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Genome Editing

for Crop Improvement

ALLEA Symposium Report | October 2020

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Lead authors
Dr. Oana Dima (VIB-UGent Center for Plant System Biology)
Emeritus Professor Dr. Hubert Bocken (Gent University, Faculty of Law and Criminology)
René Custers (VIB)
Professor Dr. Dirk Inzé (VIB-UGent Center for Plant System Biology)
Professor Dr. Pere Puigdomènech (CRAG, Centre for Research in Agricultural Genomics)

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Disclaimer

ALLEA would like to specifically thank the author and editors of this report as well as the speakers of the symposium. A programme of the event can be found in the annex. This report paid close attention to accurately reflect the discussions and presentations of the symposium held in Brussels on 7/8 November 2019.

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Contact Information

ALLEA | All European Academies
Tel +49 (0)30-3259873-72 secretariat@allea.org
Twitter: @ALLEA_academies
Website: www.allea.org

Koninklijke Vlaamse Academie van België voor Wetenschappen en Kunsten
Tel +32 02 550 23 23 info@kvab.be
Twitter: @_KVAB
Website: www.kvab.be

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Based on the ALLEA-KVAB symposium held in Brussels on 7-8 November 2019

Table of Contents

Preface

ALLEA (All European Academies) in collaboration with the Royal Flemish Academy of Belgium for Science and the Arts (*Koninklijke Vlaamse Academie van België voor Wetenschappen en Kunsten, KVAB*), organised a symposium about plant genome editing that took place in Brussels, in the Palace of the Academies, on 7th and 8th November 2019.

The ALLEA-KVAB symposium followed up on the concerns and criticisms voiced by large parts of the scientific community in response to the European Court of Justice (ECJ) decision of 25 July 2018, that organisms produced by directed mutagenesis techniques, such as genome editing with CRISPR, should be considered as genetically modified organisms (GMOs) within the meaning of the GMO Directive 2001/18. The scientific community has also voiced concerns that substantially restricting the possibility of utilising genome editing by applying the GMO legislation will have considerable negative consequences for agriculture, society and economy. More specifically, continued restrictions may hamper the selection of more productive, diverse and climate-resilient crops with a reduced environmental footprint.

Many research institutes and academies have expressed the opinion that the European legislative bodies should respond to the decision of the ECJ by clarifying that plants obtained through genome editing should not be subject to the EU GMO legislation, but should be regulated on a similar basis as plants obtained through classical breeding techniques. The features of the plant, rather than the technique used to generate it, should determine its regulatory status. This conclusion corresponds to the consensus present in the scientific community that plants that were subjected to targeted genome edits, which do not add foreign DNA, do not present any other health or environmental danger than plants obtained through classical breeding techniques, and are as safe or dangerous as the latter. Furthermore, the ECJ ruling is in sharp contrast to legislation in many other countries outside the EU that exempt genome-edited crops from their respective GMO legislations.

The symposium established a dialogue with relevant stakeholders to assess the impact of the decision of the ECJ on present research and developments in genome editing for plant breeding. Moreover, the symposium aimed at providing an overview of the scientific evidence with respect to safety of genome-edited crops and their possible potential to provide solutions to current and future agricultural problems. Other relevant aspects were considered as well, such as economic and social advantages and disadvantages, and the legal hurdles in redressing the decision of the ECJ by legislative means. Finally, the symposium also addressed issues related to the traceability of genome-edited crops and how this will likely affect international trade of food and feed. Participants of the symposium were also addressed by Hilde Crevits, the Vice Minister-president of the Flemish government and Flemish Minister for Economy, Innovation, Labour, Social Economy and Agriculture. In her closing remarks she recalled the all-encompassing nature of food production as well as the ground-breaking potential of genome editing as a contributor to solving global issues like climate change. Though supportive of the technology, she cautioned that scientific findings cannot simply be transformed into viable policies without taking public perception and ethics into account.

This summary provides European policymakers and the public with the best available scientific evidence for legislation that takes the latest scientific knowledge duly into account.

Executive summary

In a relatively short period of time, genome editing techniques have become an essential tool for our understanding of the genetic basis of biological processes and for biotechnological applications in many different fields. This is the case for crop breeding. The improvement of the plant varieties that are the base of world food production depends on the availability of plant populations that contain the largest possible variability in genes related to agronomic characters of interest. Genome-editing methods are novel because they provide a direct way to generate new variability in this category of genes. Examples of the use of these methods are increasingly being published worldwide and genome-edited varieties are expected to reach the global market at any moment, as the majority of countries have decided not to regulate them in a way different from other plant varieties.

The introduction of new components of food in the European Union is the subject of a number of Directives and Regulations with the aim of preserving the safety of food offered to European consumers. In particular, the Directive 2001/18/EC regulates the access to the European market of food products containing genetically modified (GM) components. In the way that it is presently applied, it imposes a high economic burden upon those who wish to apply for the introduction of GMO varieties. The ruling of the Court of Justice of the EU in case C-528/16 is interpreted by the European authorities to mean that genome-edited crops are subject to the provisions of Directive 2001/18/EC. If this is so, it may constitute an important economic barrier to the research and use of the new varieties obtained by genome editing in Europe. It opens a number of questions on how to enforce the provisions of traceability and labelling or how to apply the existing regulations on intellectual property upon plants and plant varieties. Different options to solve the present impasse resulting from the ECJ ruling have been explored and proposed during the ALLEA-KVAB Symposium on 'Genome Editing for Crop Improvement' that was held in Brussels, in November 2019.

1. The legal framework for genome-edited crops in Europe is not fit for purpose

Genome editing for agricultural applications unfolds in Europe because of its potential

Genome-editing methods have enabled researchers to introduce mutations in the genetic blueprint of plants with high precision and efficiency and have accelerated plant breeding. Researchers have widely adopted genome-editing methods due to the simplicity, low costs and its flexibility. This is not only the case in the academic sector; also many small and medium-sized enterprises (SMEs) and multinational corporations adopted the genome-editing technology at an unprecedented speed. There is a broad consensus that genome-edited crops will make a critical contribution in the coming years to make food systems more sustainable and more resilient to climate change.

Researchers have widely adopted genome-editing methods due to the simplicity, low costs and its flexibility.

Genome editing has already resulted in numerous crop improvements through targeted changes in the genetic blueprint of cultivated plants[1]. Valuable traits that have been introduced in genome-edited crops are improved nutritional composition, improved digestibility, lower content of anti-nutritional components, reduced allergenicity or requiring fewer resources, which have a direct benefit for our health and the environment. Many of the genome-editing applications comprise small DNA alterations, *i.e.* short insertions or deletions (indels) generated at a predefined location in the genetic blueprint.

Europe is in a leading position in terms of innovative agricultural research. This has led to the presence of dynamic biotech clusters consisting of numerous innovative start-ups and corporate partnerships. Many of these small European seed-breeding companies embrace these new technologies, as they can be implemented relatively cheap and quickly, and because they can democratise the research and development of new agricultural products.

ECJ judgment (case C-528/16)

For the past two years, the legal status of genome editing of plants has been the focus of attention. Scientific institutions and policymakers are concerned about the impact of the decision of the European Court of Justice (ECJ) of 25 July 2018 in case C-528/16[2]. In summary, the Court held that organisms obtained with techniques of mutagenesis which were developed since the adoption of the GMO Directive 2001/18 of 2001 are not covered by the Article 3, Annex IB exemption, even if no foreign DNA has been introduced. Consequently, the risk assessment and traceability, labelling and monitoring obligations provided by the GMO Directive[3] for the culture and commrcialisation of plants resulting from transgenesis procedures leading to the insertion of foreign DNA apply equally to plants obtained by genome editing.

The ECJ's decision is considered a major setback for European biotechnological research and industry and for the development of useful new agricultural products, including plants with an optimised response to climate change and providing food for a growing population. The length and cost of the authorisation process makes it, except for major industrial players, hardly possible to bring into culture and commercialise plants developed with new biotechnological breeding techniques.

There is a growing consensus that the present GMO legislation is no longer up to date. Already in November 2018, the European Commission's Group of Chief Scientific Advisors (GCSA) published a statement[4] calling for a revision of the GMO legislation to reflect on the current knowledge and scientific evidence,

in particular on genome editing and established techniques of genetic modification, while considering also other legislation relevant to food safety and environmental protection. Since then, many scientific organisations, including several academies of sciences and federations of academies, as well as many biotechnological and other research institutes, have taken a similar position[5-10] (see also ANNEX 2). Their concerns have reached the highest level of policymaking in the European Union. On November 8, 2019, the EU Council, considering that the Court of Justice ruled that new mutagenesis techniques fall within the scope of the Directive 2001/18/EC and are subject to the obligations laid down therein, called for a study to clarify the situation. It pointed to the practical questions, raised by the decision, of the national competent authorities, the Union's industry, " in particular the plant breeding sector and research". The most important question was how to ensure compliance with Directive 2001/18/EC when products obtained by means of new mutagenesis techniques cannot be distinguished, using current methods, from products resulting from "natural mutations". Subsequently, how to ensure, in such a situation, the equal treatment between imported products and products produced within the Union.

In this report, ALLEA and KVAB join other academies and federations of academies in exploring paths for bringing the GMO legislation in touch with recent scientific developments, while taking into account relevant ethical and societal considerations.

In this report, ALLEA and KVAB join other academies and federations of academies in exploring paths for bringing the GMO legislation in touch with recent scientific developments, while taking into account relevant ethical and societal considerations. Before examining the main scientific elements and ethical and societal considerations relating to genome editing, we briefly recall the background and the content of the ECJ's judgment C-528/16 of 25 July 2018 and its major implications.

