Definition of Genome Editing and Relevant Terminology

A technical report of the joint EPA / ENCA Interest Group on Genetically Modified Organisms

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About the Joint EPA / ENCA Interest Group on Genetically Modified Organisms:

The Joint EPA ENCA Interest Group on Genetically Modified Organisms (IG GMO) promotes the exchange of information and experience on environmental risk assessment and monitoring of genetically modified organisms (GMO) between the Network EPA (Network of the Heads of Environmental Protection Agencies) and ENCA (European Network of Heads of Nature Conservation Agencies). The overall aim of the IG GMO mandate is to develop joint and consolidated views and positions of the EPA and ENCA networks in order to add additional emphasis to environmental aspects in the course of GMO approval procedures, environmental risk assessment (ERA) and environmental monitoring programmes. The IG GMO is composed of members from environmental protection agencies and nature conservation agencies or institutions with competence and expertise in ERA and monitoring in different regulatory fields. The author institutions support current efforts to further develop and improve environmental risk assessment and monitoring of GMOs in Europe, particularly considering new techniques for genetic modification, while stressing the need for a stronger emphasis on the environment and nature conservation in the approval processes and during monitoring of GMOs.

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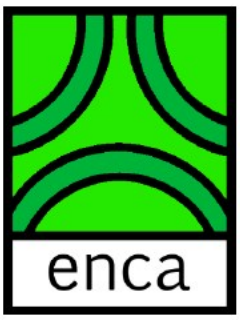
This position paper is the result of the work of the EPA Network's Interest Group on Genetically Modified Organisms. While it reflects the inputs of all participants of the Interest Group, it is only endorsed in this form by those Agencies mentioned on the front page.



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1 EXECUTIVE SUMMARY

1.1 Aim and Background

Genome editing, a new and rapidly evolving technology, is discussed worldwide since several years. The term ‘genome editing’ encompasses different techniques, CRISPR/Cas currently being the most prominent. The ongoing discussion covers amongst others the various possibilities to modify the genome and the application in different organisms, the traits envisaged, potential risks and respective environmental implications, as well as its regulation, including risk assessment requirements. Genome editing is currently legally defined neither in the European Union nor in Switzerland.

In the European Union a process for a new regulation of plants developed by certain new genomic techniques (i.e. targeted mutagenesis and cisgenesis) was started by the European Commission in 2021, with the publication of the legislative proposal in July 2023. In Switzerland, the parliament has mandated the federal administration to prepare a draft legislation for special provisions for the authorisation of certain plants developed by new genomic techniques. In the United Kingdom on the other hand, regulatory requirements were loosened in March 2023 with the Genetic Technology Act (Precision Breeding Act). This Act is currently applicable only in England. At the UN level genome editing was discussed under the Convention on Biological Diversity and the Cartagena Protocol on Biosafety in the context of gene drives and synthetic biology.

Being a controversial issue as such, the discussion is also complicated by the following issues:

- Genome editing is also discussed under other terms e.g. ‘new genomic techniques’ or ‘new mutagenesis techniques’.
- Often the terms gene editing and genome editing are used as synonyms.
- Genome editing is a technology covering different techniques (e.g. CRISPR/Cas, TALEN) and tools (e.g. the various nucleases used).
- Genome editing techniques can be used also to specifically alter the activity of genes (targeted modifications of RNA and the epigenome).
- A broad range of different modifications in the genome is possible by applying genome editing.
- The application of genome editing involves also other methods, e.g. for delivering the genome editing tool into the cell.
- Although targeted to a specific DNA sequence, unintended modifications are possible.

With this technical paper the Joint EPA/ENCA IG GMO (Interest Group on GMOs) aims to facilitate the current debate regarding genome editing. It provides background information on genome editing techniques and their application, a working definition of genome editing and a glossary of the terminology used in the context of genome editing. The glossary comprises not only technical terms derived from the technical background, but also terms used in connection with the regulatory discussions.

1.2 Content and key issues

Focussing on the development of genome editing in the context of plant breeding, characteristics of the techniques used are presented, highlighting specific aspects that are taken into account in the development of the working definition of genome editing.

The different genome editing techniques use certain genome editing tools (e.g. nucleases to cut the DNA, repair templates) to modify the genome at the location of a specific DNA sequence in a targeted way. In the beginning genome editing always involved DNA double-strand breaks. With the further development of techniques, alterations in the genome are also achieved generating single-strand breaks or no breaks at all. Thus, reducing the likelihood of unintended changes at the targeted sequences. Furthermore, genome editing can also be used to modify RNA or the epigenome, in order to alter the activity of specific genes.

Genome editing is often described in a simplified way, as being similar to conventional breeding (in particular conventional mutagenesis techniques using chemical substances or radiation), and focusing on certain types of applications (e.g. the generation of point mutations). However, a variety of modifications can be produced with genome editing from small to larger modifications, including also the introduction of one or more entire genes. In addition, specific parts of the genome are more accessible to changes and several genes can be targeted and modified at the same time. Hence, the type of changes that can be introduced by genome editing goes beyond what is possible with conventional breeding.

Taking into account these specific aspects of genome editing, the following working definition of genome editing was developed by the IG GMO:

"Genome editing comprises various techniques for the targeted modification of the genome by inducing site-specific alterations. Using respective genome editing tools and the cell's own repair mechanism a broad spectrum of modifications in the genome can be introduced."

Although genome editing modifies the genome in a targeted way at a specific sequence, unintended modifications are also possible in other parts of the genome ('off target' modifications), as well as unintended modifications at the target site ('on target' modification). It is also important to note, that in order to generate a new plant variety with the desired trait not only the genome editing technique per se is applied. Other working steps are necessary, using e.g. methods to deliver the genome editing tool into the target cell or for the regeneration of the modified cells into plants (these steps typically use *in vitro* tissue culture). Those methods used in connection with the genome editing steps are relevant since they may lead to unintended modifications.

In order to identify important terms used in connection with genome editing in the current discussions in Europe, regulatory developments in the EU, Switzerland, England, and respective discussions at the UN level were analysed.

This technical paper reflects the current state of development and regulatory discussions as of September 2023. The Joint EPA/ENCA IG GMO trusts that the information provided supports the discussions taking place in Europe.

2 INTRODUCTION

Genome editing is a new and rapidly evolving technology comprising a variety of techniques (e.g. CRISPR/Cas, TALEN), whereby further developments in the next years are to be expected. Genome editing allows for different kinds of modifications in the genome. With this a variety of traits can be developed and the use in various organisms is possible. However, this technical paper focuses on genome editing in plants. Although products are at different stages of research and development, several are expected to enter the market in the near future. In fact, a few products are already available, e.g. a high-oleic soybean produced by Calyxt (cultivated in the US) or a GABA tomato authorised for cultivation in Japan (Parisi and Rodriguez-Cerezo, 2021). This tomato contains high amounts of gamma-aminobutyric acid (GABA), an amino acid that functions as an inhibitory neurotransmitter of the central nervous system and is thus known for producing a calming effect.

Given the environmental relevance of products developed by genome editing, corresponding challenges and environmental impacts need to be addressed. These issues are intensively discussed in the European Union (EU) and Switzerland and are also on the agenda of the Convention on Biological Diversity and its Cartagena Protocol on Biosafety. In the EU, for example, the European Commission (EC) recently started a policy initiative regarding legislation for plants produced by certain new genomic techniques (European Commission, 2021b). Discussions on regulatory requirements are not only of political relevance but have the potential for major consequences for the environment, biodiversity, and nature conservation. This is especially important as genome edited products are regulated differently worldwide (Eckerstorfer et al., 2019).

Currently, there is no clear and harmonised definition of genome editing. In addition, genome editing is discussed under other terms such as ‘new genomic techniques’, ‘new gene techniques’, ‘gene editing’ ‘new breeding techniques’, or ‘new mutagenesis techniques’ which are often, but not consistently used synonymously. This lack of clarity could hinder information exchange as well as discussions on environmental impacts and associated legal issues. Thus, the aim of this technical paper is to provide background information on genome editing and terminology used in the context of this technology. This information could also be relevant for assessing different regulatory approaches, e.g. in the context of the policy initiative of the EU, regarding its coverage of genome editing techniques, modifications in the genome, or products developed by genome editing.

Based on current developments, including techniques and applications (chapter 3) as well as political discussions and regulatory developments (chapter 4 and 5), an overview of definitions used is presented and a working definition of genome editing adapted to the state of development proposed (chapter 6). Furthermore, an overview of the most relevant terminology used in the context of genome editing is provided for the sake of clarity, to facilitate the upcoming discussions (chapter 0).

