NBMA	National Biosafety Management Agency - Nigeria
NBT	new breeding techniques
NCBP	National Committee on Biosafety - the Philippines
PBI	Plant Breeding Innovations
RNA	Ribonucleic acid
SAG	Service for Agriculture and Livestock - Chile
SDN	Site-directed nucleases
SENASA	National Service of Agri-Food Health and Safety - Honduras

TALEN	transcription activator-like effector nucleases
USDA	United States Department of Agriculture
US EPA	United States Environmental Protection Agency
WHO	World Health Organization
WTO	World Trade Organization

Executive summary

Gene (or genome) editing is an umbrella term for various techniques based in molecular biology used for introducing targeted changes in the genome of living organisms. These techniques are used for numerous reasons including to breed new plant varieties, animal breeds, and microbial strains for agricultural purposes. They can potentially develop diverse traits to increase food production and quality, as well as contributing towards sustainability and climate change resilience. However, since these are innovative breeding techniques, they are also subject to scrutiny by regulatory bodies worldwide.

There are ongoing national and international discussions about the most appropriate forms of regulations to cover such techniques. Current policymaking efforts in this regard focus on the various technical issues including food safety as one of the priority areas. This report provides a review of food safety related issues in applying gene editing for food production, including the applicability of existing Codex Alimentarius principles and guidelines for relevant food safety assessments and it offers some key considerations for developing and implementing policies and regulatory criteria for products derived from gene editing.

This report also highlights areas where there are opportunities for national competent authorities to benefit from the existing and ongoing work of the Food and Agriculture Organization of the United Nations (FAO) and Codex Alimentarius and for scientific advice, capacity development, knowledge transfers and information exchanges.

Keywords: food safety, gene editing, genome editing, technology, biotechnology, regulation, new breeding techniques, CRISPR, Codex Alimentarius, standard, labelling, capacity development.

1. Introduction

1.1. Background and objectives

Gene (or genome) editing includes specific techniques that make more precise changes in the genetic makeup of living organisms, which result in the expression of new traits. The process has the potential to be easier to perform, depending on the genetic background and intended trait of interest, relatively inexpensive and is faster than using typical genetic modification techniques. As the techniques develop into practical applications in the food and agriculture sectors, many countries have started to consider if and how gene-edited foods should be regulated.

This paper aims to explain the basic scientific principles underlying gene editing. It provides readers with information related to technical issues in the area of food safety, and offers a summary of the current regulatory status of gene edited foods in different countries as well as a review of the existing documents made available by Codex Alimentarius, the international food standard setting body, that are potentially useful and relevant for any food safety assessment of gene edited foods.

1.2. Scope

As the target audience is national food safety competent authorities and relevant stakeholders, issues related to the environment and to animal feed use, as well as to those ethical or socio-economic aspects of gene editing applied to food and agriculture are not within the specific scope of this paper. However, the authors acknowledge the importance of these issues and recognize that they should be discussed at the global level. FAO has recently published an issue paper entitled "Gene editing and agrifood systems" (FAO, 2022) that presents a balanced discussion of the most pertinent aspects of gene editing, including the consequences for human hunger, human health, food safety, effects on the environment, animal welfare, socioeconomic impact and distribution of benefits.

Medical applications of gene editing are also beyond the scope of this paper for the same reason. It is also important to note that the country examples and case studies included were based on the available information and are not intended by any means, to endorse or dismiss any existing regulatory approaches to gene edited organisms and the products derived from them.

2. Gene editing basics

2.1. Gene editing techniques

Gene (or genome) editing is an umbrella term for various techniques based in molecular biology and refers to the introduction of targeted changes in the genome of living organisms. Proteins called site-directed nucleases (SDN)[1] are used to cut the two strands of Deoxyribonucleic acid (DNA) in a precise location of the genome of an organism.

There are currently four kinds of SDNs used in molecular biology: 1) meganucleases, 2) zinc-finger nucleases, 3) transcription activator-like effector nucleases (TALEN), and 4) clustered regularly interspaced short palindromic repeats (CRISPR) – CRISPER-associated (Cas) nucleases. The first two, meganucleases and zinc-finger nucleases, have been used for gene editing since the 1990s. However, the advent of CRISPR-Cas nucleases in 2013 created an increase in gene editing research and applications, because of their significant practical advantages enabling widespread use.

In the cell nucleus, DNA cuts as those performed by SDNs are mended by the endogenous DNA repair machinery. In a few cells, the repair process may lead to useful mutations, as detailed in the next section.

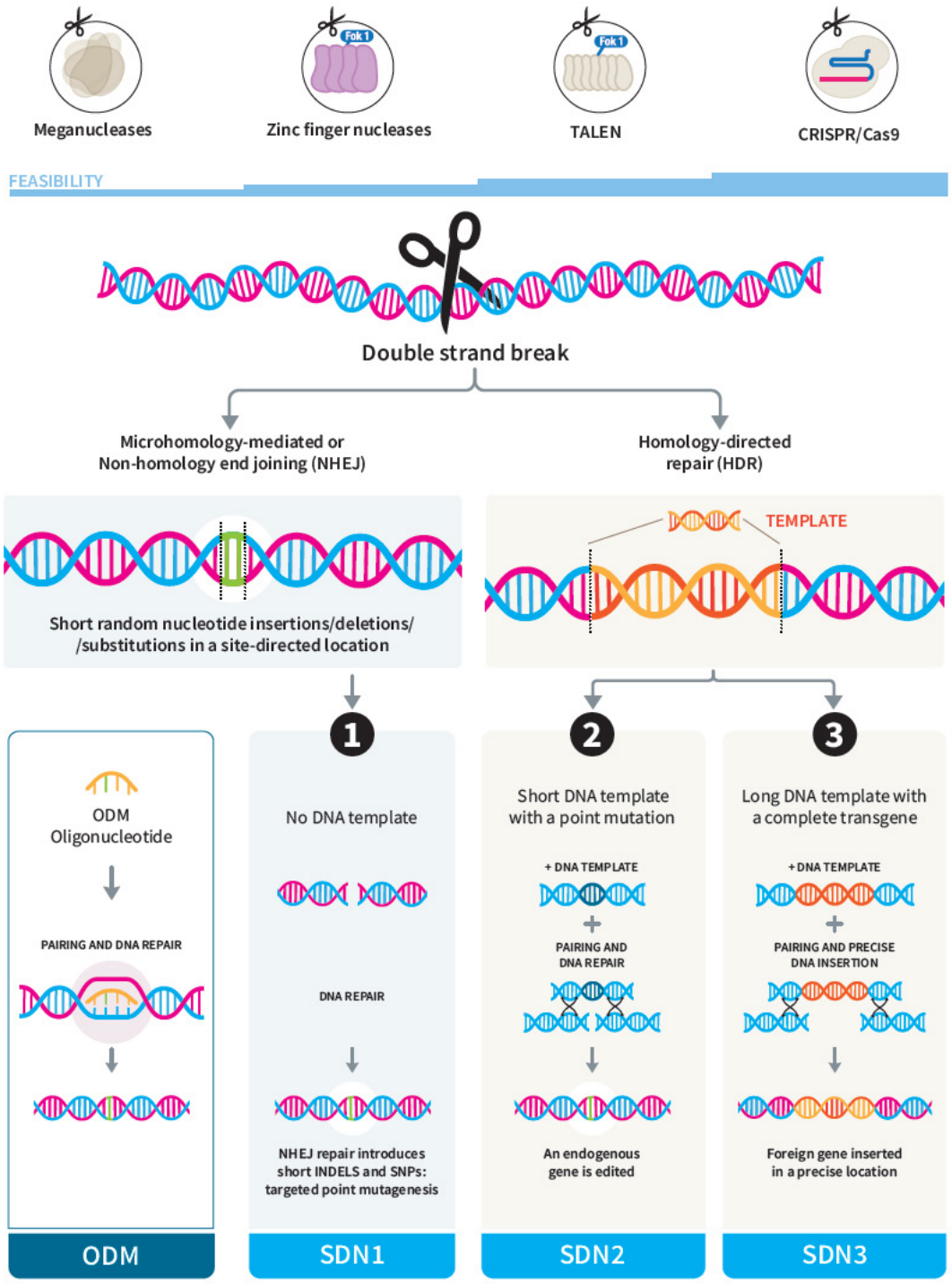
2.2. Gene editing categories

In the regulatory context, experts often group the results of SDN interventions into three categories:

- SDN1: The genome undergoes a random deletion, substitution, and/or insertion of base pairs (in a site-directed location). SDN1 is typically used to generate loss-of-function mutations.
- SDN2: A short DNA repair template is added to the process. The template has high sequence homology to the target site, but also harbours a specific point or short mutation. SDN2 is used to obtain designed mutations, such as reconstructing a gene allele from other varieties or breeds of the same species.