The issue of the regulatory status of genome editing was brought before the ECJ in the context of a reference for a preliminary ruling by the *Conseil d'État*, the highest French administrative court. The Court of Justice of the EU is the final authority for the interpretation of EU legislation. To ensure the uniform application of Union legislation, national courts can, and sometimes must, refer to it for guidance concerning the interpretation of Union law applicable in cases brought before them. The role of the ECJ, in a procedure for a preliminary ruling, is limited to answering the questions on EU law raised by the national court without questioning the facts established by the referring court. That court is to dispose of the underlying case in accordance with the ECJ's ruling - which is binding for all of Europe.

The issue of the status of genome-editing techniques arose in an action brought before the French *Conseil d'État* by *Confédération Paysanne,* a French agricultural union defending the interests of small-scale farming together with eight other NGOs[11]. Claimants were seeking to invalidate a French administrative decision authorising the cultivation of herbicide-tolerant canola varieties produced through genome editing and pursuant to Art. L 531-2 of the French environmental code. This code exempts from the GMO legislation techniques "which, in view of their natural character, are not to be considered as bringing about a genetic modification or which have conventionally been used without damage to the public health of the environment". The list of the exempted techniques (Art. D 531-2) established at the proposal of the French High Council on Biotechnology mentions mutagenesis, with the only qualification that it does not imply the use of GMOs as receptor or parental organism. Claimants argued that this exemption is in contradiction with EU law as only techniques of mutagenesis existing before the adoption of the GMO Directive could be exempted from the application of the GMO Directive. They claimed that it is also in contradiction with the precautionary principle laid down in the EU treaties, because herbicide-resistant varieties carry a significant risk as they may cause harm to the environment by leading to the development of weeds resistant to herbicides and to an increased use of herbicides.

The French *Conseil d'État* decided to submit to the Court of Justice four questions on the interpretation and validity of the EU GMO legislation, the more important one being whether organisms obtained by mutagenesis are GMOs and are subject to the obligations laid down by the GMO Directive. The other questions relating to the degree of harmonisation brought about by the GMO Directive, the application of the Directive 2002/53[12] on the common catalogue of varieties of agricultural plant species and the validity of the mutagenesis exemption in Art. 3 of the Directive in the light of the precautionary principle can be left aside here.

Before commenting on the Court's decision, the main provisions of the GMO Directive[3] may briefly be recalled. Article 2(2) defines a genetically modified organism as "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". Annex IA contains a non-exhaustive list of techniques considered to result in genetic modification as well as a limitative list of techniques that are considered not to do so. Neither list mentions mutagenesis. Article 3, however, provides an exemption for organisms obtained through the techniques of genetic modification listed in Annex IB which reads: "Techniques/methods of genetic modification yielding organisms to be excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those produced by one or more of the techniques/methods listed below are: (1) mutagenesis, (2) cell fusion (including protoplast fusion) of plant cells or organisms which can exchange genetic material through traditional breeding methods." Relevant is also recital 17 of the preamble according to which the Directive should "not apply to organisms obtained through certain techniques of genetic modification which have conventionally been used in a number of applications and have a long safety record."

The main question submitted by the *Conseil d'État* to the Court of Justice contains two parts. First, do organisms obtained by mutagenesis constitute genetically modified organisms within the meaning of the Directive 2001/18? Second, does that exemption contained in Article 3(1), read in conjunction with Annex IB to the GMO Directive, encompass all organisms obtained by mutagenesis, including those obtained by new mutagenesis techniques applied after the adoption of the GMO Directive? Or only that subset of organisms obtained by techniques existing before the GMO Directive was adopted?

The court rendered its decision after having heard an extensive and in-depth advisory opinion by Advocate General Bobek[13].

In line with the Advocate General, the Court first decided that organisms produced by mutagenesis, including genome editing, qualify as GMOs within the meaning of Directive 2001/18. Conventional mutagenesis techniques as well as new genome-editing methods both alter the genetic material of an organism in a way that does not occur naturally. The general scheme of the Directive is process-based. It distinguishes between techniques of which the use results in genetic modification and techniques which do not. It would make little sense to exempt certain forms of mutagenesis from the obligations resulting from the Directive if they would not in principle be subject to it. On the second part of the question, Advocate General Bobek found that a distinction between mutagenesis techniques developed after and before the Directive would go against the text of Art. 3(1) and Annex IB. In addition, he insisted that legal concepts, including the mutagenesis exception, should be given not a "frozen" but a dynamic interpretation, which considers the societal evolution, both technical and social[14]. Contrary to the Advocate General, the Court, however, concluded that the mutagenesis exemption does not apply to techniques or methods of mutagenesis developed since Directive 2001/18 was adopted. In accordance with general rules of interpretation, the exemption of Art. 3(1) and Annex IB, which does not specify which techniques the legislator intends to exclude from the application of the Directive, has to be given a restrictive interpretation[15], which takes into account the objective of the Directive[16]. That objective is to protect human health and the environment, in accordance with the precautionary principle referred to in Art. 1, Art. 4(1) and recital 8 of the preamble[17]. The Court refers to the general safety concerns expressed in Art. 4(1) and recitals 4, 5 and 55 of the Directive[18] and to the other concerns referred to in the decision of the *Conseil d'État*, to know that "risks associated with techniques of directed mutagenesis involving the use of genetic engineering which have been developed since the adoption of Directive 2001/18 have not thus far been established with certainty"[19], "that the direct modification of the genetic material through mutagenesis makes it possible to obtain the same effects as the introduction of a foreign gene into that organism" and that "the development of those new techniques made it possible to produce genetically modified varieties at a rate and in quantities quite unlike those resulting from the application of conventional methods of random mutagenesis"[20]. The scope of the mutagenesis derogation, according to the ECJ, is to be determined in the light of the clarification given by the legislator in recital 17 of the preamble, which states that the Directive should not apply to "organisms obtained by techniques/methods of mutagenesis which have conventionally been used in a number of applications and have a long safety record"[21]. The Court infers that the intention of the legislature was "to exclude from the scope of the Directive only organisms obtained by means of techniques/methods which have been used in a number of applications and have a long safety record"[22]. The Court does not indicate how a long safety record is to be determined, but concludes that Art. 3(1), read together with Annex IB "cannot be interpreted as excluding from the scope of the directive, organisms obtained by means of new techniques/methods of

mutagenesis which have appeared or have been mostly developed since Directive 2000/18 was adopted"[22]. Plants developed with genome-editing techniques thus are subject to the GMO Directive.

The conclusion of the litigation came on 8 February, 2020, when the French *Conseil d'État* ruled on the merits of the case brought by *Confédération Paysanne*. Adopting on all points, the position of the Court of Justice enjoined the French government to amend within six months the provisions of the *Code de l'environment* by establishing a limitative list of methods or techniques of mutagenesis which have conventionally been used in a number of applications and which have a long safety record. Considering the substance of the decision, this means a list of methods and techniques developed before the adoption of the GMO Directive. More recent mutagenesis techniques must be submitted to the GMO legislation. The *Conseil d'État* specifies that this goes not only for directed mutagenesis but also for random mutagenesis *in vitro*[23].

European plant research institutes jointly call for action

Shortly after the ECJ judgment, leading scientists representing European plant and life sciences research centres and institutes endorsed a position paper to urge European policy makers to take action in order to facilitate the potential of genome editing for agriculture, in Europe.

Scientists consider the exemption of the products of conventional mutagenesis from the provisions of the EU GMO legislation, while not exempting the products of modern, much more targeted approaches of mutagenesis as a scientifically unjustified discrimination. Moreover, scientific evidence shows that the level of uncertainty about the consequences of the mutagenesis process is much higher in conventional mutagenesis than in modern targeted forms of mutagenesis. The GMO legislation no longer correctly reflects the current state of scientific knowledge. Besides, subjecting genome-edited crops to the current EU GMO regulation will delay the development of climate-resilient crops, and hinder progress in sustainable agriculture.

Subjecting genome-edited crops to the current EU GMO regulation will delay the development of climate-resilient crops, and hinder progress in sustainable agriculture.

With a growing number of signatories, reaching currently up to 132 European research institutes and organisations, from 21 different Member States and the UK, the network EU-SAGE was launched (**Figure 1**). EU-SAGE stands for European Sustainable Agriculture through Genome Editing and aims to provide information about genome editing and to promote the development of European and EU member state policies that enable the use of genome editing for sustainable agriculture and food production. The website www.eu-sage.eu provides more information.

Figure 1. *Logo of the European network EU-SAGE: European Sustainable Agriculture through Genome Editing.*

As mentioned earlier, not only EU-SAGE voiced the concerns of scientists on the negative impact of the ECJ ruling, but also numerous other organisations and learned societies did. A selection of the different statements is listed in ANNEX 2.

Comparative elements on the legal status of genome editing

From a comparative viewpoint, the strictly process-oriented approach in the **EU** legislation which leads to submitting all genome-editing techniques to the GMO regime is an exception. Only in New Zealand, a comparable approach was adopted, after a strikingly similar regulatory and judicial development. The **New Zealand** Hazardous Substances and new Organism Act of 1996[24], Art. 2, in essence defines as genetically modified an organism in which any of the genes or other genetic material (a) have been modified by *in vitro* techniques; or (b) are inherited or otherwise derived, through any number of replications, from any genes or other genetic material which has been modified by *in vitro* techniques. A regulation of 1998[25] excludes however "organisms that result from mutagenesis that uses chemical or radiation treatments that were in use on or before 29 July 1998". In April 2013, the NZ Environmental Protection Agency decided that non-transgenic genome editing was sufficiently similar to the techniques listed in the exemption and should be similarly excluded from the application of the GMO regime. This decision was appealed in the High Court of New Zealand[26], which decided on May 20, 2014 that the 1998 list of exceptions was a closed one and that adding to the list is a political and not an administrative decision. All gene editing is currently regulated as a GM in New Zealand[27]. The Court decision has given rise to a similar criticism and call for amendment of the prevalent regulations as the ECJ decision of 25 July 2018 in Europe[28].