3 BACKGROUND INFORMATION ON TECHNIQUES & APPLICATIONS

This chapter provides background information on the development of genome editing techniques in the context of plant breeding. First, a brief insight into various techniques aiming to increase genetic variability and to introduce specific traits is presented, followed by a description of the main characteristics of genome editing techniques. Finally, specific aspects of genome editing are highlighted, which are important for a profound understanding of the discussion surrounding these techniques. In addition, information on the application of genome editing in animals and microorganisms is provided (see Box 1 and Box 2).

3.1 Development of plant varieties

Since centuries mankind has made use of selection and cross-breeding to improve plant varieties. Selected sexual crossing of varieties differing in characteristics create new recombination of traits based on the Mendelian laws of inheritance. This enables the introduction of naturally occurring traits or mutated traits of agronomic interest into elite varieties via backcrossing. Using the so called sexual hybridisation techniques the hybridisation process was optimised (e.g. via artificial pollination) and modified (e.g. *in vitro* fertilisation) expanding the range of recombination (Broothaerts et al., 2021). In addition, old techniques, such as grafting, the non-sexual combination of tissues of a scion with tissues of a root stock, are being used for certain species (e.g. fruit trees). Today, the selection of desired traits is no longer based on phenotypic properties only, but is significantly enhanced by marker-assisted selection, i.e. the identification of genomic markers associated with specific phenotypic traits. However, due to sexual incompatibility cross-breeding has its limits and thus sophisticated approaches complementing conventional breeding approaches were developed. These comprise, for example, multiplication of the number of chromosomes of a species or regeneration of double haploid plantlets from haploid gametes. With these techniques the breeders’ gene pool comprises the primary (interbreed freely), secondary (cross-breed only with difficulty) and tertiary gene pool (cross-breed only with advanced techniques) for most crop species (see glossary, chapter 7). The European Food Safety Authority (EFSA) included definitions of those gene pools in a recent statement (EFSA GMO Panel, 2022) concerning criteria for risk assessment in the context of the EC’s initiative regarding plants produced by certain new genomic techniques (also provided in the glossary). Although the breeders’ gene pool is not a new concept, further discussions regarding its definition are to be expected when applied in this specific context.

In the early 20th century, mutation breeding further increased genetic variation used in plant breeding by inducing random mutations in the genome using either chemical substances or physical radiation. Since the mutations are randomly generated in the plant genome, these methods are also called random mutagenesis techniques. While this has significantly expanded genetic variation, the screening

necessary to identify desired mutations is laborious. Thus, the development of new varieties is a lengthy process as - like with cross-breeding - multiple backcrossing steps are necessary (Gao, 2021).

At the end of last century, the development of transgenesis enabled introduction of genes from other species and classes of organisms (e.g. bacteria) into plants. With this technique the breeders' gene pool was substantially extended, while so far only the primary, secondary and tertiary gene pool were accessible for breeding purposes. Various delivery strategies are available to introduce foreign genes into cells. In classical transgenesis the transgenes are randomly integrated in the genome. This imponderability leads to specific expression patterns in a genetically modified organism (GMO) and may also entail unintended effects. E.g. depending on its integration site a transgene may interfere with the expression of other genes, resulting in changes in the phenotype (e.g. the production of a protein/toxin that normally is not produced in the non-GM variety). In transgenesis foreign DNA always remains in the modified genome and it is passed on to successive generations.

The next generation of plant breeding strategies are commonly addressed as 'new genomic techniques' or 'new plant breeding techniques'. They comprise a range of different and rapidly evolving techniques including genome editing techniques (e.g. CRISPR/Cas). The main characteristic of most of these new genomic techniques is their target-specificity for specific DNA sequences occurring in the genome. Thus, sometimes also the term targeted or site-directed mutagenesis techniques is used.

The term 'new plant breeding techniques' is also used for combinations of conventional breeding techniques with transgenesis (e.g. transgrafting, i.e. combining transgenic and unmodified plant parts via grafting) and for advances in transgenesis (e.g. cis- and intragenesis), although these techniques need not necessarily involve a site-specific editing tool (Eckerstorfer et al., 2014; European Commission, Directorate-General for Research and Innovation, 2017).

3.2 The emergence of genome editing techniques

The procedure for genome editing of plants generally involves various steps: selection of an appropriate nuclease (i.e. enzyme, which cuts nucleic acids), a means for transformation (e.g. viral vector) and a delivery method for the introduction of the genome editing agent into the plant cell. After that, the genome edited cells have to be regenerated into plants via tissue culture. Genotyping and mutant screening must be performed to identify successfully modified plants. All but the first step, i.e. the nuclease, are equally applied in transgenesis. Various methods and tools are available for the transformation and regeneration steps depending on the organisms to be modified and the intended changes (Gao, 2021). The main genome editing techniques are described below together with the respective molecular tools and their basic mode of action.

However, new techniques emerge continuously, and no commonly agreed definition exist for the terms used to describe various types of techniques, e.g. 'new genomic techniques' or 'new mutagenesis techniques'. This affects common understanding of the topic and may lead to legal ambiguity. In addition, new genomic techniques can be used in combination with conventional breeding techniques as well as with established techniques of genetic modification (e.g. transgenesis) adding further complexity to the issue.

The starting point for genome editing was the ability to design nucleases that induce double-strand breaks at specific sequences in the DNA. These breaks are being repaired by the cell's own repair system, which is error prone and may lead to alterations, i.e. small insertions or deletions. Thus, the basic mode of action consists of several steps: recognition and binding to a target sequence inducing a double-strand break and repair thereof (Broothaerts et al., 2021). Within a few years, multiple genomic tools differing in their characteristics (e.g. nucleases with different binding-specificity or cutting abilities) were developed. The application of the various molecular tools, mostly the type of nuclease used, determine the denomination of the different genome editing techniques.

3.2.1 Genome editing tools

The first set of tools explored were sequence-/site-specific nucleases, also called site-directed nucleases (SDNs), which cut the DNA at specific sites or specific sequences. In this way modifications are focussed to a targeted site in the genome instead of producing them randomly in large numbers. A range of SDNs have been identified to date, e.g. mega-nucleases, zinc-finger nucleases (ZFNs) or transcription activator-like effector nucleases (TALENs). However, the most known of these are the CRISPR/Cas nuclease systems which are derived from the "immune" system of bacteria. These nuclease systems enable the cleavage of foreign DNA and have been adapted as genome editing tools (Liu et al., 2022). In these systems specifically designed single-guide RNAs are used to direct the Cas nuclease to a complementary genomic target sequence. Since various guide RNAs may be designed and produced in simple and fast ways, the CRISPR/Cas nuclease systems offer superior versatility for an easy editing of almost all genomic sequences. In comparison ZFNs and TALENs are protein-based genome editors which recognise specific DNA target sequences via protein-DNA interactions. Adaptation of these DNA-binding domains to new target sequences is usually quite laborious and time consuming compared with CRISPR/Cas systems (Gao, 2021).

However, genome editing techniques are not limited to the applications of SDNs. Nucleic acid molecules can also be used to induce genomic changes, as for example in oligonucleotide directed mutagenesis (ODM). In a recent review published by the Joint Research Centre of the EC new genomic techniques are classified according to their action on the genome (Broothaerts et al., 2021). Here, genome editing techniques which produce double-strand breaks (e.g. SDN-mediated techniques using ZFN, TALEN, CRISPR/Cas) are differentiated from those which achieve alterations inducing only single-strand breaks or no breaks at all (e.g. ODM, base editing, prime-editing, see also chapter 3.4). Increasingly, applications which apply these techniques not only to the DNA but directly on the RNA or on the epigenome are also developed. Today, CRIPSR/Cas outweighs other genome editing techniques in research and development due to its low costs and its ease of use (Parisi and Rodriguez-Cerezo, 2021).

It is important to recognise that the type and combination of genomic tools used (e.g. nucleases, guide RNA, use of repair templates) characterise a certain genome editing technique. Its specific design determines the organisational level (e.g. DNA, RNA) it acts upon and the different kind of changes inducible with it (e.g. insertion, deletion, substitution, DNA de/methylation, chromosomal rearrangements). Thus, each genome editing technique consists of a specific set of genomic tools and has a specific range of applications (e.g. in various species, at cell or organism level). Overall, the development of new genomic tools is driven by the intention to increase and modulate the specificity and efficiency of the achieved changes (Liu et al., 2022). This constantly expands the possibilities for changes attainable in the genome and epigenome and contributes to the further development of new applications of genomic techniques.