[1] Sometimes known as "site-specific nucleases" or SSN.

FIGURE 1. Overview of gene editing techniques and categories



Source: Author's elaboration.

- SDN3: A long DNA stretch is added to the process, typically a whole gene to be inserted in the target site. SDN3 is used for site-directed transgenesis and cisgenesis (insertion of a gene from the same species, including complete allele replacement).

Site-directed small mutations can also be achieved by oligonucleotide-directed mutagenesis (ODM). ODM uses a short stretch of nucleic acid, which has a homologous sequence to the target site but contains a point or small mutation. This kind of GE gene editing is, therefore, seen as comparable to SDN2 (Sprink *et al*., 2016).

2.3. Unintended results

Gene editing techniques can quite effectively deliver the desired change in a DNA sequence. However, there are sometimes additional unintended results, including off-target changes and unintentional DNA insertions in the cell genome. An off-target change (or effect) derives from the SDN activity or homology of the oligonucleotide to genomic locations other than the target site; this could happen if the target sequence (or a similar one) is also present elsewhere in the genome. Unintended DNA insertions may result from nucleic acid fragments incorporated into the cell during the procedure. Unintended DNA insertions, including off-target changes, do not necessarily represent a food safety concern and their impact, if any, on food safety needs to be assessed on a case-by-case basis.

3. Gene editing application for food and agriculture

Gene editing can be applied to plants, animals and microorganisms for agrifood use in advanced development and commercial stages. The following are examples of such applications, although a few of the cases serve to illustrate the existing diversity in terms of species and traits.

3.1. Crops

A gene edited **high oleic soy** was launched in 2019 in the United States of America, being the first commercially available food derived from a gene edited organism (Menz *et al*., 2020). It was obtained using TALEN. The oil from these soybeans has a fatty acid profile with increased levels of oleic acid, the consumption of which has been linked to certain health benefits such as reducing low-density lipoprotein (LDL) cholesterol and triglycerides while raising high-density lipoprotein (HDL) cholesterol. This modified composition also avoids hydrogenation, an industrial process used to lengthen the shelf life and cooking stability of soybean oil. Hydrogenation creates unhealthy trans fats, and many countries have taken steps to limit their use after the World Health Organization (WHO) called for the global elimination of trans fats in foods by 2023.

In Japan, a **high gamma-aminobutyric acid (GABA) tomato** was developed using CRISPR-Cas9 gene editing technology by a start-up company partnering with a local university (Menz *et al*., 2020). It contains increased levels of GABA, an amino acid derivative believed to help lower blood pressure. In 2021, this product became commercially available through the sale of the fruit itself and in home gardening kits.

Gene edited **rice protected against bacterial blight** disease was cleared as a non-genetically modified organism (GMO) and approved for cultivation by the Colombian Agricultural Institute (ICA) in 2020. It was jointly developed by two research institutes (Luo *et al*., 2021). Bacterial blight is caused by *Xanthomonas oryzae*, a pest that activates the overproduction of sugars in the plant for its own advantage. This is a major pest of rice crops in Asia and the sub-Saharan region of West Africa, while in Colombia it is included on the list of the main pests for preventive detection tests, in an effort to keep it out of the country.

3.2. Farm and aquaculture animals

The first gene edited food animal to be categorized as "non-GMO" was a gene edited **tilapia** line known as FLT01, which developers claim to have significantly **increased the fillet yield**, growth rate, and feed conversion ratio. The status was granted by regulators in Argentina in 2018 after it was established that the fish line does not contain any foreign DNA or a new combination of genetic material.

In Brazil, the cell lines of a Red Angus female cow were gene edited and then implanted in the embryo of a surrogate mother. The resulting cloned animal, born in 2018, was a prototype to test **heat tolerance in non-adapted cattle**. The editing target was a single nucleotide conversion to replicate the "slick hair" mutation in the prolactin receptor gene initially found in Senepol, a Caribbean breed of cattle. Slick-haired animals are reported to have body temperatures about one degree lower than other cattle.

3.3. Microorganisms

In December 2020, the National Technical Biosafety Commission of Brazil (CTNBio) determined that a strain of *Klebsiella variicola* obtained with a CRISPR-Cas9 technique is not a GMO. *K. variicola* is an endophytic nitrogen-fixing bacterium that is used as a biofertilizer in maize and other crops. The genetic change in this strain optimizes its nitrogen production profile. CTNBio was previously granted a non-GMO status for a *Saccharomyces cerevisiae* (yeast) strain with increased tolerance to ethanol and harsh industrial bioreactor conditions for improved biofuel production.

3.4. An extended glimpse of the pipeline

There are a number of food and agriculture products being developed with gene editing techniques. Table 1 provides a long list of such products in alphabetical order with the applied commodities / species, relevant trait(s) and their respective developers. More examples can be found in some of the comprehensive reviews available in the Bibliography.

TABLE 1. Examples of gene-edited products in the global development pipeline

Species / commodities	Trait(s)	Developer
Alfalfa	High yield	National Institute of Agricultural Technology – Argentina
Banana	Protection against bacterial wilt, fusarium silt and banana steak virus	International Institute of Tropical Agriculture, Nigeria
Banana	Protection against bunchy top virus	Agricultural Research Council – South Africa
Banana	Biofortified with vitamin A	India National Agri-Food Biotechnology Institute
Cacao	Protection against fungal disease	Pennsylvania State University, United States of America
Cassava	Reduced cyanide levels	University of California, United States of America
Citrus	Protection against citrus canker	Chinese Academy of Sciences
Cucumber	Protection against multiple viruses	Agricultural Research Organization – Israel
Grapevine	Drought tolerance	Stellenbosch University – South Africa
Potato and sugar beet	disease-resistant varieties	Russian Academy of Sciences
Sorghum	Increased protein content	University of Queensland – Australia
Soybean	Protection against nematodes	Joint venture between Brazilian and Israeli seed and biotech companies
Wheat	Gluten-free	Wageningen University – Kingdom of the Netherlands
Wild tomato	De novo domestication – High antioxidant content	Several universities from Brazil, Germany and the United States of America
Chicken	Protected against avian leukosis virus (ALV)	Czech Academy of Sciences
Dairy cattle	Hypoallergenic milk	National Institute of Agricultural Technology – Argentina
Potato	Increased tolerance to enzymatic browning	National Institute of Agricultural Technology – Argentina
Salmon	Sterility and disease resistance	Norwegian Institute of Marine Research
Swine	Increased tolerance to cold temperatures, and leaner meat	Chinese Academy of Sciences
Swine	Protection against African Swine Fever	Edinburgh University's Roslin Institute – United Kingdom of Great Britain and Northern Ireland
Brewer's yeast	Flavour improvement in fermented beverages	Research institutions in Belgium and Brazil

Source: Author's elaboration

4. Comparison to other breeding techniques

Gene editing is one of many breeding techniques, including pre-existing breeding methods such as classical selection and cross-breeding, induced mutagenesis and transgenesis. Plant breeders typically combine these techniques in an organized iterative manner. Therefore, it is important to identify the factors that make gene editing novel or unique so that some priority areas can be established for regulatory interventions including a food safety assessment while also recognizing that some of the food products produced from gene editing could have food safety characteristics similar to foods with a long history of safe use. The comparison can focus on differences among procedures and the resulting genetic effects with an understanding that different techniques could be used to produce similar genetic effects.

Classical breeding, in its simplest form, involves the selective propagation of plants with desirable traits and the culling of those with less desirable characteristics. Another technique, often referred to as **cross-breeding**, is the deliberate interbreeding (crossing) of two individuals of the same species, where those descendants with a desired genetic combination are selected. Both techniques involve a multi-generational process that often requires five or more years of breeding to eliminate unwanted characteristics and develop the sought-after traits.