The opposite approach is followed in **Canada**[29-31] where the regulation is purely product based and does not differentiate according to the plant breeding method used. Agricultural products of biotechnology are basically regulated under the same legislation and administrative structures as agricultural products produced in more traditional ways[32]. Plants which carry a trait not previously found in the species and that thus not have a history of production and safe consumption in Canada are classified as plants with novel traits (PNTs). The technique through which they have been created is irrelevant. PNTs are submitted to a pre-market safety assessment and must be authorised prior to their release into the Canadian environment as per the Seeds Act[33] and Seeds Regulations[34]. In order to obtain an authorisation for unconfined release, proponents must demonstrate that their product is as safe for the Canadian environment as its counterpart(s). A case-by-case approach is thus prevalent. A new plant which does not present a trait not existing within normally cultivated plant populations in Canada, will, no matter how it was developed, be subjected to the normal regulatory processes. Conversely, a plant developed by mutagenesis technologies will be treated as PNT if the trait that is focused on is considered as novel[35].

In most countries, a process- and product-based approach is combined. The process used determines the applicability of the legislation, while the outcome of whether the GMO legislation is applicable or not is to a certain extent or fully determined by the characteristics of the product.

Argentina[36-38] pioneered already in 2015 with Resolution 173/2015[39], which defines a case-by-case approach to determine in a relatively simple manner " in which cases a crop obtained by new plant breeding techniques (NPBTs) using modern biotechnology does not fall under the GMO rules and regulations" (Art. 1). Decisive is "whether the result of the breeding process is a novel combination of genetic material". "A genetic change shall be regarded as a novel combination of genetic material when the assessment established the occurrence of a stable and joint insertion in the plan genome of one or more genes or DNA sequences being part of a defined genetic construct" (Art. 2). Products in which there is a permanent integration of r-DNA, thus are considered GMOs[37,40,41]. If however, the new breeding technique does not result in a new combination of genetic material, (because it makes no use at all of foreign DNA) the GMO regulation does not apply (Art. 5). The same is the case if a transient use has been made of transgenes but the final product is free thereof (Art. 5). As a result, plants derived from oligonucleotide-directed mutagenesis (ODM) and type 1 and type 2 site-directed nucleases (SDN-1 and SDN-2) are not GMOs[42]. A similar regime is applied in Colombia, Chile and Brazil and some other South American countries.

In the **USA**[43,44], the cultivation of GM crops is regulated by the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA)[45] on the basis of the Plant Protection Act of 2000[46]. Key notions are defined in section 340 of the regulations[45]. Genetic engineering is the "genetic modification of organisms by recombinant DNA techniques". Plant pests are plants or other organisms "which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants".

Whether products from genome editing and other NPBTs fall under these regulations has been the subject of debate and led during a long time to a case-by-case approach by APHIS[47]. To tackle the uncertainty, the USDA, referring to the opportunities offered by NPBTs as genome editing, published in March 2018 a statement concerning its policy on innovative plant breeding[48]. It states that "Under its biotechnology regulations, the USDA does not currently regulate, or have any plans to regulate plants that could otherwise have been developed through traditional breeding techniques as long as they are developed without the use of a plant pest as the donor or vector and they are not themselves plant pests. This can include plant varieties with the following changes: (1) deletions (the change to the plant is solely a genetic deletion of any size); (2) single base pair substitutions (the change to the plant is a single base pair substitution); (3) insertions from compatible plant relatives (the change to the plant solely introduces nucleic acid sequences from a compatible relative that could otherwise cross with the recipient organism and produce viable progeny through traditional breeding); (4) complete null segregants (off-spring of a genetically engineered plant that does not retain the change of its parent." One can conclude that, under the assumption that they are developed without the use of a plant pest as donor or vector and are not themselves plant pests, plants derived from ODM, SDN-1 and SDN-2 are not considered to be regulated, since the genetic alterations could also be induced by conventional breeding techniques or occur in nature. Regarding SDN-3, a classification on a case-by-case basis is required to determine whether the individual application of SDN-3 falls within the definition of a regulated product[49].

The basis for the **Japanese** approach[50-52] to GMOs is the Cartagena protocol on biosafety of 2003[53], which is implemented by the 2004 "Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms"[54]. The regulatory status of genome-edited plants however remained for a long time characterised by a degree of uncertainty. One important element was clarified by the exclusion in the Cartagena act itself [Art. 2(2)] of processes using nucleic acid of an organism belonging to the same species as that of the target organism or nucleic acid of an organism belonging to a species that exchanges nucleic acid with the species of the target organism[51]. This allows to conclude that products from SDN-1 methods that do not contain inserted nucleic acid or its replicated product, do not satisfy the definition of living modified organism (LMO)[52]. The remaining uncertainty, especially with respect to plants produced as a result of SDN-2 or SDN-3, was lifted in February 2019 by an authoritative interpretation of the current law by the Japanese Ministry of the Environment which is summarised as follows in a statement published in English[55]: "Any organism that inserted extracellularly processed nucleic acid (including RNA) is regarded as an LMO, even one obtained using genome-editing technologies, and is subject to the regulations stipulated in the Cartagena Act, in principle. Such organisms are subject to the Cartagena Act unless complete removal of the inserted nucleic acid (including RNA), or its replicated product, is confirmed". It can be concluded that plants obtained by means of ODM, SDN-1 and SDN-2 will not be considered to be LMOs as long as no foreign nucleic acid is integrated into the host genome. Organisms resulting from SDN-3 are considered to be LMOs[56], because a foreign gene has been integrated into the host's genome unless the complete removal of nucleic acid (including RNA) or its replicated product is confirmed.

In **Australia**[57,58], the application of GMO legislation to crops developed by NPBTs also largely depends on whether gene technology has been used in their development. The Gene Technology Act N° 169 of 2000 defines (sect. 10)[59] a genetically modified organism as an organism that has been modified by gene technology or that has inherited traits developed by gene technology in other organisms, while gene technology is defined as any technique for the modification of genes or other genetic material (with the exception of sexual reproduction and homologous recombination). The Gene Technology Regulations of 2001[60] contain schedules of techniques that are not gene technology and of organisms that are GMOs. A revision of October 2019 introduced important modifications and explicitly classifies as GMOs[61]: "(1) an organism that has had its genome modified by oligonucleotide-directed mutagenesis; (2) an organism modified by repair of single-strand or double-strand breaks of genomic DNA induced by a site-directed nuclease, if a nucleic acid template was added to guide homology-directed repair." To the list of techniques that are not gene technology[62] is added the "Introduction of RNA into an organism, if: (a) the RNA cannot be translated into a polypeptide; and (b) the introduction of the RNA cannot result in an alteration of the organism's genome sequence; and (c) the introduction of the RNA cannot give rise to an infectious agent." To the list of organisms that are not GMOs are added[63]: "an organism modified by repair of single-strand or double-strand breaks of genomic DNA induced by a site-directed nuclease, if a nucleic acid template was not added to guide homology-directed repair" and (2) "an organism that was modified by gene technology but in which the modification, and any traits that occurred because of gene technology, are no longer

present." The Australian Office of Gene Technology Regulator (OGTR) qualifies the changes as follows: One technique, SDN-1, is excluded because SDN-1 organisms present no different risk than organisms carrying naturally occurring genetic variations and cannot be distinguished from conventionally bred animals or plants. The conditions for the exemption are that "(1) no nucleic acid template was added to cells to guide genome repair following site-directed nuclease application and (2) the organism has no other traits from gene technology (*e.g.* cas9 transgene, expressed SDN protein). Techniques similar to SDN-1 but that do not meet the SDN-1 exclusion, are not excluded from regulation. Similarly, the scale of resulting nucleotide changes, whether an insertion or deletion, or whether the resulting nucleotide sequence may be found in sexually compatible species, is not a deciding factor." One may conclude that under the present Australian law, products resulting from ODM, SDN-2 and SDN-3 are considered GMOs, products from SDN-1 are not[50].

2. The science behind genome editing

On the origin of plant breeding

Plants have been domesticated by humans to be more productive and adapted to agricultural practices already since the dawn of civilization. The genetic blueprint and appearance of plants has changed dramatically in the course of this process. A spectacular example is how teosinte, a small, weedy plant endogenous to Mexico and bearing a few hard seeds, was selected to become the modern, highly productive crop that maize is today. The domestication of plants has only been possible because of spontaneous genetic changes that occur over time. Since the discovery of Gregory Mendel's laws of inheritance in 1865, plant breeding underwent many technological breakthroughs, ranging from the ability to make crosses with wild relatives, to mutation breeding in 1920 that enabled to increase the rate of genetic variation, and at the end of the 20th century, through technological advancements in molecular biology.

The domestication of plants has only been possible because of spontaneous genetic changes that occur over time.

Genetic changes – also called mutations – in the genetic blueprint or DNA are the major source of diversity we observe in plants every day. These spontaneous mutations are a result of various natural processes in a living cell (*e.g.* involving reactive oxygen species) or copying errors of the genetic blueprint during cell division (**Figure 2**). Moreover, spontaneous mutations occur in each generation of every living organism. It is estimated that for example in a single wheat plant, approximately 238 spontaneous mutations occur in each generation[64]. This implies that all individual plants in a field of crops slightly differ genetically from each other.

Spontaneous mutations occur in every living organism, including us, humans, and are essential for evolution, the process by which populations of organisms change over generations. Over centuries, we have been selecting plants for spontaneous mutations that lead to crops with desirable traits such as increased yield, fruit size, or resistance to diseases. For example, the domestication of the staple crop maize from teosinte involved spontaneous mutations in a limited number of genes, amongst which *TGA1*[65]. Variation cannot be created without mutations, and because the spontaneous mutation rate is relatively low, the ability to select new desirable traits is limited.