3.2.2 Delivery and expression of the genome editing tools

The ongoing developments are not only comprising the genomic tools that are used for genome editing, i.e. the different sequence specific nucleases, but also the ways and means of delivering and expressing these tools in target cells. Methods used for these purposes may induce unintended on-target and off-target modifications.

In many cases the genomic tools for genome editing are still expressed in the target cells from transgenic DNA constructs, which are inserted into the genome of the recipient cells by established techniques of genetic modification (Gao, 2021). The transgenic DNA construct can be segregated from the resulting plant, leaving only the introduced changes in the product. Alternatively, the genome editing tools may be expressed from extrachromosomal vectors, e.g. from plant virus vectors or plasmids, which are only transiently present in the target cells thus limiting the time of their expression. The best control of expression levels and duration of expression is achieved by two methods: 1) the introduction of RNA constructs encoding the genome editors or 2) by introduction of nuclease proteins or functionally active complexes of Cas proteins and the respective guide RNAs, which are produced and preassembled *in vitro*. However, due to technical difficulties these methods for DNA-free genome editing, which would

also reduce the chances for off-target and unintended modifications, are presently rarely used (Gao, 2021).

3.3 Characteristics of genome editing techniques

The main difference between genome editing and random mutagenesis or transgenesis is its ability to induce site-specific alterations. However, if the targeted sequence is not unique in the genome of the particular organism, the genomic tool may act on parts of the genome not intended to be modified, thus leading to off-target modifications. The further development of genomic tools and resulting genome editing techniques aims at reducing these off-target effects. Moreover, recent developments in CRISPR/Cas technology such as prime-editing rely on introducing single-strand breaks (nicks) at the target DNA sequence instead of double-strand breaks. This favours the introduction of precise sequence changes specified by the prime-editing RNA and reduces unintended effects on the targeted site resulting from the error prone repair mechanism (Kawall et al., 2020).

Another substantial characteristic of genome editing techniques is that by using multiplexing approaches several genes at different sites can be targeted and modified at once or successively. This enables the modification of polygenic traits, such as abiotic stress response or the alteration of whole metabolic pathways. Additionally, many plant species are polyploid and/or have redundant genomes/multiple copies of genes. Genome editing techniques allow the simultaneous alteration of all alleles of a certain gene or all genes of a gene family. So although the number of changes introduced by genome editing techniques is lower than in conventional mutagenesis, multiplex editing creates more alterations and different changes than achievable with transgenesis or conventional breeding (Kawall, 2019; Broothaerts et al., 2021).

Different types of alterations can be achieved with genome editing techniques: small-sized modifications of existing genes, a multitude of changes simultaneously which may substantially change an organism, and introduction of new genes (like in transgenesis but at specific sites). Genomic changes can be in the range of single nucleotides (e.g. by base editing) up to mega base pairs (e.g. by chromosomal engineering (Huang and Puchta, 2021)). In general, three different applications of site-directed nucleases are distinguished depending on the repair mechanism addressed and the optional use of repair templates: SDN-1, which mostly results in point mutations; SDN-2, which allows for base exchange, small deletions or insertions specified by repair templates, and SDN-3, which enables the integration of new genes using DNA templates.

However, this classification has been criticised for suggesting an order of different degrees of ‘foreignness’ implicitly indicating less and more problematic modifications, where in fact they are not related to potential risks arising thereof (Heinemann et al., 2021). Although the targeting specificity can be significantly improved in genome editing techniques (Liu et al., 2022), there is less control on the repair process and thus the achieved changes (Broothaerts et al., 2021). Overall, genome editing techniques can produce genetic alterations: ranging from those which may occur naturally to those that are specific to new genomic techniques (Broothaerts et al., 2021). In any case they expand the spectrum of artificially inducible alterations in the genome, in particular as they enable complex modifications in parts of the genome, which were not accessible before (Kawall, 2019). An overview of the techniques is provided in Table.

Today, genome editing techniques for current and future markets are predominantly applied in plants, currently with most activities identified in cereals, oil and fibre crops, and vegetables (Parisi and Rodriguez-Cerezo, 2021). However, the flexibility and cost-efficiency of CRISPR/Cas approaches expand the spectrum of plants accessible to modifications. In addition, multiplexing approaches broaden the range of traits targeted with genome editing techniques and thus more complex and polygenic traits are increasingly targeted in plant development (e.g. modified composition, biotic and abiotic stress tolerance) (Parisi and Rodriguez-Cerezo, 2021).

3.4 Latest developments in genome editing techniques

The toolbox of genome editing, in particular of CRISPR-based editing, is rapidly expanding in a variety of different ways. The main avenues of the recent developments are summarised in the following list. These developments are focusing on a number of several different goals, i.e. to improve the technical feasibility to conduct genome editing, to increase the range or the specificity of the introduced genetic modifications and to expand the scope of genome editing beyond introducing mutations into the nuclear DNA of the targeted organisms:

1. Newly characterised or modified Cas-nucleases were developed to ensure either a higher precision of the editing or to offer a greater versatility of application (Adli, 2018). Concerning the latter this expands the range of plants and other organisms which can be modified on the one hand and the way in which the editing is conducted on the other hand. E.g. small-sized or hypercompact Cas-variants may be transferred into target cells more easily (Zhang et al., 2021). Further developments concern the intentional fine-tuning of the activity of CRISPR-systems, e.g. by anti-CRISPR proteins for better control of genome editing (Calvache et al., 2022).
2. New methodological approaches for genome editing were developed, namely base editing and prime-editing, to improve the specificity of the editing process (Anzalone et al., 2020). The latter method may also be used to increase the efficiency of the introduction of intended sequences at a specific genomic location. Prime-editing is also able to efficiently introduce large size sequence replacements (of more than 1 kb in length) specified by an additionally supplied DNA-template (Jiang et al., 2022). Such large-scale, highly parallel genome engineering (also called genome shuffling) may be considered a first step towards application of synthetic biology in plants (Chari and Church, 2017).
3. CRISPR genome editing tools were also developed or adapted to modify other targets than nuclear DNA. Some of these systems are targeted to modify DNA contained in cellular organelles, such as mitochondria or chloroplasts (Kang et al., 2021). Other genome editing systems were developed to influence the expression of specific genes without changing (or mutating) any DNA sequences: CRISPR tools are available to directly edit specific RNA molecules in the respective cells or to modify epigenetic signals for gene expression at particular genomic loci (Adli, 2018). The latter approach is also called epigenetic engineering and may be facilitated by engineering different components of the CRISPR system: either the Cas nuclease or the respective guide RNA (Dong et al., 2022).
4. CRISPR genome editing systems are also instrumental for the ongoing development of gene drives in various organisms, including plants (Neve, 2018; Bier, 2022).

In conclusion the new genome editing tools further increase the range of possible genomic modifications as well as their complexity. This is facilitating the development of a wide variety of novel traits, including traits that are established by complex alterations of metabolic pathways resulting in substantial physiological and morphological changes. In addition, off-target activity is still observed for most of the newly developed approaches, including base editing, editing tools for organelle DNA as well as epigenetic editors (Kempton and Qi, 2019).

Box 1: Genome editing in animals

Genome editing is applied in various farm animals, including cattle, goat, sheep, pig, horse, rabbit, chicken, and quail. The traits targeted comprise different fields of applications, including animal welfare (e.g. hornlessness), disease control, performance (e.g. enhanced muscle growth, which is targeted in most of the species listed above), changes in product quality (e.g. lower lactose content in milk), or reproduction (e.g. sex determination pre-hatch in chicken). Examples are the production of hornless cattle (Carlson et al., 2016), heat tolerant cattle (Hansen, 2020), pigs resistant to African Swine Fever (Wang et al., 2022), and enhanced wool yield in sheep and goat (Li et al., 2017).

Genome editing is also applied in a variety of fish species, e.g. Tilapia, Rainbow trout, Common carp, and Atlantic salmon. The fields of application are similar to those in farm animals and include disease control, performance (e.g. enhanced muscle growth, higher feed efficiency), product quality, pigmentation, or reproduction (sterility). Examples are Nile tilapia (*Oreochromis niloticus*) resistant to *Vibrio vulnificus* infection (Chiang et al., 2020) and Common carp (Cyprinus carpio) with enhanced growth (Zhong et al., 2016).

Box 2: Genome editing in microorganisms

A scoping exercise conducted some years ago at the OECD level identified relevant areas of applications of microorganisms, including GM microorganisms, for environmental uses (OECD, Organisation for Economic Co-operation and Development, 2014). The range of possible applications included the use of GM microorganisms in agriculture, e.g. as biofertilisers or as biocontrol agents, the use of GM microalgae for purposes of production of biofuels or other compounds, and the use of GM microorganisms for bioremediation and cleaning.