These are two powerful and technically simple ways of improving the genetics of a species for the purpose of human societies; they have been used over centuries with great success in most domesticated species. However, their main drawback is that improvement is restricted to what is already available in the gene pool of the same species (which can extend to related interfertile species for certain crops), and breeders must wait for useful spontaneous mutations to appear slowly. In turn, this may lead to a progressive narrowing of genetic diversity and small improvement gap in new varieties developed over time.

Induced mutation breeding Induced mutation breeding began to be applied widely in the early twentieth century, based on technical and scientific advancements in chemistry,

physics, and tissue culture. It consists of generating conditions that temporarily increase the frequency of errors in the DNA repair or copy mechanisms, leading to random deletions or insertions in the nucleotide sequence of the next generation. The same kind of mutations could also appear spontaneously in nature by very similar processes, but with far fewer chances of actually occurring and being found within a commercially viable timeframe.

Mutations may be induced by **chemical mutagens**, **ionizing radiation** or certain **tissue culture techniques**. In all cases, they are generated randomly. Therefore, a great number of attempts and extensive screening are usually required. Quite often, attempts to obtain a specific phenotype using these methods fail. Since the methods involve creating numerous and random mutations, their application is limited to plants and microorganisms. It is impossible to apply these methods in higher animals because of economic and ethical reasons.

FAO, jointly with the International Atomic Energy Agency (IAEA), assists its Members to develop and implement technologies that, using **gamma irradiation and X-rays**, can induce the mutation of plants and thereby considerably speed up the breeding process. This can also involve using certain biotechnologies (tissue culture, molecular markers) to identify and select the desired mutations.

By the end of the twentieth century, **modern biotechnology** enabled a new way of modifying the genome of living organisms by introducing DNA molecules of one species into the cells of another (transgenesis). This technology can often be applied only to specific varieties whose genetic background is not optimal for the breeder´s purposes. Therefore, it is often combined later with cross-breeding to incorporate the trait/genotype of interest into an adequate genetic background.

Modern biotechnology made possible to confer new desired traits that were not previously present in the genetic pool of the target species. However, the use of such a powerful tool comes with great responsibility. Therefore, relevant international protocols, treaties, agreements and Codex guidelines have been produced to help countries implement regulations in their country contexts. Consumer acceptance is still not high in many countries, and the application of modern biotechnology particularly in food has sometimes led to heated and emotional debate.

Mutagenesis and **transgenesis** constitute focused genetic changes. These techniques can often be applied only to specific varieties where the genetic background is not optimal for growing in a defined geographic region. Therefore, these techniques must usually be combined later with cross-breeding to incorporate the trait/genotype of interest into an adequate genetic background suitable for commercialization.

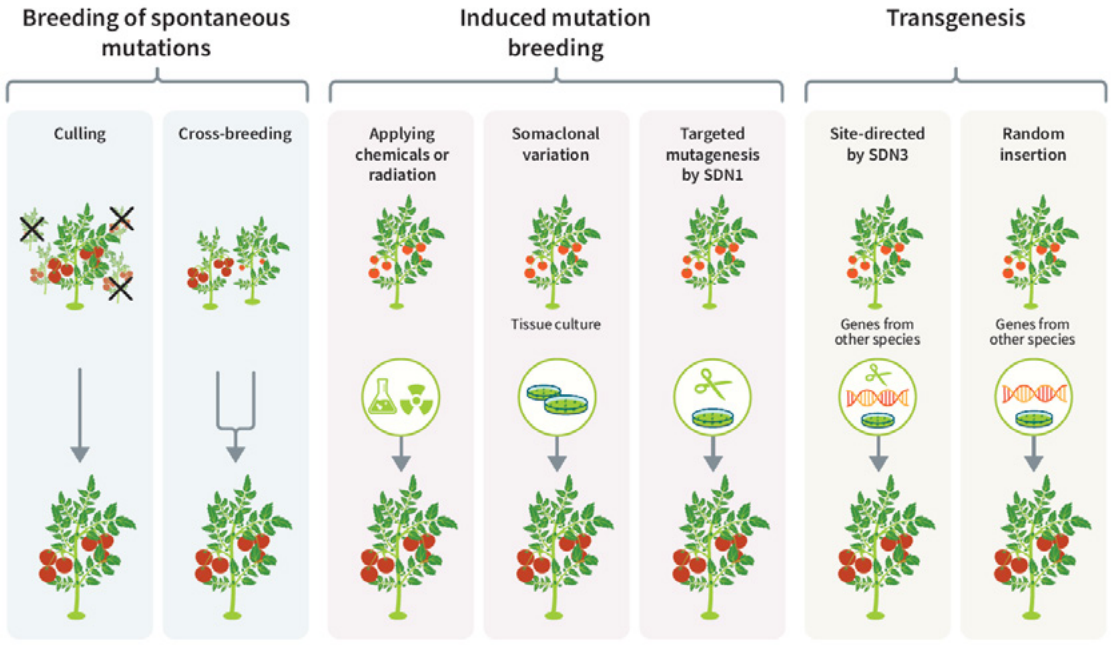
At the beginning of the twenty-first century, **gene editing** emerged as another breeding technique. Its simpler embodiment, **SDN1** induces mutations limited to only a few basepairs like earlier mutation breeding techniques. An important difference, however, is that chemical mutagens or radiation create mutations in random locations, while gene editing is site-directed, thus improving specificity and the ability to target specific genomic regions for modification. reducing uncertainty, time and difficulty. Also, like earlier mutation breeding

techniques, SDN1 is mostly used to "knock out" or turn off endogenous genes; however, in some cases functionality can be maintained with some enhancement, as with herbicide-tolerant mutants.

SDN2 and **ODM** could also be compared with earlier mutagenesis techniques, with the added advantage that the changes in the target sequence can be designed. In most cases, it would be possible to modulate biological functions by changing expression levels or by generating tolerance to an inhibitor such as an herbicide. In many instances, SDN2 is used to recreate or introduce an allele that exists in other varieties (differing in a few mutations with the allele in the recipient line). In that sense, SDN2 results are also comparable with cross-breeding.

Finally, **SDN3** can be used to replace a complete gene allele with another one that pre-exists in other varieties or breeds (possibly having more extensive sequence differences). Applied in that particular way, its results are also comparable with cross-breeding. However, SDN3 can also be used more broadly to introduce genes from unrelated species in site-directed locations of the genome, this is, to obtain a transgenic organism.

FIGURE 2. Comparison between breeding techniques



Source: Author's elaboration.

5. Regulatory approaches for gene editing

5.1. Regulatory timeline

The following is a list of regulatory actions and situations in various countries around the world regarding gene edited organisms for food and agriculture. Such regulatory approaches from various countries are presented in chronological order to illustrate how the global landscape has evolved over time. The chronological order refers only to the start time of a regulatory measure and further developments in the same country are kept in the same section, even if they occurred in a later year.

2013

A **New Zealand** research institute requested the Environmental Protection Authority (EPA) to determine how gene edited organisms would be regulated. Initially, the EPA compared the use of site-directed nucleases with chemical mutagenesis. Since mutagenesis is included in a list of techniques excluded from being regulated as GMOs under the local laws, the EPA interpreted that mutagenesis using site-directed nucleases was also excluded. However, that decision was challenged in the High Court in 2013. The court finally concluded that the list of exempted techniques is closed; therefore, approval from the EPA is required unless the law is modified.

2015

In **Argentina**, the Ministry of Agriculture, Livestock and Fisheries issued Resolution 176/2015, which introduced a procedure for classifying whether or not products from new breeding techniques (including gene edited organisms) were GMOs or not. The procedure is based on the definition of a living modified organism (LMO) from the Cartagena Protocol on Biosafety under the Convention on Biological Diversity. According to this definition, an LMO (GMO in the Argentine domestic regulations) "*possesses*

a novel combination of genetic material obtained through the use of modern biotechnology" (Argentine Secretariat of Foodstuffs, Bioeconomy and Land Development, 2021). This resolution does not create any new product category or special regulatory treatment.

- Since then, several gene edited plant and animal lines developed for food and agricultural purposes have been classified as non-GMO in Argentina. In almost every case, the decision was made because the resulting organism was not considered to possess a novel combination of genetic material.
- In 2019, Argentina on behalf of a coalition of countries, presented a joint statement to the World Trade Organization (WTO) that highlighted the potential benefits of applying gene editing to food and agriculture and stated that governments should avoid arbitrar and unjustifiable distinctions between gene edited organisms and those obtained by other breeding methods.