Random mutation techniques accelerate plant breeding

As a result of scientific advances in the fields of genetics and physics, new methods became established in the middle of the 20th century that enabled us to increase the rate of genetic variation or mutations by treatment with factors such as ionising radiation (*e.g.* UV, X-ray, gamma) or chemicals (*e.g.* ethyl methane sulfonate (EMS)) (**Figure 2**).

This process, called mutation breeding, produces thousands of random mutations in the genetic blueprint of a plant. While this process is still faster than relying on spontaneous mutations, time-consuming selection and backcrossing are still necessary to isolate a desired new trait and select against the thousands of mutations, some of which are deleterious.

For over 70 years, mutation breeding has been a key resource to improve the varieties that were available. The Joint FAO/IAEA Mutant Variety Database (**mvd.iaea.org**) currently compiles more than 3000 crop varieties that have been produced through mutation breeding. It is no longer possible to fully trace with which of today's crops these mutagenised crop varieties are crossed. Many of the plants that we consume nowadays, from cereals to vegetables and fruits, are derived from mutation breeding. An example is barley

that is resistant against powdery mildew infection because of a mutation introduced in the *MLO* gene through mutation breeding[66]. Current beer and whiskey production would be virtually impossible without this induced mutation.

It is no longer possible to fully trace with which of today's crops these mutagenized crop varieties are crossed. Many of the plants that we consume nowadays, from cereals to vegetables and fruits, are derived from mutation breeding.

From the end of the 20th century, technological advancements in molecular genetic analysis methods, such as high-throughput sequencing, made it possible to identify the genetic units of hereditary genes that code for a certain trait. This has contributed to a considerable improvement in the procedure of breeding and selection of mutations specifying desired traits and has further accelerated the breeding process.

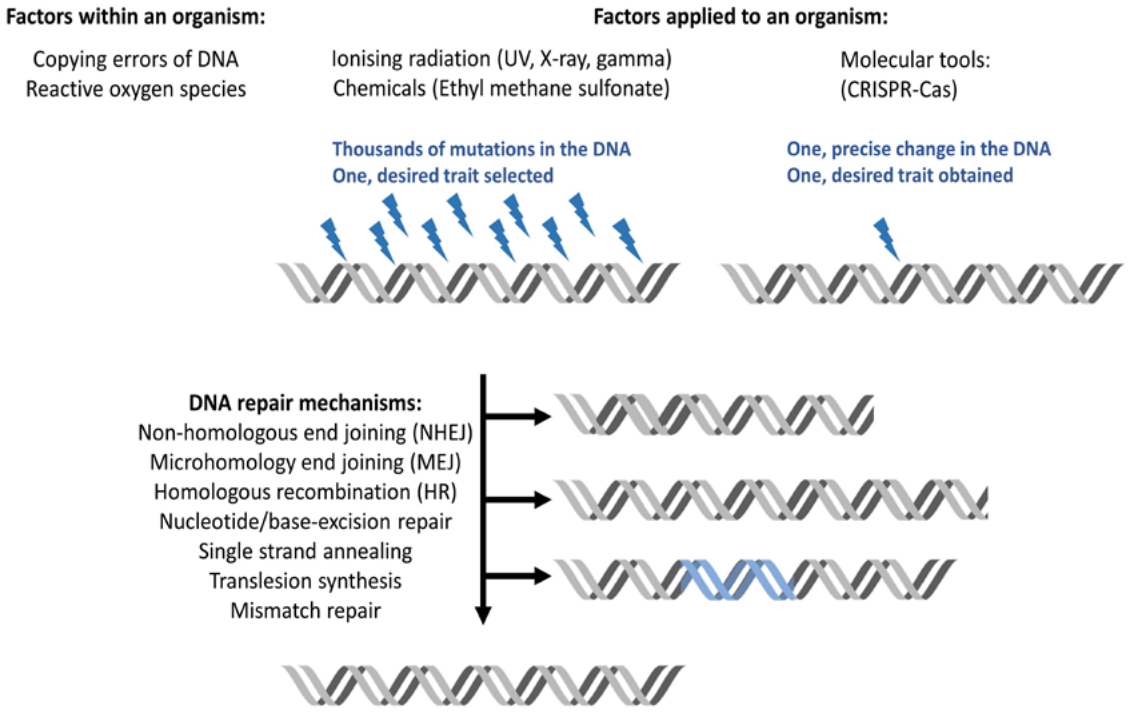

Figure 2. Overview of factors within an organism or factors that can be applied to an organism and which can result in changes in the genetic blueprint. *Mutations within an organism occur spontaneously as a result of, for example, exposure to reactive oxygen species or copying errors of the genetic blueprint. Factors originating from outside the organism such as ionising radiation or chemicals can also induce mutations. Subsequently, DNA damage is repaired by various DNA repair mechanisms present in the cell. However, occasionally these repair systems make errors, resulting in genetically inheritable DNA changes, respectively depicted in the figure as a deletion, insertion or substitution (blue).*

Genome editing is a revolutionary tool for plant breeding

Over the past 20 years, several new plant breeding techniques (NPBTs) have been developed[67]. NPBTs make specific changes within the genetic blueprint of the plant in order to change its traits, and these modifications can vary in scale from a small alteration to inserting or removing one or more genes. There are various methods for achieving these changes, which include for example: deploying processes that alter gene activity without altering the genetic blueprint itself (epigenetic methods), grafting of unaltered plant pieces onto a genetically modified root stock or modifying the genetic blueprint during the DNA repair process (genome editing).

Genome editing encompasses the efficient, precise and time-saving introduction of mutations in the genetic blueprint of cultivated plants by making use of one of a variety of targeted molecular editors. One precondition is that the target gene in the recipient plant is known. A frequently used metaphor for NPBTs is a word processor with a 'find and replace' tool: it is possible to search for a specific word throughout the whole text and use the 'replace' tool to install a targeted change *only* in that word.

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Currently, there are many different molecular editors available, such as Mega nucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) or clustered regularly interspaced short palindromic repeats (CRISPR)-associated systems (Cas), which are all called site-directed nucleases (SDNs). The CRISPR/Cas technology was only introduced in 2013 but it is now by far the most popular tool for creating targeted changes in the genetic blueprint due to its simplicity[1]. For this reason, the ALLEA-KVAB symposium largely focused on genome editing using CRISPR-Cas (**Figure 3**), further referred to as **genome editing**.

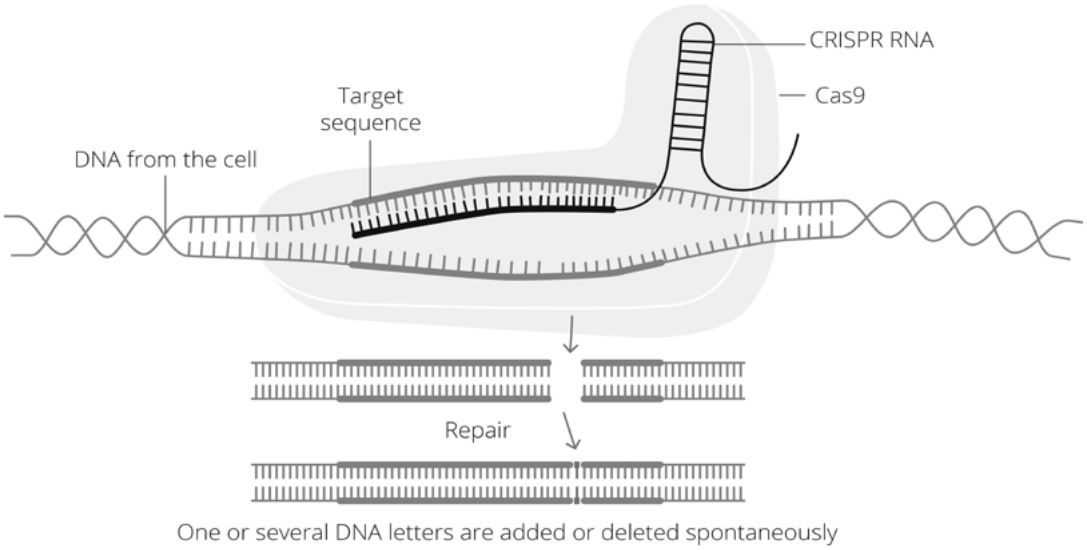

Figure 3. CRISPR-Cas genome editing in a nutshell. *The CRISPR-Cas system is the most recent platform developed to make highly specific changes to the DNA of organisms. CRISPR stands for 'clustered regularly interspaced short palindromic repeats' and 'Cas' for the CRISPR-associated system. Compared to other platforms for engineered nucleases, the DNA-recognition is based on RNA-DNA interactions, enabling a fast and cost-effective engineering of the DNA-recognition module. The CRISPR RNA and trans-activating CRISPR RNA form a complex that acts as the guide RNA (gRNA) for the Cas9 endonuclease. The gRNA binds the Cas9 endonuclease and directs cleavage of a unique target sequence in the DNA, based on a matching genomic sequence. Subsequently, the double-strand break in the DNA is recognised by the endogenous DNA repair systems, which occasionally make errors, resulting in inheritable changes to the DNA. Adopted from VIB Fact Series "CRISPR-Cas Genome editing in plants"[68].*

Genome editing of plants requires the delivery of the molecular editor into cultured cells or whole plants. To perform the genome editing, two components need to be present: an endonuclease (*e.g.* Cas9) and a guide RNA (gRNA) that directs the endonuclease to the DNA sequence that will be modified.

Generally, two strategies are exploited: stable transformation or transient transformation. In the case of stable transformation, genes that encode the molecular editor are introduced and integrated in the genetic blueprint of the plant of interest using genetic modification *e.g. Agrobacterium tumefaciens*-mediated transformation. Subsequently, the plant cells use the instructions to produce the molecular editor and

generate the targeted change to the genetic blueprint. Plants without the module containing the molecular editor but with the desired DNA change, can be generated by outcrossing. In the end, the product only contains the intended change in the DNA without any foreign DNA.