Commonly non-modified microorganisms are used for the above-mentioned purposes. In addition, established techniques of genetic modification (i.e. transgenesis) and - more recently - genome editing approaches are explored to improve microorganisms used for these types of applications. Both approaches are used in complementary ways. Transgenesis is used for the creation of new metabolic or physiological characteristics by introduction of new gene functions. Genome editing is used for the modification of expression of endogenous genes via gene knockouts or by modification of regulatory elements for gene expression. Multiplexed genome editing, i.e. the simultaneous editing of multiple gene targets, is frequently used in microorganisms to facilitate complex engineering of metabolic pathways (Adiego-Pérez et al., 2019).

In microalgae genome editing was used in a number of species, including *Chlamydomonas sp*. and *Chlorella sp*. as well as in a few diatoms, such as *Nannochloropsis sp*. and *Phaeodactylum tricornutum,* to optimize the biosynthetic pathways for photosynthetic efficiency and increased biomass production as well as for increased production of biofuel lipids and other high-value compounds, e.g. carotenoids (Patel et al., 2019; Kumar et al., 2020).

Metabolic engineering by transgenesis and genome editing is also explored for microbial applications in bioremediation (Sharma and Shukla, 2022). These applications in a variety of bacteria target environmental pollutants such as heavy metal contaminations and other pollutants, e.g. synthetic dyes and pesticides (Jaiswal et al., 2019).

Genome edited rhizobacteria, e.g. *Bradyrhizobium elkanii*, *Sinorhizobium sp*. and *Rhizobium spp*., are also developed as plant growth promoting agents to aid phytoremediation (Basu et al., 2018). In general plant promoting microorganisms, such as rhizobacteria and soil-associated fungi may be used as biofertilizers to increase agricultural productivity (Pirttilä et al., 2021).

Biotechnological modification is also explored to improve microbial biocontrol agents, developed from a range of viruses, bacteria including *Bacillus thuringiensis,* and entomopathogenic fungi, e.g. *Beauveria bassiana* and *Metarhizium anisopliae* (Leung et al., 2020).

Table: Comparison of techniques ("not applicable": there is no specific target site)

		Conventional breeding techniques		Established techniques of genetic modification	New genomic techniques				
								Genome Editing	
		Cross-breeding	Random mutagenesis	Transgenesis	Cisgenesis	Intragenesis	(SDN3)	Targeted mutagenesis (SDN2)	Targeted mutagenesis (ODM, SDN1)
Intended modifications	Gene insertions (large insertions)	No	No	Yes (untargeted)	Yes	Yes	Yes	No	No
	Deletions and/or small insertions	No	Yes *(untargeted)*	No	No	No	No	Yes *(targeted)*	Yes *(targeted)*
	Point mutations	No	Yes *(untargeted)*	No	No	No	No	Yes *(targeted)*	Yes *(targeted)*
	Targeted multiplexing (multiple genomic changes)	No	No	No	No	No	Yes	Yes	Yes
	Site specific modification	No	No	No	No	No	Yes	Yes	Yes
	Modification of RNA	No	No	No	No	No	No	No	Yes
	Modification of gene expression	No	Yes *(untargeted)*	Yes	No	Yes	Yes	Yes	Yes
	Modification of epigenome	No	No	Yes (untargeted)	No	No	No	No	Yes
	General unintended modifications	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Unintended on-target modifications	Not applicable	Not applicable	Not applicable	Yes	Yes	Yes	Yes	Yes
	Presence of exogenous DNA in product	No	No	Yes	Yes	Yes	Yes	Yes	No
	Selection marker	No	No	Yes	Yes	Yes	No	No	No
	Delivery method needed	No	No	Yes	Yes	Yes	Yes	Yes	Yes
	Regeneration via tissue culture	No	No (*in vivo*) Yes (*in vitro*)	Yes	Yes	Yes	Yes	Yes	Yes

4 REGULATORY DEVELOPMENTS IN EUROPE

In order to identify important terms used in connection with new gene technologies and genome editing, an overview is provided on current respective discussions and regulatory developments in Europe. Terms identified are addressed in the glossary (see chapter 0), where also (legal) definitions are provided if available.

4.1 Developments in the EU

According to the Directive 2001/18/EC a GMO *"means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination*" (Directive 2001/18/EC, 2001). The Directive also includes a non-exhaustive list of techniques, that result in genetic modifications (Directive 2001/18/EC, Annex I A, part 1).

In the EU new gene technologies were first discussed on scientific level and under the term 'new breeding techniques'. The High Level Group on Scientific Advisors referred to the following techniques: genome editing technologies, cisgenesis, intragenesis, agro-infiltration, RNA-dependent DNA methylation (for epigenetic modification), grafting and reverse breeding (European Commission, Directorate-General for Research and Innovation, 2017). The application of new breeding techniques in synthetic biology and gene drives was also discussed.

The legal discussions on new gene technologies started with a preliminary ruling procedure where the French Conseil d'État (Council of State) requested the Court of Justice of the European Union to determine whether the application of mutagenesis techniques results in GMOs and whether they fall under the Directive 2001/18/EC. The respective judgment was published on 25 July 2018 clarifying that organisms produced by mutagenesis techniques are to be considered GMOs and that organisms produced by new mutagenesis techniques have to meet the requirements of the Directive 2001/18/EC. Exempted from those requirements are organisms produced by mutagenesis techniques "*which have conventionally been used in a number of applications and have a long safety record*" (Court of Justice of the European Union, 2018). New mutagenesis techniques are referred to as techniques of directed mutagenesis that have emerged since the adoption of the Directive 2001/18/EC.

Although the ruling brought clarity regarding new mutagenesis techniques, discussions regarding the practical consequences went on, also at the Council of the European Union. Thus, in November 2019 the EC was requested by a respective Council Decision to submit a study regarding the status of 'novel genomic techniques' under the EU law. If seen necessary according to the study outcomes, a legislative proposal was to be submitted accordingly (Council of the European Union, 2019). In the decision the Council also referred to new breeding techniques. It was highlighted that the Directive 2001/18/EC was drafted on the basis of breeding techniques available at that time and that the development of new breeding techniques led to uncertainty whether those are covered by the definition of GMO and the scope of the Directive 2001/18/EC.

For the context of the study, the EC defined *'*new genomic techniques' (NGT) as "*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 legislation on genetically modified organisms (GMOs) was adopted*" (European Commission, 2021a). In the study the EC provided also a glossary with selected terms, defined for the context of the study. Those include e.g.:

- conventional GMOs: "*GMOs resulting from established genomic techniques. Conventional GMOs that have been authorised to date in the EU are transgenic*".
- established genomic techniques: "*Genomic techniques developed prior to 2001, when the existing GMO legislation was adopted*".
- mutagenesis*: "Creation of mutation(s) in an organism without insertion of foreign genetic material*".

- conventional or random mutagenesis techniques: "*An umbrella term used to describe older techniques of mutagenesis that have been used since the 1950s; they involve irradiation or treatment with chemicals in order to produce random mutations, and typically involve screening of a large number of mutants to select one with desirable properties. Organisms obtained with such techniques are GMOs that are exempted from the scope of the EU GMO legislation*".
- targeted mutagenesis or site-directed mutagenesis technique: "*An umbrella term used to describe newer techniques of mutagenesis that induce mutation(s) in selected target locations of the genome without insertion of genetic material. The process usually results in a 'knock-out', i.e. the disruption of the functioning of a gene that is responsible for an unwanted effect, or in modifications of the expressed protein or of regulatory elements of a gene*".
- genome-editing (gene-editing) techniques: "*A subset of NGTs that allows precise modification of DNA in the target genome in a variety of ways. Genome editing encompasses a variety of techniques, which may be applied in mutagenesis, cisgenesis, intragenesis or transgenesis".*

Definitions of several terms used in the context of new genomic techniques were also provided by the EFSA in a recent statement concerning criteria for risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis (EFSA GMO Panel, 2022).

In view of the outcomes of the study published in 2021, the EC started a policy initiative on plants produced by targeted mutagenesis and cisgenesis (including derived food and feed). The respective inception impact assessment "legislation for plants produced by certain new genomic techniques" was published in September 2021 (European Commission, 2021b). The legislative proposal and the accompanying impact assessment were published in July 2023 (European Commission, 2023a,b).

As described above, various terms were used in the EU in the context of new gene technologies. Except GMO, none of the terms has been legally defined, but several of the terms are used synonymously.