2017

- The Service for Agriculture and Livestock (SAG) of **Chile** issued an official clarification on the applicability of its previous Resolution No. 1523/2001 for propagation material developed by new plant breeding techniques, including gene editing, which is done on a case-by-case basis. For this purpose, SAG uses a standardized form. Firstly, the form requires information to identify the material intended to be introduced into the environment (i.e. species, variety/line, phenotype and developer). Secondly, it covers the breeding process used and the technique´s characteristics. Thirdly, it requests information on previous releases and permits in other countries.
- In **Israel**, the Plant Protection Services Administration published a decision by the National Committee for Transgenic Plants, establishing that the progeny gene edited plants will not be subject to GMO regulations when foreign DNA sequences are not found to have been incorporated into the plant genome. This decision, however, only applies to field trials for the time being.
- In **the United States of America**, the United States Department of Agriculture (USDA) regulates genetically engineered and gene edited plants based on plant pest risk. The USDA's regulations include a set of explicit regulatory exclusions for plants containing a single genetic modification that is a (1) change resulting from the cellular repair of a targeted DNA break in the absence of an externally provided repair template, (2) targeted single base-pair substitutions, or (3) the introduction or reconstruction of a gene already present in the plant's gene pool. Since 2011, those regulations provide a process for exempting additional plants with modifications that could be achieved through conventional breeding. In addition, plants are also exempt if they have the same plant, trait, and mechanism-of-action as a plant that had undergone assessment by USDA and were found unlikely to pose an increased plant pest risk.

- In 2017, Food and Drug Administration (FDA) published a revised draft Guidance for Industry #187 on its regulation of intentional genomic alterations in animals in which the scope of FDA's regulation was clarified. The scope now covers both rDNA technology and genome editing.
- In 2019, the United States Environmental Protection Agency (US EPA) proposed exemptions under its pesticide regulations for certain plant-incorporated protectants (PIPs) based on sexually compatible plants created through biotechnology in October 2020. In the same year, FDA completed a voluntary premarket consultation on the safety of food from a genome edited soybean produced using TALENs (https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=NewPlantVarietyConsultations&id=FAD2KO). In March 2022, FDA made a low-risk determination for genome-edited Slick cattle, which represents FDA's first low-risk determination for an intentional genomic alterations in an animal for food use.

2018

- The National Technical Biosafety Commission in **Brazil** (CTNBio) issued its Normative Resolution Nº 16/2018. The principle of this resolution is to determine, through a case-by-case consultation system, whether a product generated by NBTs should or not be classified as GMO by CTNBio. For this consultation, the developer institution must provide information about the original organism and the product, including the methods used to generate it, and its molecular analysis. The classification of a product as non-GMO (for legislation purposes) is based on the following criteria: (I) absence of recombinant DNA/RNA; (II) presence of genetic elements that could be obtained by crossing; (III) presence of induced mutations that could also be obtained by established techniques, such as exposure to radiation or chemicals; and (IV) the presence of mutations that could occur naturally.
- The Brazilian regulation also includes lists of techniques and genetic interventions that are not considered to produce a GMO as examples. In general, products obtained by SDN1 mutation or SDN2 mutation and that meet the conditions established in RN16, could be designated as non-GMO, in a case-by-case analysis. In contrast, transgene inserts by SDN3 mutation will normally be classified as GMO, in a case-by-case analysis, according to the RN16. If the product is designated as GMO, the developer must comply with all biosafety requirements and will be approved only after CTNBio's GMO risk assessment. If the product is not classified as a GMO, it can be registered using existing conventional procedures. RN16 applies to all types of organisms, including plants, animals, and microorganisms, in the research and/or commercial release phase. (Vieira *et al*., 2021).
- **Colombia** notified the WTO of its Agricultural Institute (ICA) Resolution no. 29299/2018 "*Setting out the applicable procedure for crops where any stages over the plant-breeding process incorporate innovative phyto-improvement techniques through modern biotechnology and the final product does not contain any foreign genetic material*" (Colombian Agricultural Institute, 2018). Its text is quite similar to the Argentine Resolution 176/2015. Since then,

ICA has processed a few petitions for gene edited rice and corn lines, which ended up classified as non-GMO.

- The Court of Justice of the **European Union** (CJEU) ruled that all organisms obtained by mutagenesis (regardless of the technique used) are GMOs according to the Directive 2001/18/EC, and only organisms derived from mutagenesis techniques that have conventionally been used in a number of applications and have a long safety record, are exempt from the Directive. Therefore, organisms obtained by new mutagenesis techniques, such as genome editing, are GMOs subject to the requirements of the GMO legislation.
- Following a request from the Council of the European Union, the Commission issued in 2021 a study regarding the status of new genomic techniques under European Union law (European Commission, 2021). The term "new genomic techniques" is used in this context to refer to techniques that are capable of altering the genetic material of an organism and that have emerged or have been developed since 2001, when the current European Union legislation on genetically modified organisms was adopted. This term includes gene editing.
- Based on the conclusion of the study on new genomic techniques, the European Commission announced the preparation of a policy initiative on plants obtained by targeted mutagenesis and cisgenesis which would also cover food and feed derived from such plants. The initiative aims at an appropriate regulatory oversight for the concerned plant products, ensuring a high level of protection of human and animal health and the environment, and enabling innovation and the contribution of plants obtained by new genomic techniques to the objectives of the European Green Deal and the Farm to Fork Strategy. In the context of this initiative, the European Commission may propose legislation in 2023.
- In **Switzerland**, Federal Council confirmed, in response to a Parliamentary interpellation, that gene edited organisms fall under the definition of GMOs according to the local Gene Technology Act.

2019

- **Australia** amended its GMO regulation law to address gene editing. The amendment consists of an expanded list of "organisms that are not GMOs" to include those "modified by repair of single-strand or double-strand breaks of genomic DNA induced by site-directed nuclease, if a nucleic acid template was not added to guide repair" (Australian government. Office of the Gene Technology Regulator, 2019) (i.e. SDN1).
- **Ecuador** clarified in its environmental code regulations so that organisms not possessing recombinant or foreign DNA are excluded from the GMO biosafety regulations.
- **Guatemala** and **Honduras** signed their bilateral Resolution No. 60/2019 where both countries agreed to harmonize their GMO regulation, in

connection with the establishment of a common market. That resolution settled criteria for distinguishing which gene edited products should be treated as GMOs and which as conventional new varieties in both countries. The criteria were later implemented domestically by the Honduran National Service of Agri-Food Health and Safety (SENASA) in its regulation 8/2019 and by the Guatemala Ministry of Agriculture, Livestock, and Foodstuff through its Agreement no. 271/2019. These implementing regulations are based on a specific definition for a "novel combination of genetic material", (Honduran National Service of Agri-Food Health and Safety, 2019, and the final product characteristics compared to conventional breeding products. They also pay special attention to attaining harmonization with other countries.

- **Nigeria** amended the National Biosafety Management Agency (NBMA) Act to include emerging agricultural biotechnologies. The amendment even defines gene editing as "a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism" (Nigeria National Biosafety Management Agency Amendment Act, 2019). Subsequently, in 2020 the NBMA published detailed guidelines for gene editing regulations in particular. When the gene edited product does not have a novel combination of genetic material, a non-GMO regulatory classification is applied.
- The Ministry of Agriculture and Livestock of **Paraguay** published Resolution No. 565/2019, which approves a form for "Prior Consultation for products obtained through new breeding techniques ..." (Paraguay Ministry of Agriculture and Livestock, 2019). It is quite similar to the Argentine regulation, while at the same time, it contains a list of techniques like the Brazilian regulation. The Paraguay National Commission on Agricultural and Forestry Biosafety is responsible for analysing applications using this form, although no case has yet been presented.
- In **the Philippines**, the Department of Agriculture (DA) issued a Memorandum Circular No. 8 series of 2022 titled "Rules and procedure to evaluate and determine when products of Plant Breeding Innovations (PBIs) are covered under Joint Department Circular No. 1 (JDC1), s2021 based on the National Committee on Biosafety of the Philippines (NCBP) Resolution No. 1, s2020. As defined in the NCBP resolution No. 1 series of 2020, PBIs are "a new set of molecular, genomics and cellular tools that enable the targeted and efficient development of new varieties of crops with desired traits or characteristics in a way that is faster and more precise than conventional plant breeding techniques. These PBI include site-directed nucleases (SDN), oligonucleotide-directed mutagenesis, cisgenesis and intragenesis, RNA-dependent DNA methylation (RdDM), grafting with GM material, reverse breeding, agroinfiltration, synthetic genomics, and other upcoming techniques, with the potential to produce both GM and non-GM plants as final products.
- In **Japan**, The Ministry of Environment (MoE) and the Ministry of Health, Labour and Welfare (MHLW) published procedures and guidelines to clarify

when their GMO regulations apply to genome-edited products. In 2020, the Ministry of Agriculture, Forestry and Fisheries (MAFF) also published implementing guidelines on the same topic.