In the case of transient transfection, the molecular editor is only temporarily introduced into the plant cells to generate the targeted change to the genetic blueprint. This can be conducted through the temporary introduction of a DNA module that encodes the molecular editor or by delivery of the molecular editor itself (as protein-RNA complex) into the plant cells. As a result, the genome-edited plant is directly obtained without integration of the module into the genetic blueprint of the plant.

It should be noted that there are many variants of genome editing using different types of Cas enzymes. Furthermore, this is an extremely rapidly evolving field with new applications being published almost daily. Depending on the tool used for genome editing and the repair mechanism of the host plant, the change in the genetic blueprint may be simple or complex. Applications of genome editing are generally grouped in different categories: SDN-1, SDN-2, SDN-3 and base or prime editing (**Figure 4**).

SDN-1: mutations consisting of changes in a few bases, short insertions or deletions (indels) are generated by SDN-1 in a predefined location in the genetic blueprint as a result of a non-homologous end joining (NHEJ) repair mechanism of the cell.

SDN-2: specific point mutations or small indels are generated as a result of the introduction into the cell of a DNA repair template homologous to the targeted area. By means of homologous recombination (HR), precise and small genetic modification can be achieved.

Base or prime editing: more recent technology with additional functionalities that are engineered with the nuclease linked to a reverse transcriptase for prime editing (PE) or a deaminase for base editing (BE). In contrast with SDN-1 and SDN-2, double-strand DNA breaks are not required for these techniques.

SDN-3: genes are inserted into a predefined location in the genetic blueprint. This is enabled through the introduction of a large stretch of DNA molecule. The insertion of the gene in the genetic blueprint can take place either by HR or by NHEJ. The introduced genes can be derived from plants (*cis*-genesis) or from other organisms (*trans*-genesis). SDN-3 modifications resemble classical genetic modifications and were not under consideration in the ALLEA-KVAB symposium.

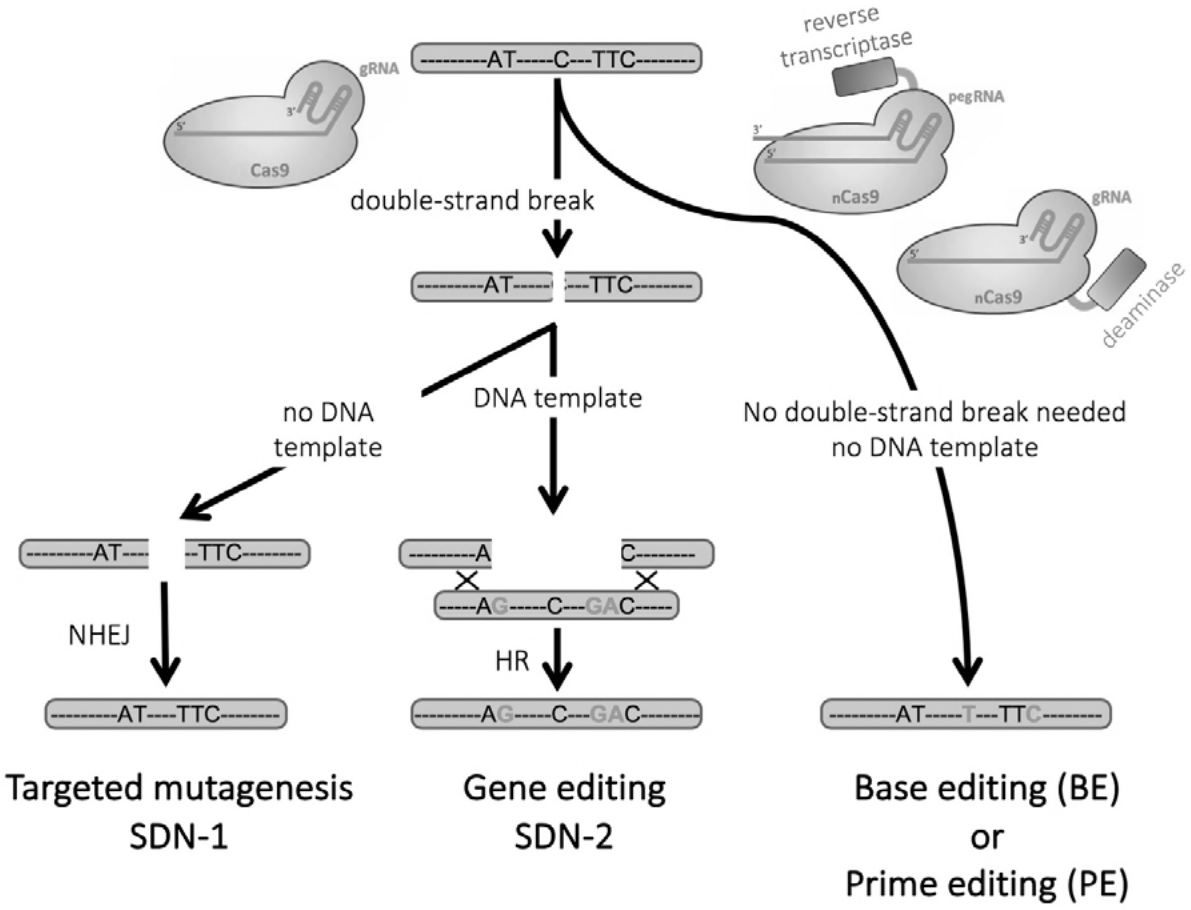

Figure 4. Overview of the applications of genome editing and the outcomes in the genetic blueprint. *Applications of genome editing are generally grouped in different categories: site directed nuclease SDN-1, SDN-2, SDN-3 and base or prime editing. In the case of SDN-1, no DNA template is introduced in the cell and the double-strand break is repaired through non-homologous end joining (NHEJ), which occasionally makes errors, resulting in inheritable DNA changes. SDN-2 requires the introduction of a DNA template in the cell which, except for a few changes in the DNA, is identical to the sequence in which the double-strand break is introduced. Subsequently, the double-strand break is repaired through homologous recombination (HR), which causes a targeted, inheritable DNA change. SDN-3 is not depicted because this application was not under the scope of this symposium summary. Base editing comprises a modified SDN linked with an enzyme that catalyses changes in the genetic blueprint without the need for double-strand breaks or DNA templates. Prime editing consists of a modified SDN linked with a reverse transcriptase enzyme. It mediates targeted insertions, deletions and conversions without the need for double-strand breaks or DNA templates. Figure adapted from the presentation 'Risk assessment and regulation of genome-edited crops' by Dr. Fabien Nogué, INRAE Center of Versailles (France).*

Important to highlight is that at the molecular level, the type of changes in the genetic blueprint obtained by SDN-1, SDN-2, BE or PE are similar to what can be obtained by mutation breeding or by spontaneous mutations and are consequently not distinguishable from them.

Important to highlight is that at the molecular level, the type of changes in the genetic blueprint obtained by SDN-1, SDN-2, BE or PE are similar to what can be obtained by mutation breeding or by spontaneous mutations and are consequently not distinguishable from them. Spontaneous mutations occur in each generation of every living organism. As mentioned earlier, it is estimated that for example in wheat, with its large genome, approximately 238 spontaneous mutations occur in each generation[64]. For other crops, the number of mutations that arise in each generation is estimated approximately 32 in maize, 16 in soybean and 13 in tomato based on extrapolation from sequence analysis in the model organism *Arabidopsis* (**Figure 5**)[64]. Random mutation breeding increases the mutation rate and is estimated to produce 1 mutation for

each 3,220 kb in the DNA of tomato upon treatment with 0.1% EMS[69]. As a consequence, this will result in more than 600 randomly introduced mutations in the genetic blueprint of tomato per generation. This is in huge contrast with the introduction of a hypothetical single genome-edited mutation specifying a desired trait (**Figure 5**). In conclusion, mutations introduced through genome editing contribute barely to the increase of the overall mutation rate in plants.