4.2 Situation in other European countries

4.2.1 Switzerland

In Switzerland GMOs are regulated according to the Swiss Gene Technology Act. In its Article 5 (2) GMOs are defined as "*organisms in which the genetic material has been altered in a way that does not occur under natural conditions by crossing or natural recombination"* (Gene Technology Act, 2022).

The Swiss Gene Technology Act includes in Article 37*a* provisions regarding a transition period for placing on the market of certain GMOs (the so-called GMO moratorium). Until 31 December 2025 GM plants (including plant parts, seeds and other propagation material) as well as GM animals for agricultural, horticultural or silvicultural purposes are not to be authorised for placing on the market (Gene Technology Act, 2022).

With the arrival of new gene technologies discussions regarding the scope of the regulatory framework as well as the definition of a GMO started also in Switzerland. In February 2023 a report, approved by the Swiss Federal Council, was published in response to various questions raised by the parliament (Swiss National Council, 2020; Swiss Council of States, 2021; Swiss National Council, 2021). Based on the legal examination, the report states that products produced by new gene technologies are GMOs and fall under the scope of the Swiss Gene Technology Act. An exemption of certain techniques would have to respect the constitutional framework and require a respective adaptation of the Act by the Parliament. The report also refers to the existing scope of action to simplify the authorisation procedure or grant exemptions for certain GMOs. It is also noted that the existing regulation provides sufficient flexibility to also assess GMOs produced by new gene technologies (Federal Department of the Environment, Transport, Energy and Communications, 2023). The report addressed also the provisions accompanying the last extension of the Swiss GMO moratorium (Gene Technology Act, Art. 37a, (2)). The Swiss Federal Council has to provide by mid-2024 a draft legislation for a risk-based authorisation regime for non-transgenic GM plants (including plant parts, seed and other plant propagating material)

for agricultural, horticultural or silvicultural purposes that have been developed using new breeding techniques and that have a proven added value for agriculture, the environmental or consumers' benefits compared with conventional breeding techniques (Gene Technology Act, 2022).

Also a separate discussion is going on regarding the legal status of the TEgenesis technique. TEgenesis is a mutagenesis technique where, caused by chemicals, mobile genetic elements of the plant (so called transposable elements) are mobilised and subsequently integrated in new places of the plant genome. A legal opinion provided by the Federal Office of Justice concluded that products produced by TEgenesis fall under the Swiss Gene Technology Act (Federal Office of Justice FOJ, 2021). This is in line with the conclusions on the legal status of plants produced by new breeding techniques in the report in response to the postulates. In the EU this technique was discussed under the topic "Epibreed procedure" and a parliamentary question was sent to the EC, asking whether this technique falls under the scope of the Directive 2001/18/EC (European Parliament, 2020). In their response the EC referred to the judgement of the Court of Justice of the European Union and states that in their opinion organisms produced by the "Epibreed procedure" fall under the provisions of the EU GMO law (European Commission, 2020).

4.2.2 Norway

In 2018 and in the context of discussions on new genomic techniques the Norwegian Biotechnology Advisory Board published a report discussing amongst others whether all GMOs should be covered by GMO legislation, and what requirements should apply (including requirements for labelling, traceability and monitoring). The report also proposes three regulatory options: (1) retain current distinction between organisms produced by gene technology and conventional methods, (2) include currently exempt organisms/methods under GMO regulations, (3) exempt certain organisms produced by gene technology from GMO regulations. Although the Biotechnology Advisory Board did not recommend to exempt GMOs with permanent, heritable changes from regulation, approval and assessment requirements should be differentiated (The Norwegian Biotechnology Advisory Board, 2018).

In 2020 the Norwegian Government appointed the Norwegian Public Committee on Gene Technology to discuss the scientific developments and propose changes to the current GMO legislation. In 2023 a respective report was published, available in Norwegian language only (Norwegian Public Committee on Gene Technology, 2023). In addition, recommendations from the majority of the Committee (7 out of 11 members) are available in English language (NCE Heidner Biocluster, 2023), proposing amongst others differentiated regulation (four levels of regulation) based on the type of genetic change ("precision bred" organisms with changes within the species' gene pool, and GMOs with changes outside the species' gene pool) and knowledge on the trait (two categories). This is seen as operationalisation of the EFSAs proposal for risk assessment criteria (EFSA GMO Panel, 2022). Thus, the debate going on in the EU is reflected in the Norwegian document.

4.2.3 England

In March 2023 the Precision Breeding Act was enacted in the United Kingdom, currently being applicable in England only, but not in Scotland, Wales or Northern Ireland. This act regulates precision bred plants and animals and removes these organisms from the definition of GMO (Food Standards Agency, 2023). Precision bred plants and animals are those that have been produced by modern biotechnologies (which includes genome editing) and where the changes in the genome could have resulted from traditional processes (e.g. induced mutagenesis) or occur naturally. Plants and animals with changes that could not have occurred naturally or by traditional process are still regulated as GMOs.

Based on the Precision Breeding Act the Food Standards Agency will develop a respective authorisation process (Food Standards Agency, 2023). The aim is to regulate the products "proportionate to the risk" and as such apply simpler regulatory requirements (Department for Environment, Food and Rural Affairs, 2022).

5 DISCUSSIONS AT THE UN LEVEL

In this chapter an overview is provided on current discussions in the context of genome editing on UN level under the Convention on Biological Diversity (CBD) and the Cartagena Protocol on Biosafety (CPB). Important terms identified are also available in the glossary (see chapter 0).

In the UN context GMOs are discussed under the term 'living modified organism' (LMO). According to Article 3 of the CPB a LMO means "*any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology".* Modern biotechnology is defined as "*the application of: a) In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or b) Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection*" (Secretariat of the Convention on Biological Diversity, 2000).

The CPB is a treaty under the CBD and regulates safe handling, transport and use of LMOs on the international level. Within its framework genome editing is particularly relevant regarding the implementation of its articles 15 (risk assessment) and 16 (risk management). So far genome editing was discussed in the context of 'new developments in modern biotechnology' and gene drives (Secretariat of the Convention on Biological Diversity, 2020). However, Parties to the CPB did not so far decide to develop a definition of genome editing.

Genome editing is also discussed in the context of synthetic biology in the framework of the CBD. A respective operational definition was developed by the Ad Hoc Technical Expert Group (AHTEG) on Synthetic Biology in 2015: *"Synthetic biology is a further development and new dimension of modern biotechnology that combines science, technology and engineering to facilitate and accelerate the understanding, design, redesign, manufacture and/or modification of genetic materials, living organisms and biological systems."* (Secretariat of the Convention on Biological Diversity, 2015).

In 2018 Parties at CBD COP 14 decided that the AHTEG on Synthetic Biology shall "*take stock of new technological developments in synthetic biology since the last meeting of the Ad Hoc Technical Expert Group, including the consideration, among other things, of concrete applications of genome editing if they relate to synthetic biology, in order to support a broad and regular horizon scanning process*" (Secretariat of the Convention on Biological Diversity, 2018). This process was established at CBD COP 15 (Secretariat of the Convention on Biological Diversity, 2022a). The mandate of the AHTEG did not include the development of a definition of genome editing. However, a definition of genome editing is provided in a report on synthetic biology published by the Secretariat of the CBD. Genome editing is defined as "*a suite of tools (oligonucleotide mutagenesis and site-directed nucleases) that can facilitate targeted changes to the genome. CRISPR/Cas is an example of a widely known tool for genome editing*" (Secretariat of the Convention on Biological Diversity, 2022b). This report also provides information on genome editing techniques and respective applications.

6 WORKING DEFINITION OF GENOME EDITING

Neither in the EU nor in Switzerland genome editing is legally defined. Examples for definitions have been provided in the study of the EC (European Commission, 2021a) and in the report on synthetic biology published by the Secretariat of the CBD (Secretariat of the Convention on Biological Diversity, 2022b) as referred to in chapters 4.1 and 5.

Genome editing is a fast and constantly evolving technology with further developments to be expected. Thus, no final definition is possible since future developments cannot be envisaged. However, as described in chapter 3 in more detail, the following elements are considered significant features of genome editing taking into account the current status of development:

- Genome editing is a fast-developing technology, comprising various techniques
- Various genome editing techniques (e.g. TALEN, CRISPR/Cas) have been developed using different editing tools (e.g. nucleases)

- Genome editing uses a targeted approach inducing site-specific alterations
- Genome editing techniques involve double-strand breaks, single-strand breaks, or no breaks
- Genome editing techniques allow a broad range of modifications of DNA, RNA and the epigenome
- A broad spectrum of modifications is possible (e.g. insertions, deletions, point mutations, insertions of genes, DNA de/methylation, multiplexing)

These aspects could be summarised by the following working definition:

Genome editing comprises various techniques for the targeted modification of the genome by inducing site-specific alterations. Using respective genome editing tools and the cell`s own repair mechanism a broad spectrum of modifications in the genome can be introduced.