- The MoE criteria are centred on analysing the products to see if they fall outside the scope of the LMO definition in the applicable law, which is based on the Cartagena Protocol. Besides, it clarifies that organisms (a) do not have integrations of "extracellularly processed nucleic acids", or (b) only incorporated genetic material that comes from the same or sexually compatible species are both excluded.
- Conversely, MHLW criteria state that foods derived from gene edited organisms presenting the same level of risk as those from conventional breeding are not subject to the GMO food safety assessment process. The MHLW criteria for identifying products that do not require a GMO safety assessment include (a) absence of foreign DNA in the final product, and (b) changes induced by a site-directed enzyme that results in deletions, substitutions, or the spontaneous insertion of one or more nucleotides. Regarding feeds derived from gene edited plants, the MAFF guidelines are closely aligned with the approach taken by MHLW over foodstuffs.
- A locally developed gene edited tomato with increased gamma-aminobutyric acid content (for health benefits) was the first product to receive confirmation of non-GMO status. Other products followed, including a sea bream (fish) line that was gene edited using CRISPR technology to knock out the myostatin gene; it was also developed by a start-up incubated by a local university. Both the tomato and the fish became commercially available to the public in 2021.

2021

- Health **Canada** determined that a high amylopectin starch ("waxy" phenotype) corn obtained by SDN1 technique was not a novel food product, and therefore it did not require pre-market safety assessment as a novel food. The rationale for this decision was that the product has the same phenotype as pre-existing commercial corn varieties with a similar spontaneous mutation and having a history of safe use as food. In contrast, the following year a gene-edited high oleic soybean was determined to be a novel food, and hence it was subjected to a food safety assessment based on WHO/FAO expert consultations.
- The Canadian regulatory approach is based on the characteristics of the final product, regardless of how it was obtained. As with conventional breeding and recombinant DNA techniques, gene editing techniques have the potential to develop both novel and non-novel traits. In Canada, only those gene edited products that are deemed to have a novel trait require pre-market safety assessments.
- Furthermore, in 2022 Health Canada published a scientific opinion on the regulation of gene-edited plant products. It states that novel food products from any breeding technique that can present a food safety hazard will require a food safety assessment, to be done according to domestic

guidance based in the Codex Guidance framework for the food safety assessments of foods derived from Biotechnology.

- In **South Africa**, the legal definition of a GMO is "*an organism the genes or genetic material of which has been modified in a way that does not occur naturally through mating or natural recombination or both*" (Entine *et al.,* 2021). Based on that definition, the Executive Council of the GMO Act, which is administered by the Department of Agriculture, Land Reform and Rural Development, concluded that the risk assessment framework for GMOs would also apply to new breeding techniques (NBTs), and modified its applications forms accordingly to allow a tiered assessment approach.
- In Argentina, the Biosafety Commission updated the regulations for products obtained by new breeding techniques and issued Resolution 21/2021 (Argentine Secretariat of Foods, Bioeconomy and Land Development, 2021).

2022

- In **China**, the Ministry of Agriculture and Rural Affairs (MARA) issued guidelines for safety evaluation of genetically engineered plants for agricultural use. These guidelines apply gene-edited plants on which no exogenous genes were introduced, with differential treatments according to risk levels. For those genetically engineered plants whose traits do no elicit food or environmental risk hypothesis, the guidelines establish a simplified registration procedure with respect to transgenic plants.
- The Government of **India** exempted the Genome Edited plants falling under the categories of SDN1 and SDN2, which are free from exogenous DNA, from the provisions of Rules 7 to 11 (both inclusive) of the Manufacture, Use, Import, and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells, Rules, 1989 of the Environment Protection Act, 1986. With this exemption, the process of genome editing of plants (SDN1 and SDN2) being carried out under containment, until free from exogenous introduced DNA, to be regulated by the Institutional Biosafety Committee following guidelines issued by Central Government under information to Review Committee on Genetic Manipulation (RCGM). The "Guidelines for the Safety Assessment of Genome Edited Plants, 2022"' were notified by the Department of Biotechnology, Government of India on 17th May 2022 and are available at https://dbtindia.gov.in/latest-announcement/guidelines-safety-assessment-genome-edited-plants2022.
- The National Biosafety Authority (NBA) of **Kenya** issued guidelines for determining the regulatory process of genome edited organisms and products, following stakeholder consultations.
- The guidelines established that the following categories of genome editing techniques and derived products are not regulated under the biosafety act: "*(i) All modifications by inserting genes from sexually compatible species and where regulatory elements (promoters and terminators) are also from the*

same species; (ii) All deletions/knock outs provided that there is no insertion of foreign genetic material in the end-product: and (iii) Processed products whose inserted foreign genetic material cannot be detected" (National Biosafety Authority Kenya, 2022). NBA has already reviewed several applications using new plant breeding techniques in contained facilities (biosafety laboratories and greenhouses).

- When the United Kingdom of Great Britain and Northern Ireland officially left the European Union in 2020, all the relevant European Union regulations were retained. However, after a parliamentary debate on gene editing, the Department for Environment, Food and Rural Affairs performed a public consultation regarding the update of the regulation of genetic technologies. In response to the consultation result, the United Kingdom Government outlined a plan to change regulation for certain genome-edited plants in two stages: first to exempt them from GMO field trial regulation in England (implemented in 2022), and then from the regulatory definition of a GMO (United Kingdom Parliament Post, 2022).

Many other countries are currently conducting policy-making processes to develop regulatory criteria for gene editing applied to agriculture. In some cases, the content of these processes is documented in publicly available documents.

- In Costa Rica, the State Phytosanitary Service has recently proposed a draft national legal framework for new plant breeding techniques, but it is still under consideration. It consists of a procedure to define whether or not a crop derived from these techniques is an LMO, quite in line with the other Latin American countries mentioned.
- Food Standards Australia New Zealand (FSANZ) is performing an ongoing review of how the binational Food Standards Code applies to foods derived from NBTs. This includes a proposal, not yet completed, to revise and update the definitions in the binational food code that determine what foods are regulated as GM foods. FSANZ has proposed that foods derived from NBTs should not be regulated as GM foods if they are equivalent in characteristics and risk to conventional foods with a history of safe use.
- Norway is currently following the European Union's authorization procedures. However, after public surveys and parliamentary debates, the Norwegian Biotechnology Advisory Board has presented recommendations for how GMOs should be regulated, including to exempt or expedite the safety assessment of gene-edited organisms.

5.2. Differences and similarities among countries

Overall, most of the governments that are enacting policies for gene editing applied in food and agriculture are using their national regulatory frameworks on modern biotechnology, novel foods or GMOs as a comparative model. In some cases, countries are relying on existing laws used to oversee the safety of food in general, regardless of the technique used

to produce the food, and clarifying how existing regulatory provisions apply to products produced using gene editing. A few countries' policies focused specifically on gene editing.

To date, no country has created a new and separate category for the regulation of gene edited food products. Gene edited organisms and the food derived from them are treated in the same way as either novel foods, GMOs or conventional products. Some countries require a case-by-case consideration of each product in order to establish the proper regulatory frameworks. In some cases, every gene edited product is considered to be a GMO.

Despite the variety of regulatory approaches taken by different countries and jurisdictions with regard to foods derived from gene edited organisms, almost all governments share some general key objectives, which are to protect the health of consumers and to ensure fair practices in food trade. Regardless of how gene edited organisms are classified or handled in their respective regulatory frameworks and in different contexts, many experts believe that relevant Codex guidelines can be useful in supporting these policy approaches.

6. Applicability of existing Codex guidelines

6.1. Codex Alimentarius and food safety risk analysis

Codex Alimentarius is a collection of standards, guidelines and codes of practice adopted by the Codex Alimentarius Commission (CAC). CAC is the central part of the Joint FAO/WHO Food Standards Programme.