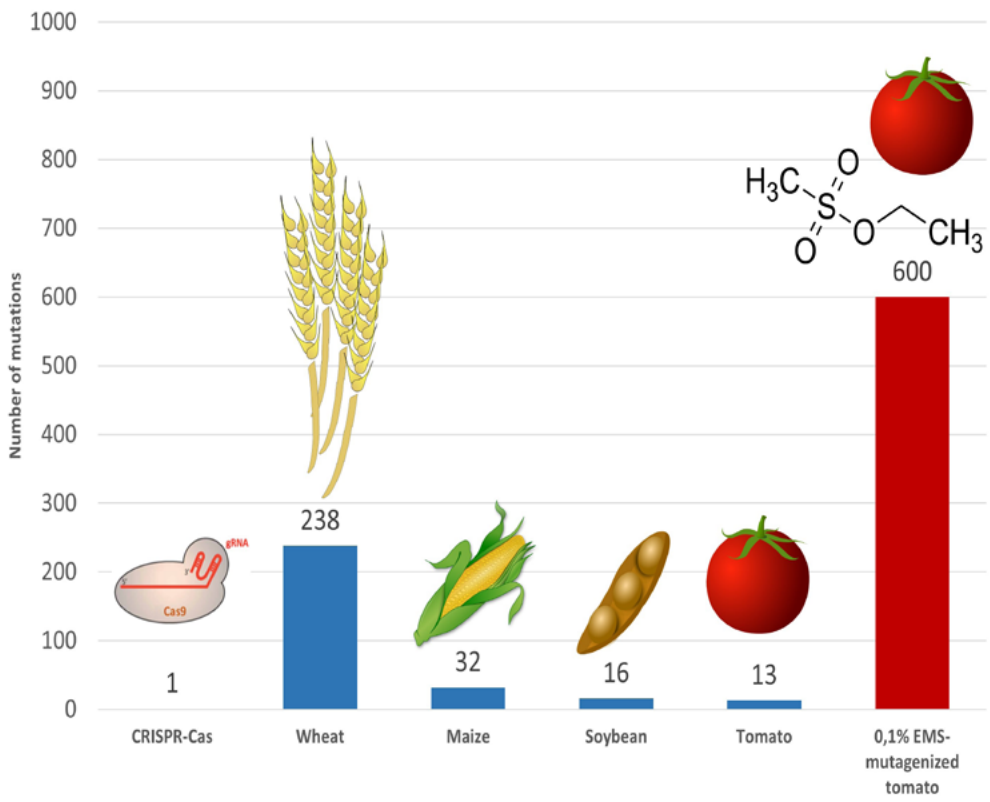

Figure 5. Estimated number of spontaneous mutations that occur in every individual plant compared to a hypothetical single change in the genetic blueprint introduced using CRISPR-Cas genome editing or EMS-mutagenesis. Spontaneous mutations occur in every living organism. *It has been determined that in the model plant Arabidopsis, approximately two spontaneous mutations will arise in each individual plant in each generation[64]. The number of spontaneous mutations that occur is correlated with the size of the genome. This implies that for example for wheat, maize, soybean and tomato, a different number of spontaneous mutations is estimated based on extrapolation of the mutation rate in Arabidopsis and with respect to the size of the genome. On the other hand, it is estimated that mutation breeding through 0.1% EMS treatment produces one mutation for each 3,220 kb in tomato[69]. Based on the size of the genome of tomato (950 Mb), it is estimated that EMS mutagenesis causes approx. 600 mutations. This is in huge contrast with the introduction of a hypothetical single mutation with CRISPR-Cas genome editing. It is important to note that the indicated number of spontaneous mutations will occur in every individual generation while the genome-edited mutation or EMS mutation will be introduced only once during the development of a variety. Figure adapted from the presentation 'Risk assessment and regulation of genome-edited crops' by Dr. Fabien Nogué, INRAE Center of Versailles (France).*

Genome editing is being widely adopted by researchers and plant breeders

Genome-editing methods have enabled researchers to introduce mutations in the genetic blueprint of plants with high precision and efficiency and have accelerated molecular breeding. Researchers have widely adopted genome-editing methods due to its simplicity, low costs and flexibility.

Genome editing is an innovative technology for crop improvement that is:

» **specific:** no longer reliant on randomly induced genetic variation using mutation breeding;

» **precise:** showing the highest level of control over the changes introduced in the genetic blueprint;

» **time efficient:** feasible in one or two generations of a plant;

» **multitudinous:** enabling simultaneous editing of several locations in the genetic blueprint.

Till now, there have been over 1,500 articles published in scientific journals about genome editing in plants (based on a search in the Web of Science using the terms 'CRISPR' and 'plants') and this number is rapidly increasing.

Genome editing has also become part of a plant breeder's toolbox. Genetic diversity is the fuel for plant breeding and breeders are continuously looking for more genetic diversity, which allows them to improve plants. Furthermore, more and more genes underlying interesting traits are being identified and genome editing accelerates the selection of novel variants (alleles) of such genes with high efficiency.

Genome editing provides high potential to solve some of the problems that breeders are currently facing. For example, classical breeding often encounters the problem that a favourable trait is closely linked to a negative trait. In some cases it is virtually impossible to separate these two traits. Using genome editing, however, it is straightforward to disable the unwanted genetic trait. Having said this, plant breeding will continue to rely on crossing with other varieties and subsequent selection, which implies taking into account the genetic blueprint of the plant of interest and all the characteristics that are associated with the respective hereditary units. Sometimes it is perceived that genome editing enables scientists and breeders to develop crops that can go directly from the lab to the field. In fact, this is not the case: genome editing is part of the breeding cycle and breeders still need to go to the field and analyse plants with all their characteristics over many years, on several locations and finally select the plants with favourable traits.

Classical breeding often encounters the problem that a favourable trait is closely linked to a negative trait. In some cases it is virtually impossible to separate these two traits. Using genome editing, however, it is straightforward to disable the unwanted genetic trait.

Genome editing is now part of the innovation history that plant breeding developed. Since the discovery of Gregory Mendel's laws of inheritance, plant breeding underwent many technological breakthroughs, ranging from the ability to make crosses with wild relatives, polyploidisation, embryo rescue, double haploid technology, *in vitro* culture, mutation breeding, marker-selected breeding, genome selection and now genome editing. Genome editing is one of the latest tools, but probably not the last tool that will become available for plant breeders.

3. The potential of genome editing for agriculture, society and environment

The demand for food and resources will continue to grow worldwide, while the natural resources required for food and biomass systems will become limited, and ecologically valuable natural landscapes contributing to biodiversity are lost at increasing pace. The climate crisis is upon us, and its impacts are getting more severe with each passing year. Global actions to slow down climate change are promising but likely insufficient. More substantial investment in efforts to adapt to conditions like higher temperatures, longer periods of drought and more unpredictable rainfall are needed.

Solving the global food and resource problem requires multidisciplinary approaches because plant production comprises many aspects such as inputs, management practices, plant protection, soil management and plant varieties. Breeders, geneticists and biotechnologists focus mainly on improving plant varieties through breeding. Genome editing enables accelerated breeding because it is feasible to introduce a genome-edited mutation specifying a certain favourable trait in one or two generations of a plant. However, such genome-edited plants will need to become part of the normal breeding efforts of companies and have to undergo, like any other new variety, multi-year field trials at many different locations before being released to the market.

Scientists state that new molecular breeding techniques such as genome editing will make a critical contribution in the coming years to make food systems more sustainable and more resilient to climate change. We need to reduce inputs, increase yields, better ensure our food security and help stabilise food prices during unpredictable climates[70-74].

Scientists state that new molecular breeding techniques such as genome editing will make a critical contribution in the coming years to make food systems more sustainable and more resilient to climate change.

This view is confirmed by numerous reports that have been published by international organisations such as the Global Commission on Adaptation[75], the Food and Agriculture Organisations of the United Nations[76], the Intergovernmental Panel on Climate Change[77] and the European Environment Agency[78]. Moreover, it is highlighted that developments should not be limited to a small number of particularly widespread crop species, which were the main focus of genetic engineering in the past. A greater diversity of crop species is not only desirable, but of central importance for both sustainable agriculture and healthy nutrition. The use of more varieties of crop species is considered to increase the resilience to climate change.

Genome editing has already resulted in numerous improvements in crops through targeted changes in the genetic blueprint of cultivated plants. By now, there are more than 100 applications of genome-editing methods on at least 28 different plant species, which are all documented in scientific publications[1]. These are genome-edited crops with scientific evidence for the respective change in the genetic blueprint. For some of the genome-edited crops, field trials still need to be conducted, while others are already on the market (**Table 1**). Which developments will be conducted in the future in the breeding sector and public research institutes and which applications will eventually be authorised for release on the market, depends largely on the economic and legal framework.

A few examples of genome editing for crop improvement are discussed below.

Bread wheat (*Triticum aestivum* L.) is a major staple crop worldwide. Given the importance of wheat, new traits have continuously been sought to improve its yield and quality. Bread wheat incurs critical yield losses from powdery mildew, a major disease caused by the fungus *Blumeria graminis* f. sp. *Tritici* (**Figure 6**). Currently, farmers heavily rely on fungicides to control the disease. In order to make the cultivation of

wheat more sustainable, researchers have looked into the potential of genome-editing tools to improve resistance to powdery mildew in wheat[79].

Loss of function of the gene *MLO* in barley, *Arabidopsis* and tomato leads to broad-spectrum and durable resistance to the fungal pathogens that cause powdery mildew[79]. However, to date, no wheat varieties with loss of function of the *MLO* gene have been reported. This highlights the limitations of conventional breeding methods, including mutation breeding. The reason is that the genetic blueprint of wheat is highly complex consisting of three different diploid genomes. Each of the three diploid genomes contains two copies of the gene of interest. This so-called hexaploidy nature of wheat makes that, for most genes, there are six copies.

Genome editing with CRISPR enabled scientists to simultaneously change all six *MLO* alleles of wheat, hence resulting in resistance to powdery mildew. This research presents a successful example of the use of genome editing for innovation breeding of wheat. The rapidity and precision with which changes can be achieved by this approach will help to improve wheat at a rate sufficient to improve global food security.

Figure 6. Cultivation of wheat and powdery mildew on a wheat leaf. *Bread wheat incurs critical yield losses from powdery mildew, a major disease caused by the fungus Blumeria graminis f. sp. Tritici. An infected leaf is depicted on the right panel. Farmers currently heavily rely on fungicides to control the disease. In order to make the cultivation of wheat more sustainable, researchers have successfully used genome-editing tools to improve resistance to powdery mildew in wheat.*

Another example is the improvement of cold storage and processing traits of potato through genome editing[80]. *Solanum tuberosum* or potato is the world's third most important food crop and is used by processors to produce crisps, French fries, etc. (**Figure 7**).

Cold storage of potato tubers is commonly used to reduce sprouting and extend the postharvest shelf life. However, cold temperature stimulates the accumulation of reducing sugars in potato tubers. Upon high-temperature processing, these reducing sugars react with free amino acids, resulting in brown, bitter-tasting products and elevated levels of acrylamide, which is potentially unhealthy.

To address these trade-offs, Calyxt Inc used genome-editing technology to inactivate four copies of the vacuolar invertase gene (VInv) in the genetic blueprint of the commercial potato variety 'Ranger Russet' with the aim to reduce the production of these reducing sugars. Tubers from these genome-edited plants had undetectable levels of reducing sugars, and processed chips contained lower levels of acrylamide and were lightly coloured.

The improved potato variety brings additional benefits to the consumers of French fries and crisps. The first field trials of this genome-edited potato were completed in 2015 and certified planting material is underway to facilitate a commercial launch. Moreover, these results provide a framework for using genome editing to quickly improve traits in relevant potato cultivars.