7 GLOSSARY OF TERMS USED IN CONNECTION WITH GENOME EDITING

This glossary includes the most relevant terms used in connection with genome editing, e.g. in the current political and legal discussions. Covered are also those terms that were mentioned in chapters 3 to 6 above.

Agro-infiltration: Agroinfiltration *sensu strictu* and agroinfection are targeted to transient expression of the introduced transgenic elements or to modification of the expression of endogenous crop plant genes, typically by silencing them (Eckerstorfer et al., 2014).

Base editing: used to exchange single bases in the genome without inducing double-strand breaks.

Breeders' gene pool: according to EFSA "*the sources of genes/alleles available for conventional plant breeding […]. Breeders distinguish between primary, secondary and tertiary gene pools. Each primary gene pool comprises one cultivated species and other taxonomic species with which it can interbreed freely. The secondary gene pool includes species that can be cross-bred only with difficulty with a member of the primary gene pool but which produce at least some fertile hybrids. The tertiary gene pool comprises those species that are more distantly related to a member of the primary gene pool, but which can be cross-bred only using advanced techniques such as embryo rescue, induced polyploidy and bridge crosses. Breeders are continually expanding the tertiary gene pool and will continue to do so in the future* (EFSA GMO Panel, 2022)".

Cisgenesis: according to EFSA "*a genetic modification involving genetic material obtained from the breeders' gene pool and transferred to the host using various delivery strategies, the incorporated sequences contain an exact copy of the sequence already present in the breeders' gene pool* (EFSA GMO Panel, 2022)".

Conventional breeding techniques: defined in the context of the respective document "*as methods used by plant breeders for the improvement of commercial varieties and where the resulting plants/varieties are not covered by the definitions of genetic modification in Directive 2001/18/EC*" (EFSA, 2012).

Conventional GMO: in the context of the respective study defined by the EC as "*GMOs resulting from established genomic techniques. Conventional GMOs that have been authorised to date in the EU are transgenic*" (European Commission, 2021a).

Conventional mutagenesis techniques: in the context of the respective study defined by the EC an "*umbrella term used to describe older techniques of mutagenesis that have been used since the 1950s; they involve irradiation or treatment with chemicals in order to produce random mutations, and typically involve screening of a large number of mutants to select one with desirable properties. Organisms obtained with such techniques are GMOs that are exempted from the scope of the EU GMO legislation"* (European Commission, 2021a).

CRISPR/Cas - Clustered Regularly Interspaced Short Palindromic Repeats: Synthetic nuclease complexes, developed from the bacterial nuclease Cas9 (CRISPR associated 9), which is a component of the adaptive immunity system in bacteria aimed to recognize and destruct foreign DNA (Eckerstorfer et al., 2014). This system can be modified and used as a technique to introduce sequence-specific DNA breaks.

Delivery method: method for the introduction of the genome editing agent into the plant cell

Epigenome: inheritable chemical modifications of DNA and Histone-proteins. Changes in the epigenome can alter the activity of genes (Pflanzenforschung.de, 2023).

Established genomic techniques: according to EFSA "*genomic techniques developed before 2001, when the Directive 2001/18/EC was adopted* (EFSA GMO Panel, 2022)". See also 'conventional GMO'.

Genetically modified organism (GMO): according to the Directive 2001/18/EC "*an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination* (Directive 2001/18/EC, 2001)".

Gene drives (engineered): Gene drives can be designed to introduce and spread a genetic modification permanently into a whole population or species. Those gene drives are referred to as modification drives (Dolezel et al., 2019).

Gene drives organisms: are designed to spread genetically engineered traits into wild populations (Dolezel et al., 2019).

Genome: according to the High-Level Group of Scientific Advisors *"(1) The entire complement of genetic material (including coding and noncoding sequences) present in a cell of an organism, a virus, or an organelle, (2) The complete set of chromosomes (hence of genes) inherited as a unit from one parent* (European Commission, Directorate-General for Research and Innovation, 2017)."

Genome editing: Genome editing comprises various techniques for the targeted modification of the genome inducing site-specific alterations. Using respective genome editing tools and the cells own repair mechanism a broad spectrum of modifications in the genome can be introduced (chapter 6).

Genome editing methods: synonym for 'genome editing techniques'.

Genome editing techniques: Techniques applied in genome editing e.g. TALEN, CRISPR/Cas.

Genome editing tool: Genomic tool used in the application of a genome editing technique, e.g. nucleases to cut the DNA.

Genomic tool: molecular tool, e.g. guide RNA, nuclease.

Genome engineering: synonym for 'genome editing'.

Genome shuffling: introduction of large size sequence replacements (of more than 1 kb in length) specified by an additionally supplied DNA-template.

Grafting: joining together of plant parts by means of tissue regeneration. Grafting is the act of placing a portion of one plant (bud or scion) into or on a stem, root, or branch of another (stock) in such a way that an union will be formed and the partners will continue to grow (Enzyclopedia Britannica, 2023).

Intragenesis: according to EFSA "*a genetic modification involving genetic material obtained from the breeders' gene pool and transferred to the host using various delivery strategies; the incorporated sequences contain a re-arranged copy of sequences already present in the breeders' gene pool* (EFSA GMO Panel, 2022)".

Living modified organism (LMO): according to the Cartagena Protocol on Biosafety, means "*any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology* (Secretariat of the Convention on Biological Diversity, 2000)".

Meganucleases: Naturally occurring, rare cutting endodeoxyribonucleases that are characterised by a DNA recognition site of typically 20–30 nucleotides (Eckerstorfer et al., 2014).

Modern biotechnology: according to the Cartagena Protocol on Biosafety, means "*the application of: a) In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or b) Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection* (Secretariat of the Convention on Biological Diversity, 2000)".

Mutagenesis: in the context of the respective study defined by the EC as the "*creation of mutation(s) in an organism without insertion of foreign genetic material*" (European Commission, 2021a).

Mutagenesis techniques: techniques producing mutations in the genome, see also 'conventional mutagenesis techniques' and 'new mutagenesis techniques'.

Multiplexing: application of genome editing where several genes at different sites are targeted and modified at once or successively.

New (plant) breeding techniques: umbrella term used for gene technologies developed after 2001 see also ‘new genomic techniques’.

New gene technologies: synonym for ‘new genomic techniques’.

New genomic techniques: in the context of the respective study defined by the EC as “*An umbrella term used to describe a variety of techniques that can alter the genetic material of an organism and that have emerged or have been developed since 2001, when the existing GMO legislation was adopted*” (European Commission, 2021a).

New mutagenesis techniques: Mutagenesis techniques are techniques of directed mutagenesis that have emerged since the adoption of the Directive 2001/18/EC, see also ‘targeted mutagenesis techniques’.

Novel genomic techniques: synonym for ‘new genomic techniques’.

ODM - oligonucleotide directed mutagenesis: according to EFSA “*an approach which is based on the use of oligonucleotides for the introduction of targeted mutations in the genome, usually of one or a few adjacent nucleotides […]* (EFSA GMO Panel, 2022)”.

Prime-editing: Modification of the CRISPR/Cas system introducing only single-strand breaks.

Random mutagenesis techniques: synonym for ‘conventional mutagenesis techniques’.

Reverse breeding: “*a novel plant breeding technique designed to directly produce parental lines for any heterozygous plant*” (Dirks et al., 2009).

RNA-dependent DNA methylation: “*RNA-dependent DNA methylation (RdDM) is a small interfering RNA (siRNA) based epigenetic modification which induces gene silencing […]* (Ages, 2013).

Site-directed mutagenesis technique: synonym for ‘targeted mutagenesis technique’.

Site-directed nucleases: synthetic nucleases with the general aim to introduce double-strand breaks at specific sites of the genomic DNA (Eckerstorfer et al., 2014).

Site-specific nucleases: synonym for ‘site-directed nuclease’.

Synthetic biology: is, according to an operational definition developed by the AHTEG on Synthetic Biology “*a further development and new dimension of modern biotechnology that combines science, technology and engineering to facilitate and accelerate the understanding, design, redesign, manufacture and/or modification of genetic materials, living organisms and biological systems* (Secretariat of the Convention on Biological Diversity, 2015)“.

TALEN - transcription activator-like effector nucleases: dimeric enzymes with a structure which is related to ZFNs, i.e. composed of a nuclease domain fused to a DNA-binding domain (Eckerstorfer et al., 2014). This system can be modified and used as a technique to introduce sequence-specific DNA breaks.