The food safety risk analysis is guided and documented by CAC and various Codex texts have been published, such as the "Statements of principle concerning the role of science in the Codex decision-making process and the extent to which other factors are taken into account" and the "Statements of principle relating to the role of food safety risk assessment (Codex Alimentarius Commission Procedural Manual)". Moreover, it is also guided by the "Working Principles for Risk Analysis for Application in the Framework of the Codex Alimentarius (Codex Alimentarius Commission Procedural Manual)" and the "Working Principles for Risk Analysis for Food Safety for Application by Governments (CAC/GL 62-2007)".

In addition, different specific Codex guidelines are now widely used in regulations applied to foods derived from modern biotechnology in particular. They are the result of technical consensus among experts from numerous governments, achieved after several rounds of exchanges in specific Codex committees and the Codex Ad Hoc Intergovernmental Task Force on Food Derived from Biotechnology (TFFBT). They were published between 2003 and 2011, but their guidance is robust and they are broadly applicable, and they continue to be valid after more than a decade.

6.2. Potentially relevant Codex guidelines

6.2.1. Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (CXG 44-2003)

The purpose of documenting the principles for the risk analysis of foods derived from modern biotechnology is to provide a basic framework for undertaking risk analysis on the

safety and nutritional aspects of foods derived from modern biotechnology. It introduces three components of risk analysis: risk assessment (including food safety assessment), risk management and risk communication. The document also explains the uniqueness of this particular application of risk analysis to safety assessments of whole foods, in contrast to other food safety assessments of single substances or microorganisms in food.

In general, the risk assessment process follows four steps: hazard identification, hazard characterization, exposure assessment and risk characterization. In other words, the whole process of risk assessment starts once a hazard is properly identified. In the case of whole foods, it is appropriate to call the process a "food safety" assessment rather than a "risk" assessment, as the point is to identify whether a hazard or other possible food safety concern is present.

One of the key principles described in the Codex document is the comparative approach. As there is no single target to examine, the safety assessment compares the food derived from modern biotechnology to its conventional counterpart, focusing on the similarities and differences because traditional toxicological animal studies cannot be readily applied to whole foods. If the safety assessment identifies a new hazard, a compositional (nutritional) alteration, or other safety concern, its impact on human health through consumption must be determined.

Applicability: The document adopts the definition of modern biotechnology from the Cartagena Protocol (Box 1). Codex's "Principles" are broad enough to apply to the risk analysis of foods derived from gene edited organisms, as long as there was an application of *in vitro* nucleic acid techniques, which is most likely valid for all known gene editing techniques.

BOX 1. The definitions below apply to the Codex Principles.[a]

"Modern biotechnology" means the application of:

- *in vitro* nucleic acid techniques, including recombinant Deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles; or
- fusion of cells beyond the taxonomic family

"that overcome natural physiological reproductive or recombinant barriers and that are not techniques used in traditional breeding and selection."

Note: a) Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (CXG 44-2003).

Most gene editing techniques involve the introduction of nucleic acids (either DNA and/or Ribonucleic acid, RNA) in target cells that will be used to develop a gene edited organism. It is debatable whether the term "injection" should be interpreted broadly as a synonym of "introduction" or in a literal way (mostly animal cell techniques use actual DNA injection by micromanipulation, while techniques for plants use other introduction methods). Nevertheless, all techniques involve the use of recombinant-DNA at some stage. In some cases, recombinant-DNA is introduced in the target cells; in others, a recombinant-DNA is used to express a protein in a bacterial expression system, and then the protein is inserted in the target cell (along with RNA molecules in the case of CRISPR-Cas).

Unlike the Cartagena Protocol, which is focused on the concept of a "Living" Modified Organism (LMO) in the context of environmental risk assessment and addresses "novel combinations of genetic material", Codex guideline do not make a distinction regarding novel combinations of genetic material.[2]

6.2.2. Guidelines for the Conduct of Food Safety Assessment of Foods Derived from recombinant-DNA Plants, Animals and Microorganisms (CXG 45-2003, CXG 68-2008, and CXG 46-2003, respectively)

These guidelines focus on the first and possibly foremost step of the risk analysis framework presented by the "Principles" (CXG 44-2003), which is the food safety assessment. If a new or altered hazard, nutritional or other food safety concern is identified by the safety assessment, the risk associated with it would first be assessed to determine its relevance to human health. Following the safety assessment, and if necessary further risk assessment, the food would be subjected to risk management considerations before it is considered for commercial distribution.

The guidelines describe the recommended approach to conducting safety assessments of foods derived from recombinant-DNA organisms where a conventional counterpart exists, through the comparative approach using the concept of substantial equivalence. Substantial equivalence analysis is a starting point to structure the safety assessment of a "new" food item, based on similarities and differences against its conventional counterpart.

The guidelines are based on a framework of food safety assessment that follows a stepwise process starting with the descriptions of: 1) the molecular characterization of the recombinant-DNA plant / animal / microorganism; 2) the host plant / animal / microorganism and its use as food; 3) the donor organisms; and 4) the genetic modification. Then followed by safety assessment steps with information on: 1) expressed substances; 2) compositional and nutritional analyses; 3) evaluation of metabolites; 4) food processing; and 5) intended and unintended effects and other considerations.

Applicability: the title of the documents mentioning "recombinant-DNA" plants, animals and microorganisms may give the impression that gene edited food is beyond its scope, although the term "recombinant-DNA" has not been defined even though the guidelines repeatedly employ the term. At the same time, these guidelines state that while they are designed for foods derived from recombinant-DNA organisms, the approach they describe could generally be applied to **foods derived from organisms that have been altered by other techniques**. Therefore, it can be concluded that these guidelines contain science-based provisions that may be applied to gene edited foods regardless of the specific definitions.

While the scope of gene editing may match that of the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology, it does not mean that Codex guidelines imply that gene edited organisms are GMOs. Codex guidelines do not include anything that could

[2] Convention on Biological Diversity (CBD) and Codex may not necessarily share all the same technical definitions. Given the non-binding nature of Codex guidelines, countries may have local definitions and languages that may not exactly match those of Codex's.

be taken as a technical recommendation for governments to decide under which kind of regulatory framework gene edited organisms should be handled. Even if a government deals with gene edited organisms under the regulatory framework for conventional new varieties/breeds/strains, it may be possible that a novel trait elicits a specific food safety concern or hypothesis, or a general decision is made to perform a food safety assessment for foods derived from gene edited organisms. In this context, these Codex guidelines would be applicable to gene edited products.

Section-wise applicability and non-applicability: the provisions on newly expressed substances (mostly referring to the toxicity and allergenicity of recombinant proteins) may not apply to gene editing interventions of type SDN1 and SDN2, which are unlikely to generate the expression of a new substance. Conversely, the section on compositional analysis can be applicable in all cases. Compositional analysis can reveal the formation of new or changed patterns of metabolites, which in turn help to detect unintended effects. In conclusion, the applicability of specific elements of the safety assessment needs to be determined on a case-by-case basis.

6.2.3. Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods (CAC/GL 74-2010)

The objective of these guidelines is to support the establishment of molecular and immunological methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods, which produce results with comparable reproducibility when performed at different laboratories. The guidelines are intended to provide guidance on how to establish methods to detect and identify specific DNA sequences and proteins in food by defining appropriate validation criteria, and whether a method complies with these criteria based on its performance characteristics.

Applicability: the molecular and immunological methods described in the guidelines apply to a wide range of uses, such as tests for biomarkers in foods, including those derived from modern biotechnology and food authentication. They may be used by laboratories responsible for food analysis.

While some simple gene editing interventions such as SDN1 may result in changes that may not be different from those generated by random mutations, the methods of analysis covered by the guidelines, particularly those based on DNA sequences, may be technically applicable to foods derived from all sorts of gene edited organisms. Therefore, these guidelines could eventually be applied to validating the methods to detect and quantify foods derived from gene edited organisms.

6.2.4. Compilation of Codex Texts Relevant to Labelling of Foods Derived from Modern Biotechnology (CAC/GL 76-2011)

The purpose of this compilation is to assemble in a single document some key elements of guidance from Codex texts, which are relevant to labelling foods derived from modern biotechnology. In addition to the guidelines described previously, this compilation refers to specific sections of Codex standards for (a) labelling of pre-packaged foods, (b) general

guidelines on claims, (c) use of nutrition and health claims, (d) organically produced foods, (e) use of the term "Halal", and (f) working principles for risk analysis for food safety for application by governments.