Figure 7. Cultivation, storage and processed products of potatoes.

Table 1. A selection of genome-edited crops categorised based on improved food and feed quality, reduction of pesticide use, water consumption and crop losses, or agronomic importance[1,81].

Plant	Beneficial trait	Genome-editing technique	Research study
Traits related to improved food/feed quality			
Alfalfa	Reduced lignin content	TALEN	APHIS* database[47]
Canola	Improved fatty acid composition	CRISPR-Cas	Okuzaki *et al.*, 2018[82]
Peanut	Improved fatty acid content	TALEN	Wen *et al.*, 2018[83]
Rice	Increased amylose content	CRISPR-Cas	Sun *et al.*, 2017[84]
Tomato	Increased lycopene content	CRISPR-Cas	Li *et al.*, 2018[85]
Wheat	Increased fibre content	TALEN	APHIS* database[47]
Wheat	Reduced gluten content	CRISPR-Cas	Sánchez-León *et al.*, 2017[86]
Soybean	Improved oil quality	TALEN	Haun *et al.*, 2014[87] Demorest *et al.*, 2016[88] APHIS* database[47]
Sage	Reduced phenolic acid content	CRISPR-Cas	Zhou *et al.*, 2018[89]
Maize	Improved starch production	CRISPR-Cas	APHIS* database[47]
Lettuce	Increased vitamin C content	CRISPR-Cas	Zhang *et al.*, 2018[90]
Traits related to reduced crop losses, pesticide use or water consumption			
Cacao	Resistance to Phytophthora tropicalis	CRISPR-Cas	Fister *et al.*, 2018[91]
Cucumber	Broad resistance to viruses	CRISPR-Cas	Chandrasekaran *et al.*, 2016[92]
Grapefruit	Resistance to citrus canker	CRISPR-Cas	Jia *et al.*, 2015[93] Jia *et al.*, 2017[94]
Orange	Resistance to citrus canker	CRISPR-Cas	Peng *et al.*, 2017[95]
Grapevine	Resistance to Botrytis cinerea	CRISPR-Cas	Wang *et al.*, 2018[96]
Tomato	Broad resistance to bacterial infections	CRISPR-Cas	de Toledo Thomazella *et al.*, 2016[97]
Wheat	Resistance to powdery mildew	TALEN/CRISPR-Cas	Wang *et al.*, 2014[79] Zhang *et al.*, 2017[98] APHIS* database[47]
Soybean	Drought and salt tolerance	CRISPR-Cas	APHIS* database[47]
Maize	Drought tolerance	CRISPR-Cas	Njuguna *et al.*, 2017[99]
Potato	Resistance to Potato Virus Y (PVY)	CRISPR-Cas	Zhan *et al.*, 2019[100]
Traits related to agronomic importance			
Rice	Increased seed weight	CRISPR-Cas	Li *et al.*, 2016[101]
Canola	Increased shatter resistance and seeds number per husk	CRISPR-Cas	Braatz *et al.*, 2017[102] Yang *et al.*, 2018[103]
Lettuce	Germination at high temperature	CRISPR-Cas	Bertier *et al.*, 2018[104]
Wheat	Increased grain weight	CRISPR-Cas	Wang *et al.*, 2018[105]
Potato	Improved cold storage and processing traits	TALEN	Clasen *et al.*, 2015[80]
Tomato	Increased fruit size	CRISPR-Cas	Rodríguez-Leal *et al.*, 2017[106]

**The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) provides a database "Am I regulated?" of genome-edited crops, which have been evaluated for their regulatory status in the US.*

4. Risk considerations of genome-edited crops

The European Court of Justice rules in its judgment in case C-528/16 that genome-edited crops are subject to the provisions of the GMO Directive[2]. As a consequence, genome-edited crops have to be regulated as GMOs and must be risk-assessed before market release[3]. Applicants have to submit a dossier to the European Food Safety Agency (EFSA), which will evaluate the results of the analysis of the genome-edited crop in a thorough risk assessment procedure.

The risk assessment of GM plants in Europe is mainly based on a comparative approach: a comparison between the conventional crop and its GM counterpart. It consists of a molecular characterisation of the GM plant, a comparative analysis of the compositional phenotypic and agronomic properties, a safety assessment for humans and animals (allergenicity, nutritional value, toxicology) and a safety assessment of the environment. The current status and future challenges of risk assessment and regulation of genome-edited crops are extensively reviewed in Schiemann *et al.*, 2019[107] and Lassoued *et al.*, 2019[72].

Two decades of experience with the market introduction of GMOs in Europe has proven that regulating genome-edited organisms as GMOs *de facto* blocks the development and market introduction of such crops in Europe, in particular for cultivation. Opinions about GMOs are sharply divided across the EU and many are arguing that mechanisms for performing an environmental impact assessment of genome-edited crops should take into account not only risks but also benefits and risks for the environment of not facilitating the market introduction of genome-edited crops.

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It should be noted that there are many different applications of genome editing of crops which are generally grouped in different categories: SDN-1, SDN-2, SDN-3 and base or prime editing (**Figure 4**). In the context of risk assessment, it is important to highlight that the type of changes in the genetic blueprint of a crop obtained by SDN-1, SDN-2 and base or prime editing is similar to what can be obtained by mutation breeding or by spontaneous mutations, and are consequently not distinguishable from them.

There is a growing consensus that risk assessment should differentiate between genome-edited crops that have DNA changes which can also occur spontaneously in nature or as a result of conventional breeding, and genome-edited crops that contain changes in the genome which cannot occur in nature or as a result of conventional breeding methods, for example the insertion of a foreign gene into a predefined location in the genome (SDN-3).

Genome-edited crops with DNA changes that can as well spontaneously occur in nature or result from mutation breeding methods are considered to be generally as safe as crops with the same DNA changes obtained through conventional methods. In other words, a genome-edited crop with a specific mutation is as safe as a conventional crop containing the same mutation. In addition, crops with a specific mutation developed through genome editing are considered safer than crops with that same mutation resulting from mutation breeding. The reasoning is that in the latter case, the crop will contain many additional random mutations of which the effect is not known and cannot be predicted. As a result, the conventional crop has a much higher degree of uncertainty about its safety compared to the genome-edited crop.

With the use of genome editing, plant breeding becomes much more knowledge based. Plant breeding thereby transitions from a sometimes blind or random approach to a much more targeted and precise approach. Genome editing reduces the amount of uncertainties, which contributes to safety[72]. Crops that

have the same likelihood of being safe should be treated by the biosafety legislation in the same manner, otherwise the legislation would be unjustifiably discriminatory.

The use of a particular technology will not determine whether or not a certain crop is safe, but the introduced characteristics will determine its safety. In terms of biosafety legislation, the approach should not be based on whether or not a crop developed through genome editing could possibly create a risk and as such should be subject to the GMO legislation. There is a general misconception that genome-edited crops would not be regulated in case they would not be subjected to the provisions of the GMO directive. As confirmed by Advocate General Bobek in his opinion of 18 January 2018 about case C-528-16[13], many legislative safeguards for the protection of human health and the environment are in place at EU level, which also cover the development, production, commercialisation and consumption of non-GMO crops produced by NPBTs[108,109]. If the development of the new product involves GMOs or GM micro-organisms, the Contained Use Directive 2009/41/EC[110] will be applicable. The production and commercialisation of plant reproductive material is subject to the Common Catalogue Directive 2002/53/EC[12] and several Sectoral Seed Marketing Directives as well as to the General Product Safety Directive 2001/95/EC[111]. Crops for consumption are subject to the General Food law Regulation (EC) n°178/2002[112] and the Novel Food Regulation (EU) 2015/2283[113]. The Official Controls Regulation (EU) N° 2017/625[114] sets a framework for compliance control by member states. The Product Liability Directive 85/374/EEC[115] provides harmonised liability rules in the event of damage to consumers, while the Environmental Liability Directive 2004/35/EC[116] makes preventive and remedial measures possible in the event of damage to the environment.

There is a limited chance that genome editing results in off-target modifications in the genome of a crop. In order to reduce the chance of off-target modifications, scientists are continuously working on improvements of genome editing to raise the specificity of the technology to a very high level. In case an off-target modification has occurred in the genome of a crop as result of genome editing, one has the option to cross out the off-target modification or to select another crop without the off-target modification.

In plants, the possibility of the off-target modifications is a relative discussion when one realises that conventional random mutagenesis creates much more off-target changes to the genome. These additional off-target modifications resulting from mutation breeding methods are generally neither identified, nor are their effects determined. Additionally, it is important to be aware that depending on the size of the genome of the crop, from one generation to the other, already tens to several hundreds of spontaneous mutations will occur in the genome of the crop. Genome editing is continuously being improved to increase efficiency and decrease the frequency of off-target modifications[72]. Moreover, scientists and plant breeders have the ability to cross and select plants in which only the desired DNA alteration is present, without off-target changes or spontaneous mutations[117].

In summary, the judgment of the ECJ rules that genome-edited crops are subject to the provisions of the GMO Directive[2]. As a consequence, genome-edited crops have to be regulated as GMOs and must be risk assessed[3]. This is an unjustified discrimination for the applications of genome editing such as SDN-1, SDN-2 and base or prime editing because this regulation does not apply to crops that could also be generated through conventional methods of breeding. In Chapter 9, several policy options are presented to rectify this discrepancy.

5. Traceability issues of genome-edited crops in a globalised world

The scientific assessment and validation of detection methods for GM food and feed

The Joint Research Centre of the European Commission (JRC) is providing the scientific and technical support for the implementation and enforcement of EU legislations and has been working on GMOs since the early 1990s. In the last 20 years, the JRC has focused on traceability issues related to GMOs and GM food and feed products. The term 'traceability' implies the possibility to track GMOs and GM food and feed products at all stages of the supply chain and encompasses detection, identification and quantification[3].