Targeted mutagenesis techniques: in the context of the respective study defined by the EC an “*umbrella term used to describe newer techniques of mutagenesis that induce mutation(s) in selected target locations of the genome without insertion of genetic material. The process usually results in a ‘knock-out’, i.e. the disruption of the functioning of a gene that is responsible for an unwanted effect, or in modifications of the expressed protein or of regulatory elements of a gene*” (European Commission, 2021a).

TEgenesis: “*a new plant breeding technique, using chemicals to activate mobile genetic elements specific to the plant. These transposable elements (TEs) are moved around within the genome, enabling some plants to adapt to changed environmental conditions*.” (Swiss Expert Committee for Biosafety SECB, 2020).

Trait: a new characteristic introduced to the organism.

Transgenesis: according to EFSA "*the process of stably introducing gene(s) from any sexually incompatible species, or any synthetic gene non existing in nature, into the genome of a given cell and the propagation of such gene(s) thereafter* (EFSA GMO Panel, 2022)".

Transgrafting: a technique combining transgenic and unmodified plant parts via grafting. See also 'grafting'.

ZFN - zinc-finger nucleases: Protein dimers consisting of two subunits, composed of a Zinc-Finger DNA-binding domain and a nuclease domain (Eckerstorfer et al., 2014). This system can be modified and used as a technique to introduce sequence-specific DNA breaks.

8 REFERENCES

Adiego-Pérez, B., Randazzo, P., Daran, J. M., Verwaal, R., Roubos, J. A., Daran-Lapujade, P., et al. (2019). Multiplex genome editing of microorganisms using CRISPR-Cas. *FEMS Microbiol Lett* 366. doi: 10.1093/femsle/fnz086

Adli, M. (2018). The CRISPR tool kit for genome editing and beyond. *Nat Commun* 9, 1911. doi: 10.1038/s41467-018-04252-2

Ages (2013). *New plant breeding techniques. RNA-dependent DNA methylation, Reverse breeding, Grafting*.

Basu, S., Rabara, R. C., Negi, S., and Shukla, P. (2018). Engineering PGPMOs through Gene Editing and Systems Biology: A Solution for Phytoremediation? *Trends Biotechnol* 36, 499–510. doi: 10.1016/j.tibtech.2018.01.011

Bier, E. (2022). Gene drives gaining speed. *Nat Rev Genet* 23, 5–22. doi: 10.1038/s41576-021-00386-0

Broothaerts, W., Jacchia, S., Angers, A., Petrillo, M., Querci, M., Savini, C., et al. (2021). *New genomic techniques: State of the Art Review*. Publications Office of the European Union.

Calvache, C., Vazquez-Vilar, M., Selma, S., Uranga, M., Fernández-Del-Carmen, A., Daròs, J.-A., et al. (2022). Strong and tunable anti-CRISPR/Cas activities in plants. *Plant Biotechnol J* 20, 399–408. doi: 10.1111/pbi.13723

Carlson, D. F., Lancto, C. A., Zang, B., Kim, E.-S., Walton, M., Oldeschulte, D., et al. (2016). Production of hornless dairy cattle from genome-edited cell lines. *Nat Biotechnol* 34, 479–481. doi: 10.1038/nbt.3560

Chari, R., and Church, G. M. (2017). Beyond editing to writing large genomes. *Nat Rev Genet* 18, 749–760. doi: 10.1038/nrg.2017.59

Chiang, K.-Y., Lin, W.-C., Tsai, T.-Y., Lin, C.-W., Huang, S.-J., Huang, C.-Y., et al. (2020). Dual expression of transgenic delta-5 and delta-6 desaturase in tilapia alters gut microbiota and enhances resistance to Vibrio vulnificus infection. *PLOS ONE* 15, e0236601. doi: 10.1371/journal.pone.0236601

Council of the European Union (2019). Council Decision (EU) 2019/1904 of 8 November 2019 requesting the Commission to submit a study in light of the Court of Justice's judgment in Case C-528/16 regarding the status of novel genomic techniques under Union law, and a proposal, if appropriate in view of the outcomes of the study. *Official Journal of the European Union* L 293/103.

Court of Justice of the European Union (2018). Judgement of the Court (Grand Chamber) of 25 July 2018 in Case C-528/16 concerning the request for a preliminary ruling under Article 267 TFEU from the Conseil d'État (Council of State, France). Accessed July 01, 2022, https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:62016CJ0528

Department for Environment, Food and Rural Affairs (2022). *Genetic technology (precision breeding) bill. Factsheet 1 - Overview*.

Directive 2001/18/EC (2001). *Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment or genetically modified organisms repealing Council Directive 90/220/EEC*.

Dirks, R., van Dun, K., Snoo, C. B. de, van den Berg, M., Lelivelt, C. L. C., Voermans, W., et al. (2009). Reverse breeding: a novel breeding approach based on engineered meiosis. *Plant Biotechnol J* 7, 837–845. doi: 10.1111/j.1467-7652.2009.00450.x

Dolezel, M., Simon, S., Otto, M., Engelhard, M., and Züghart, W. (2019). *Gene drive organisms - Implications for the environment and nature conservation: A joint technical report of the EPA/ENCA Interest Group on Risk Assessment and Monitoring of GMOs*.

Dong, C., Gou, Y., and Lian, J. (2022). SgRNA engineering for improved genome editing and expanded functional assays. *Curr Opin Biotechnol* 75, 102697. doi: 10.1016/j.copbio.2022.102697

Eckerstorfer, M. F., Engelhard, M., Heissenberger, A., Simon, S., and Teichmann, H. (2019). Plants Developed by New Genetic Modification Techniques-Comparison of Existing Regulatory Frameworks in the EU and Non-EU Countries. *Front. Bioeng. Biotechnol.* 7, 26. doi: 10.3389/fbioe.2019.00026

Eckerstorfer, M. F., Miklau M., and Gaugitsch H. (2014). *New plant breeding Techniques: Risks Associated with their Application*. Vienna: Umweltbundesamt GmbH.
EFSA (2012). Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis. *EFSA Journal* 10, 2561. doi: 10.2903/j.efsa.2012.2561
EFSA GMO Panel (2022). Criteria for risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis. *EFSA J* 20, e07618. doi: 10.2903/j.efsa.2022.7618
Enzyclopedia Britannica (2023). *graft*, https://www.britannica.com/topic/graft
European Commission (2020). *Answer given by Ms Kyriakides on behalf of the European Commission. Parliamentary question - P-003885/2020(ASW)*. Accessed April 05, 2023, https://www.europarl.europa.eu/doceo/document/P-9-2020-003885-ASW_EN.html
European Commission (2021a). *Commission staff working document: 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*. SWD(2021) 92 final.
European Commission (2021b). Inception impact assessment. Legislation for plants produced by certain new genomic techniques: Ref. Ares(2021)5835503-24/09/2021. Accessed July 01, 2022, https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/13119-Legislation-for-plants-produced-by-certain-new-genomic-techniques_en
European Commission (2023a). Proposal for a regulation – COM(2023)411. Legislation for plants produced by certain new genomic techniques. Accessed August 30, 2023, https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/13119-Legislation-for-plants-produced-by-certain-new-genomic-techniques_en
European Commission (2023b). Impact assessment report – SWD(2023)412. Legislation for plants produced by certain new genomic techniques. Accessed August 30, 2023, https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/13119-Legislation-for-plants-produced-by-certain-new-genomic-techniques_en
European Commission, Directorate-General for Research and Innovation (2017). *New techniques in agricultural biotechnology*. Luxembourg: Publications Office.
European Parliament (2020). *Is the Epibreed procedure governed by the EU's GMO law? Parliamentary question - P-003885/2020*. Accessed April 05, 2023, https://www.europarl.europa.eu/doceo/document/P-9-2020-003885_EN.html
Federal Department of the Environment, Transport, Energy and Communications (2023). *Regulierung der Gentechnik im Ausserhumanbereich: Bericht des Bundesrates in Erfüllung der Postulate 20.4211 Chevalley: Gentechnik. Welcher Geltungsbereich?; 21.3980 WBK-N: GVO-Moratorium. Belastbare Informationen als Grundlage für gute Entscheide; 21.4345 WBK-S: Züchtungsverfarhen mit Genom-Editierungsmethoden*.
Federal Office of Justice FOJ (2021). *L'applicabilité de l'exception de l'Annexe 1, al. 3, let. a ODE à la méthode « TEgenesis »*.
Food Standards Agency (2023). *Precision breeding: What precision breeding is and its potential applications*. Accessed May 30, 2023, https://www.food.gov.uk/safety-hygiene/precision-breeding#:~:text=The%20Genetic%20Technology%20(Precision%20Breeding,bred%20plants%20will%20come%20first.
Gao, C. (2021). Genome engineering for crop improvement and future agriculture. *Cell* 184, 1621–1635. doi: 10.1016/j.cell.2021.01.005
Gene Technology Act (2022). *Federal Act on Non-Human Gene Technology: Gene Technology Act, GTA*.
Hansen, P. J. (2020). Prospects for gene introgression or gene editing as a strategy for reduction of the impact of heat stress on production and reproduction in cattle. *Theriogenology* 154, 190–202. doi: 10.1016/j.theriogenology.2020.05.010
Heinemann, J. A., Paull, D. J., Walker, S., and Kurenbach, B. (2021). Differentiated impacts of human interventions on nature. *Elementa: Science of the Anthropocene* 9. doi: 10.1525/elementa.2021.00086
Huang, T.-K., and Puchta, H. (2021). Novel CRISPR/Cas applications in plants: from prime editing to chromosome engineering. *Transgenic Res* 30, 529–549. doi: 10.1007/s11248-021-00238-x