Applicability: This compilation is not intended to suggest or imply that foods derived from modern biotechnology are to be labelled, nor are they necessarily different from conventional foods. It is intended to simply address the needs of different governments to obtain practice-based guidance and possible options that fit various regulatory approaches regarding the labelling of foods derived from modern biotechnology. If foods derived from gene edited organisms are subject to any kinds of food labelling requirements, specific to the use of gene editing or biotechnology, this compilation may serve to assist national governments, as the approach is consistent with the relevant Codex guidelines and provisions.

6.2.5. Other guidelines

There are two additional Codex guidelines that are possibly relevant to apply to foods derived from gene edited organisms:

- The "Guidelines on the Judgement of Equivalence of Sanitary Measures associated with Food Inspection and Certification Systems (CXG 53-2003)" may be useful in situations where Codex members have a different regulatory classification system or regulatory approach for gene edited products.
- Along the same line, the "Principles and guidelines for the exchange of information between importing and exporting countries to support the trade in food (CXG 89-2016)" could be more relevant, since the diverse approaches by different governments may require good communication to clarify the presence of these products in international shipments.

7. Potential relevance to the work of FAO and Codex Alimentarius

7.1. Potential need for discussion fora

Although a series of existing Codex guidelines may suffice to address safety and other aspects of foods derived from gene edited organisms, countries may wish to have a neutral and transparent platform to discuss the relevant issues as the technology and techniques advance.

7.2. Potential need for scientific and policy advice

FAO/Codex members, particularly low- and middle-income countries (LMICs) may request technical guidance on specific food safety assessments of gene edited foods. Such guidance can include:

- specific sections of existing guidelines that do/do not apply to foods derived from gene edited organisms;
- technical guidance for the assessment of off-target effects and unintended DNA insertions; and
- information exchange mechanisms such as meetings/workshops, hosted by global organizations like FAO, where practical experience on food safety assessment associated with foods derived from gene editing can be shared.

7.3. Opportunities for capacity development

FAO has published a specific training tool for conducting a safety assessment of foods derived from recombinant DNA entitled "GM Food Safety Assessment: tools for trainers" (FAO, 2009). The publication is composed of three parts. Principles, the first part, provides guidance for implementing an effective framework for the safety assessment of foods derived from modern biotechnology. Tools and techniques for trainers, the second part,

offers a practical guide for preparing and delivering a workshop on the topic of the safety assessment of foods derived from recombinant-DNA plants. This section contains various checklists and forms, a sample workshop agenda, a sample workshop evaluation sheet, and five helpful presentation modules for trainers. Case studies, part three, presents three safety assessment dossiers summarized for training purposes.

The training tools focus particularly on GM food safety and so some parts of the tool, particularly the third part, may not directly apply to training on the safety assessment of a gene edited food. Therefore, international organizations may consider incorporating the following elements into the tool so that an overarching capacity development tool for assessing the safety of whole foods, including foods derived from recombinant-DNA, gene edited foods and other whole food products developed through new technologies in the future can be developed:

- latest scientific and technological developments;
- differences among techniques and applications;
- practical applications of relevant Codex guidelines; and
- case studies on gene edited foods.

8. Discussion

Many gene editing applications, such as inserting traits for pest protection or drought tolerance, have the potential to increase productivity, resilience, and sustainability, thus eventually contributing to improved food security worldwide. Some specific applications aim to introduce traits that change the food composition to increase the safety and/or quality to interest consumers; for instance, by attempting to increase healthy fatty acids, reduce gluten content, or lower cyanide levels. At the same time, it is important to note that regardless of the types and methodologies, any breeding technique may occasionally generate food safety concerns, and gene editing is not an exception.

To aid countries that need international guidance, the review of the Codex guidelines shows that existing protocols and paradigms such as food safety risk analysis and guidance on whole food safety assessment processes can be easily tailored and applied to the safety assessment of gene edited foods if governments deem such assessment necessary.

From the regulatory perspective, it is national governments that eventually set the levels of consumer protection and trade related measures. Subsumed to that principle, it is also desirable to avoid setting rules and regulations discriminating arbitrarily in terms of process and production methods, where such methods are not the direct indicators of product safety. Including onerous requirements in the regulatory frameworks without a scientific basis should be avoided, otherwise the implementation of such regulations can become a burdensome compliance issue rather than the ultimate objective of consumer protection. The review of the state of the art of regulatory approaches in different countries shows that it is not essential to create a brand-new set of regulations for gene editing for the purpose of food safety. While approaches may vary, many countries have found a way to include gene edited foods in an existing regulatory category to manage food safety issues.

Gene editing is an innovative and versatile set of breeding tools, generating genetic improvements more efficiently and precisely than many earlier breeding methods. Since the CRISPR-Cas technique is relatively simple, a great number of public research institutions and local entrepreneurs are applying the technology to obtain products that could be used by small-scale farmers in many parts of the world. Some gene edited foods are already

commercialized and many are in the pipeline. This period of growth is in contrast with the situation of GMOs, where the initial development was led by a few multinational companies that can bear the high regulatory costs. Compensating for such costs leads to a focus on large-scale crops, such as staple crops.

In conclusion, the possible effects of gene editing on food safety, quality and trade are not expected to be much different from such effects on foods derived from pre-existing breeding techniques. Therefore, the relevant guidelines developed by Codex Alimentarius remain applicable for addressing the safety and fair trade of foods derived from gene editing, consistent with international obligations. Although there could be room to provide a specific discussion fora, generating tailored scientific guidance on specific operative aspects, such as off-target effects, and providing capacity development opportunities for LMICs with gene editing related case studies, most of the support that FAO, Codex Alimentarius and many other international organizations continuously provide to their members is sufficient to meet current needs, and may focus on how to apply existing guidelines according to the particular implementation of regulatory actions on gene editing.

Bibliography

Codex guidelines

FAO (Food and Agriculture Organization of the United Nations) & WHO (World Health Organization). 2018. Codex Alimentarius Commission Procedural Manual, Twenty-sixthedition. Rome, FAO. https://www.fao.org/3/i8608en/I8608EN.pdf

FAO & WHO. 2010a. Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods. Rome, FAO. https://www.fao.org/fileadmin/user_upload/gmfp/resources/CXG_074e.pdf

FAO & WHO. 2010b. Compilation of Codex texts relevant to the labelling of foods derived from modern biotechnology. Codex Alimentarius. Rome, FAO. http://www.fao.org/fao-whocodexalimentarius/shproxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B76-2011%252FCXG_076e.pdf

FAO & WHO. 2008. Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants. Codex Alimentarius. Rome, FAO. http://www.fao.org/fao-whocodexalimentarius/shproxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B45-2003%252FCXG_045e.pdf

FAO & WHO. 2003. Principles for the Risk Analysis of Foods Derived from Modern Biotechnology. Codex Alimentarius. Rome, FAO. http://www.fao.org/fao-whocodexalimentarius/shproxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B44-2003%252FCXG_044e.pdf

Gene editing techniques and their applications

Blix, T.B., Dalmo, R.A., Wargelius, A. & Myhr, A.I. 2021. Genome editing on finfish: Current status and implications for sustainability. *Reviews in Aquaculture* 13, 2344–2363. https://doi.org/10.1111/raq.12571

Jansing, J., Schiermeyer, A., Schillberg, S., Fischer, R. & Bortesi, L. 2019. Genome editing in agriculture: technical and practical considerations. *International Journal of Molecular Sciences*, 20(12), 2888. https://doi.org/10.3390/ijms20122888

Laible, G., Wei, J. & Wagner, S. 2015. Improving livestock for agriculture – technological progress from random transgenesis to precision genome editing heralds a new era. *Biotechnology journal*, 10(1), 109–120 https://doi.org/10.1002/biot.201400193

Lillico, S. 2019. Agricultural applications of genome editing in farmed animals. *Transgenic research* 28(2), 57–60. https://doi.org/10.1007/s11248-019-00134-5

Ricroch, A. 2019. Global developments of genome editing in agriculture. *Transgenic research* 28(2), 45–52. https://doi.org/10.1007/s11248-019-00133-6

Country approaches to the regulations

Dederer, H.G. & Hamburger, D. 2019. *Regulation of Genome Editing in Plant Biotechnology*. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-17119-3