Jaiswal, S., Singh, D. K., and Shukla, P. (2019). Gene Editing and Systems Biology Tools for Pesticide Bioremediation: A Review. *Front Microbiol* 10, 87. doi: 10.3389/fmicb.2019.00087
Kang, B.-C., Bae, S.-J., Lee, S., Lee, J. S., Kim, A., Lee, H., et al. (2021). Chloroplast and mitochondrial DNA editing in plants. *Nat Plants* 7, 899–905. doi: 10.1038/s41477-021-00943-9
Kawall, K. (2019). New Possibilities on the Horizon: Genome Editing Makes the Whole Genome Accessible for Changes. *Front Plant Sci* 10, 525. doi: 10.3389/fpls.2019.00525
Kawall, K., Cotter, J., and Then, C. (2020). Broadening the GMO risk assessment in the EU for genome editing technologies in agriculture. *Environ Sci Eur* 32. doi: 10.1186/s12302-020-00361-2
Kempton, H. R., and Qi, L. S. (2019). When genome editing goes off-target. *Science* 364, 234–236. doi: 10.1126/science.aax1827
Kumar, G., Shekh, A., Jakhu, S., Sharma, Y., Kapoor, R., and Sharma, T. R. (2020). Bioengineering of Microalgae: Recent Advances, Perspectives, and Regulatory Challenges for Industrial Application. *Front. Bioeng. Biotechnol.* 8, 914. doi: 10.3389/fbioe.2020.00914
Leung, K., Ras, E., Ferguson, K. B., Ariëns, S., Babendreier, D., Bijma, P., et al. (2020). Next-generation biological control: the need for integrating genetics and genomics. *Biological Reviews* 95, 1838–1854. doi: 10.1111/brv.12641
Li, W.-R., Liu, C.-X., Zhang, X.-M., Chen, L., Peng, X.-R., He, S.-G., et al. (2017). CRISPR/Cas9-mediated loss of FGF5 function increases wool staple length in sheep. *FEBS J* 284, 2764–2773. doi: 10.1111/febs.14144
Liu, G., Lin, Q., Jin, S., and Gao, C. (2022). The CRISPR-Cas toolbox and gene editing technologies. *Mol Cell* 82, 333–347. doi: 10.1016/j.molcel.2021.12.002
NCE Heidner Biocluster (2023). *A new political course for gene technology in the food system - recommendations from the majority of the the Norwegian Public Committee on Gene Technology (Genteknologiutvalget)*.
Neve, P. (2018). Gene drive systems: do they have a place in agricultural weed management? *Pest Manag Sci* 74, 2671–2679. doi: 10.1002/ps.5137
Norwegian Public Committee on Gene Technology (2023). *Genteknologi i en bærekraftig fremti*.
OECD, Organisation for Economic Co-operation and Development (2014). *Biosafety and the Environmental Uses of Micro-Organisms: Conference Proceedings*. Paris, London: Organization for Economic Cooperation & Development; Stationery Office, The [distributor].
Parisi, C., and Rodriguez-Cerezo, E. (2021). *Current and future market applications of new genomic techniques*. Publications Office of the European Union.
Patel, V. K., Soni, N., Prasad, V., Sapre, A., Dasgupta, S., and Bhadra, B. (2019). CRISPR-Cas9 System for Genome Engineering of Photosynthetic Microalgae. *Mol Biotechnol* 61, 541–561. doi: 10.1007/s12033-019-00185-3
Pflanzenforschung.de (2023). *Epigenome*. Accessed May 30, 2023, https://www.pflanzenforschung.de/de/pflanzenwissen/lexikon-a-z/epigenom-2237
Pirttilä, A. M., Mohammad Parast Tabas, H., Baruah, N., and Koskimäki, J. J. (2021). Biofertilizers and Biocontrol Agents for Agriculture: How to Identify and Develop New Potent Microbial Strains and Traits. *Microorganisms* 9. doi: 10.3390/microorganisms9040817
Precision Breeding Act. *Gene technology (precision breeding) act 2023*.
Secretariat of the Convention on Biological Diversity (2000). *Cartagena Protocol on Biosafety to the Convention on Biological Diversity: text and annexes*. Montreal.
Secretariat of the Convention on Biological Diversity (2015). *Report of the Ad Hoc Technical Expert Group on Synthetic Biology: UNEP/CBD/SYNBIO/AHTEG/2015/1/3*.
Secretariat of the Convention on Biological Diversity (2018). *Decision adopted by the Conference of the Parties to the Convention on Biological Diversity: 14/19 Synthetic biology. CBD/COP/DEC/14/19*.
Secretariat of the Convention on Biological Diversity (2020). *Biosafety - preparing for the post-2020 agenda: Decisions of the Ninth Meeting of the Conference of the Parties to the Convention on Biological Diversity serving as the meeting of the Parties to the Cartagena Protocol on Biosafety. Sharm El-Sheikh, Egypt 17 to 29 November 2018*.

Secretariat of the Convention on Biological Diversity (2022a). *Decision adopted by the Conference of the Parties to the Convention on Biological Diversity: 15/31 Synthetic biology. CBD/COP/DEC/15/31*.

Secretariat of the Convention on Biological Diversity (2022b). *Synthetic Biology. CBD Technical Series No. 100*. Montreal.

Sharma, B., and Shukla, P. (2022). Futuristic avenues of metabolic engineering techniques in bioremediation. *Biotechnol Appl Biochem* 69, 51–60. doi: 10.1002/bab.2080

Swiss Council of States (2021). *Postulat 21.4345: Züchtungsverfahren mit Genom-Editierungsmethoden*. Accessed July 01, 2022, https://www.parlament.ch/de/ratsbetrieb/suche-curia-vista/geschaeft?AffairId=20214345

Swiss Expert Committee for Biosafety SECB (2020). *SECB opinion on TEgenesis, December 2020*, https://www.efbs.admin.ch/en/recommendations/opinions-of-the-secb

Swiss National Council (2020). *Postulat 20.4211: Gentechnikgesetz. Welcher Geltungsbereich?* Accessed July 01, 2022, https://www.parlament.ch/de/ratsbetrieb/suche-curia-vista/geschaeft?AffairId=20204211

Swiss National Council (2021). *Postulat 21.3980: GVO Moratorium. Belastbare Informationen als Grundlage für gute Entscheide*. Accessed July 01, 2022, https://www.parlament.ch/de/ratsbetrieb/suche-curia-vista/geschaeft?AffairId=20213980

The Norwegian Biotechnology Advisory Board (2018). *Proposal for relaxation of Norwegian regulations for deliberate release of genetically modified organisms (GMO), with applicability also for EU regulation*.

Wang, S., Qu, Z., Huang, Q., Zhang, J., Lin, S., Yang, Y., et al. (2022). Application of Gene Editing Technology in Resistance Breeding of Livestock. *Life* 12, 1070. doi: 10.3390/life12071070

Zhang, Y., Iaffaldano, B., and Qi, Y. (2021). CRISPR ribonucleoprotein-mediated genetic engineering in plants. *Plant Commun* 2, 100168. doi: 10.1016/j.xplc.2021.100168

Zhong, Z., Niu, P., Wang, M., Huang, G., Xu, S., Sun, Y., et al. (2016). Targeted disruption of sp7 and myostatin with CRISPR-Cas9 results in severe bone defects and more muscular cells in common carp. *Sci Rep* 6, 22953. doi: 10.1038/srep22953