Entine, J., Felipe, M.S.S., Groenewald, J.H., Kershen, D.L., Lema, M., McHughen & Wray-Cahen, D. 2021. Regulatory approaches for genome edited agricultural plants in select countries and jurisdictions around the world. *Transgenic Research* 30, 551–584. https://doi.org/10.1007/s11248-021-00257-8

Friedrichs, S., Takasu, Y., Kearns, P., Dagallier, B., Oshima, R., Schofield, J. & Moreddu, C. 2019. Meeting report of the OECD conference on "genome editing: applications in agriculture—implications for health, environment and regulation". *Transgenic Research* 28, 419–463. https://doi.org/10.1007/s11248-019-00154-1

Gatica-Arias, A. 2020. The regulatory current status of plant breeding technologies in some Latin American and the Caribbean countries. *Plant Cell, Tissue and Organ Culture* 141(2), 229–242. https://doi.org/10.1007/s11240-020-01799-1

Jenkins, D., Dobert, R., Atanassova, A. & Pavely, C. 2021. Impacts of the regulatory environment for gene editing on delivering beneficial products. *In Vitro Cellular & Developmental Biology-Plant*, 1–18. https://doi.org/10.1007/s11627-021-10201-4

Schmidt, S.M., Belisle, M. & Frommer, W.B. 2020. The evolving landscape around genome editing in agriculture: many countries have exempted or move to exempt forms of genome editing from GMO regulation of crop plants. *EMBO reports*, 21(6), e50680. https://doi.org/10.15252/embr.202050680

Specific topics

Detection methods applied to products derived from gene edited organisms

Bennett, E.P., Petersen, B.L., Johansen, I.E., Niu, Y., Yang, Z., Chamberlain, C.A. & Frödin, M. 2020. INDEL detection, the 'Achilles heel' of precise genome editing: a survey of methods for accurate profiling of gene editing induced indels. *Nucleic acids research*, 48(21), 11958–11981. https://doi.org/10.1093/nar/gkaa975

Shillito, R.D., Whitt, S., Ross, M., Ghavami, F., De Vleesschauwer, D., D'Halluin, K. & Meulewaeter, F. 2021. Detection of genome edits in plants—from editing to seed. *In Vitro Cellular & Developmental Biology - Plant*, 1–14. https://doi.org/10.1007/s11627-021-10214-z

Off-target changes and unintended effects

Burgio, G. & Teboul, L. 2020. Anticipating and identifying collateral damage in genome editing. Trends in Genetics 36(12), 905–914. https://doi.org/10.1016/j.tig.2020.09.011

Lema, M. 2021. Regulatory assessment of off-target changes and spurious DNA insertions in gene-edited organisms for Agri-food use**.** *Journal of Regulatory Science* 9(1), 1–15. https://doi.org/10.21423/jrs-v09i1lema

References

Argentine Secretariat of Foodstuffs, Bioeconomy and Land Development. 2021. Resolution no. 31/2021, Annex I Section A "Definitions" Page 2. Available at https://magyp.gob.ar/sitio/areas/biotecnologia/conabia/_pdf/RES_032-2021_3%20anexos.pdf

Argentine Secretariat of Foods, Bioeconomy and Land Development. 2021. Resolution no. 21/2021. Available at https://www.boletinoficial.gob.ar/detalleAviso/primera/240526/20210208.

Australian government. Office of the Gene Technology Regulator. 2019. Gene Technology Amendment (2019 Measures No. 1) Regulations 2019. Available at https://www.legislation.gov.au/Details/F2019L00573

Colombian Agricultural Institute. 2018. Resolution 29299/2018, Page 1. Available at https://www.ica.gov.co/getattachment/2d02cc52-d1c5-4123-8a5a-aea9ad2ce926/2018R29299.aspx

Entine, J., Felipe, M., Groenewald, J.H., Kershen, D.L., Lema, M., McHughen, A., Nepomuceno, A.L., Ohsawa, R., Ordonio, R.L., Parrott, W.A., Quemada, H., Ramage, C., Slamet-Loedin, I., Smyth, S.J., & Wray-Cahen, D. 2021. Regulatory approaches for genome edited agricultural plants in select countries and jurisdictions around the world. Transgenic research, 30(4), 551–584. https://doi.org/10.1007/s11248-021-00257-8

European Commission. 2021. Study on the status of new genomic techniques under Union law and in light of the Court of Justice ruling in Case C-528/16. https://food.ec.europa.eu/plants/genetically-modified-organisms/new-techniques-biotechnology/ec-study-new-genomic-techniques_enbiotechnology/ec-study-new-genomic-techniques_en

FAO (Food and Agriculture Organization of the United Nations). 2022. Gene editing and agrifood systems. Rome. 86 pp. https://doi.org/10.4060/cc3579en

FAO. 2009. GM food safety assessment tools for trainers. Rome, FAO. https://www.fao.org/3/i0110e/i0110e.pdf

FAO (Food and Agriculture Organization of the United Nations) & WHO (World Health Organization). 2019. Codex Alimentarius Commission – Procedural Manual twenty-seventh edition. Rome. 254 pp. https://www.fao.org/3/ca2329en/ca2329en.pdf

FAO & WHO. 2016. Principles and guidelines for the exchange of information between importing and exporting countries to support the trade in food (CXG 89-2016). https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B89-2016%252FCXG_089e.pdf

FAO & WHO. 2011. Compilation of Codex Texts Relevant to Labelling of Foods Derived from Modern Biotechnology (CAC/GL 76-2011). https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B76-2011%252FCXG_076e.pdf

FAO & WHO. 2010. Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods (CAC/GL 74-2010). https://www.fao.org/fileadmin/user_upload/gmfp/resources/CXG_074e.pdf

FAO & WHO. 2008. Guidelines for the Conduct of Food Safety Assessment of Foods Derived from recombinant-DNA Animals (CXG 68-2008). https://www.who.int/docs/default-source/food-safety/food-genetically-modified/cxg-068e.pdf?sfvrsn=c9de948e_2

FAO & WHO. 2007. Working Principles for Risk Analysis for Food Safety for Application by Governments (CAC/GL 62-2007). https://www.fao.org/3/a1550t/a1550t.pdf

FAO & WHO. 2003a. Guidelines for the Conduct of Food Safety Assessment of Foods Derived from recombinant-DNA Plants (CXG 45-2003). https://cban.ca/wp-content/uploads/Codex-Food-Safety-Guidelines.pdf

FAO & WHO. 2003b. Guidelines for the Conduct of Food Safety Assessment of Foods Derived from recombinant-DNA Microorganisms (CXG 46-2003). https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B46-2003%252FCXG_046f.pdf

FAO & WHO. 2003c. Guidelines on the Judgement of Equivalence of Sanitary Measures associated with Food Inspection and Certification Systems (CXG 53-2003). https://www.fao.org/3/y6396e/y6396e05.htm

FAO & WHO. 2003d. Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (CXG 44-2003). https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B44-2003%252FCXG_044e.pdf

Honduran National Service of Agri-Food Health and Safety (SENASA). 2019. Regulation 8/2019 and the Guatemala Ministry of Agriculture, Livestock, and Foodstuff Agreement no. 271/2019.

Luo, D., Huguet-Tapia, J.C., Raborn, R.T., White, F. Brendel, V.P. & Yang, B. 2021. The Xa7 resistance gene guards the rice susceptibility gene SWEET14 against exploitation by the bacterial blight pathogen. *Plant Communications* 19;2(3):100164. https://doi.org/10.1016/j.xplc.2021.100164

Menz, J., Modrzejewski, D., Hartung, F., Wilhelm, R. & Sprink, T. 2020. Genome Edited Crops Touch the Market: A View on the Global Development and Regulatory Environment. *Frontiers in Plant Science,* 11:1–17. https://doi.org/10.3389/fpls.2020.586027

Gene (or genome) editing includes specific techniques that make more precise changes in the genetic makeup of living organisms, which result in the expression of new traits. As the techniques develop into practical applications in the food and agriculture sectors, many countries have started to consider if and how gene-edited foods should be regulated.

This paper aims to explain the basic scientific principles underlying gene editing. It provides readers with information related to technical issues in the area of food safety and offers a summary of the current regulatory status of gene edited foods in different countries as well as a review of the existing documents made available by Codex Alimentarius, the international food standard setting body, that are potentially useful and relevant for any food safety assessment of gene edited foods.

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