In Europe, national reference laboratories are responsible for the implementation of EU legislation and need to have the set of tools to safeguard that GM food and feed products comply with the legal requirements. The reference laboratories are tasked to test and validate the method for detection, including sampling and identification of the GMO transformation event and, where applicable, for the detection and identification of the GMO transformation event in food or feed.

The JRC is responsible for the method validation and works together with reference laboratories, which are also responsible for the general control of the food market in their territories. This is the instrument that has been put in place to make sure that all GMOs that are submitted for commercialisation have a suitable, validated method for detection and quantification[3].

The European Reference Laboratories for GM Food and Feed (EURL GMFF) have built up a lot of experience regarding the scientific assessment and validation of detection methods for GM food and feed. They have determined for example the minimum performance requirements for analytical methods of GMO testing and provide what the method, regardless of the application (*e.g.* genome-edited product), needs to comply with in order to be acceptable for releasing a product on the market[118].

The issue of 'uniqueness' of a DNA sequence

An important aspect to consider related to detection and identification methods, is the issue of 'uniqueness' of a DNA sequence. The genetic blueprint or genome of any living organism consists of sequences of the four DNA building blocks or bases (A, G, C, and T) of which the order determines the characteristics of the organism. For example, the genetic blueprint of maize contains approximately 2.5 billion bases. At what point can a certain string or sequence of bases be considered as species-specific? Recent studies show that a sequence of 14–17 bases, depending on the genome size of the respective organism, is theoretically expected to be unique[119]. More detailed information can be found in **Figure 8**.

The size of the genome of an organism needs to be taken into account because the larger the genome, the higher the probability that a certain DNA sequence of a given length will occur randomly.

On theoretical grounds, it is possible to calculate the probability to have a unique DNA sequence within a genome if DNA sequences are random (graph A). Moreover, the size of the genome of an organism needs to be taken into account because the larger the genome, the higher the probability that a certain DNA sequence of a given length will occur randomly. For larger genomes, such as wheat (13 billion bases), the DNA sequence needs to be minimum 25 bases long in order to be identified as unique (graph A).

However, DNA sequences in genomes are not random. To evaluate the eventual impact of this, the JRC generated 10,000 random DNA sequences for each size between 6 and 30 bases and tested what percentage of these sequences were identified with 100% similarity in the published genetic blueprint of rice. The results show as predicted that the probability not to detect a unique DNA sequence of size 'n' starts to decrease at lower 'n' values (graph B). However, in this approach the difference in minimum length is smaller (17 bases) compared to the random analyses (18-19 bases).

With the advances of next generation DNA sequencing technologies, there is a better view on the genetic diversity between species and within a given species. Platforms such as Ensembl (**http://www.ensembl.org/**) or Transplant infrastructure (**http://www.transplantdb.eu/**) collect and compile sets of variations for different crop plants, like barley, tomato, wheat. Another example is the Rice Genomes Project, which includes rice data derived from more than 3,000 different rice cultivars[120]. A comparative sequence analysis learned that the vast majority of the spontaneously occurring DNA changes in different varieties of the same species are short: depending on the crop, 95% of them are shorter than 10 bases, and 99% are shorter than 17 to 23 bases.

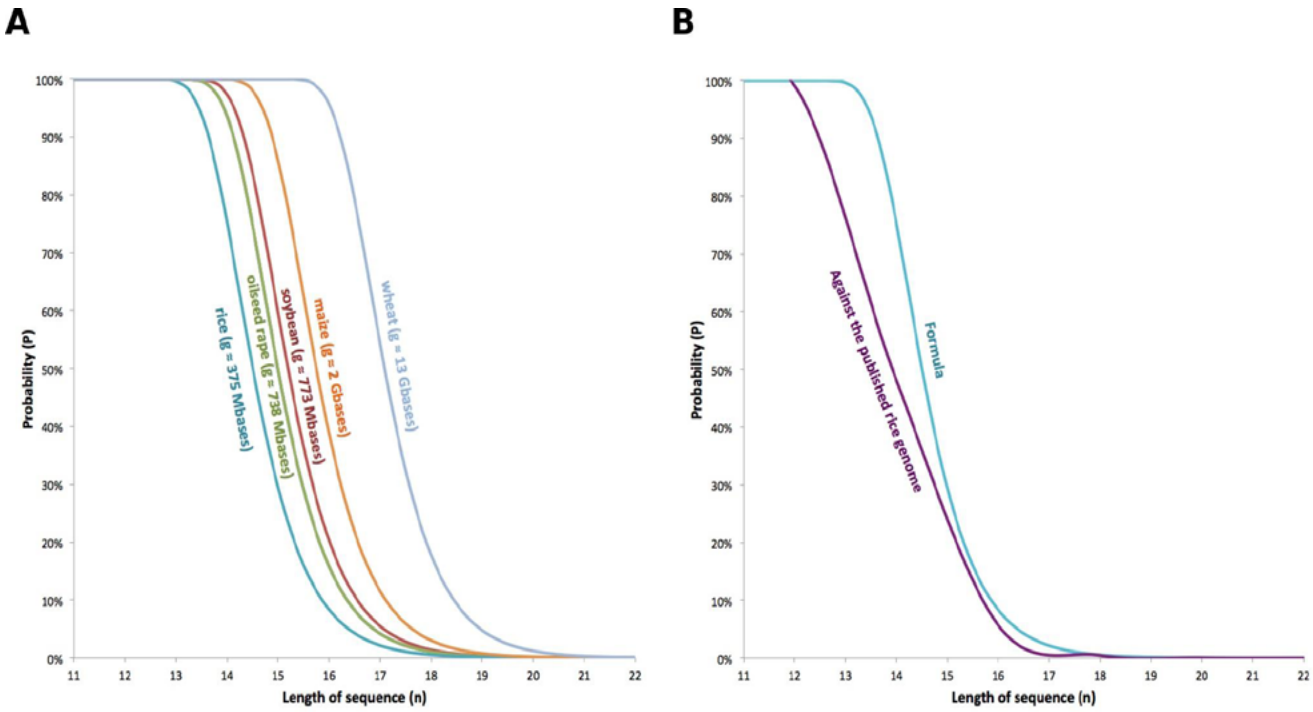

Figure 8. The probability to find a unique DNA sequence in function of the length of the sequence. *Figure adopted from the presentation 'Traceability Issues' by Guy Van den Eede.*

Currently, there is also sequencing data available for crop varieties that were obtained through mutation breeding[121]. The whole-genome sequences of rice plants, a few generations after damaging their DNA with ionising radiations, revealed that deletions are more common and are generally bigger in size than insertions. The majority of the deletions (85%) are 25 bases and less; no insertions larger than 26 bases have been observed.

Detection methods for food and feed from genome-edited crops?

In the explanatory note released by the Group of Chief Scientific Advisors (GCSA) of the European Commission's Scientific Advice Mechanism (SAM)[122], the section on detection specifies the issues related to the detection of small DNA changes, which is considered problematic based on a number of case-by-case studies. The smaller the DNA change, the more difficult it is to comply with detection requirements referring to the minimum performance requirements for analytical methods of GMO testing.

The European Network of GMO Laboratories (ENGL) has reviewed the possibilities and challenges for the detection of food and feed plant products obtained by genome editing[123]. The focus is on applications of genome editing (SDN-1, SDN-2, base or prime editing) that do not contain any inserted recombinant DNA in the final plant. By analogy to the term 'transformation event' used in GMO legislation, it was proposed to use 'genome-edited event' to refer to the altered DNA sequence at a specific location in the genome as a result of genome editing.

They conclude that without prior knowledge it is technically impossible to detect small DNA changes introduced by genome editing and to distinguish genome-edited plants from plants selected for certain spontaneous mutations or plants that are obtained through mutation breeding. Applications of genome editing comprise small deletions, small insertions or single base changes and could also occur spontaneously. Based on the assessment of the probability to identify a unique DNA sequence in a certain plant genome, the size estimate of changes that renders detection problematical is estimated to be smaller than 25 bases.

They conclude that without prior knowledge it is technically impossible to detect small DNA changes introduced by genome editing and to distinguish genome-edited plants from plants selected for certain spontaneous mutations or plants that are obtained through mutation breeding.

In conclusion, evaluation can only be conducted on a case-by-case basis. In that respect, the most critical aspects for consideration include practicability, specificity and sensitivity of the method. Whereas some of these issues may be circumvented during the application for market authorisation of certain genome-edited food or feed products, the lack of traceability would make the control and enforcement of the legislation impossible. This implies that genome-edited organisms have limited marketability if they cannot be distinguished and as such cannot comply with the current regulations.

Impact on the international trade of agricultural commodities

The ruling of the ECJ can result in significant trade disruptions. Products derived from crops that are subject to the EU GMO Directive are not allowed for import into the EU until they have been submitted for approval for import and processing. Furthermore, in most cases these products need to be labelled as GMOs. The problem is that many of the products of genome editing are, without prior knowledge of the type of changes made, technically impossible to trace, which is one of the reasons why the European Council has requested the European Commission to conduct a study in light of the Court of Justice's judgment in case C-528/16 regarding the status of novel genomic techniques under the Union law on the impact of the ECJ ruling.

The regulatory approach for genome-edited crops in Europe is completely out of line with the regulations existing in other continents across the world that have adopted more fit for purpose regulations.

The regulatory approach for genome-edited crops in Europe is completely out of line with the regulations existing in other continents across the world that have adopted more fit for purpose regulations (**Figure 9**). For example, the United States Department of Agriculture (USDA) does not regulate or has any plans to regulate plants that could otherwise have been developed through conventional breeding techniques, as long as they are not plant pests or developed using plant pests[48]. The lack of regulatory harmonisation worldwide will lead to challenges in global trade and in the seed sector, where breeding of parental lines can be performed in Europe and production of seeds in some other parts of the world[124,125].

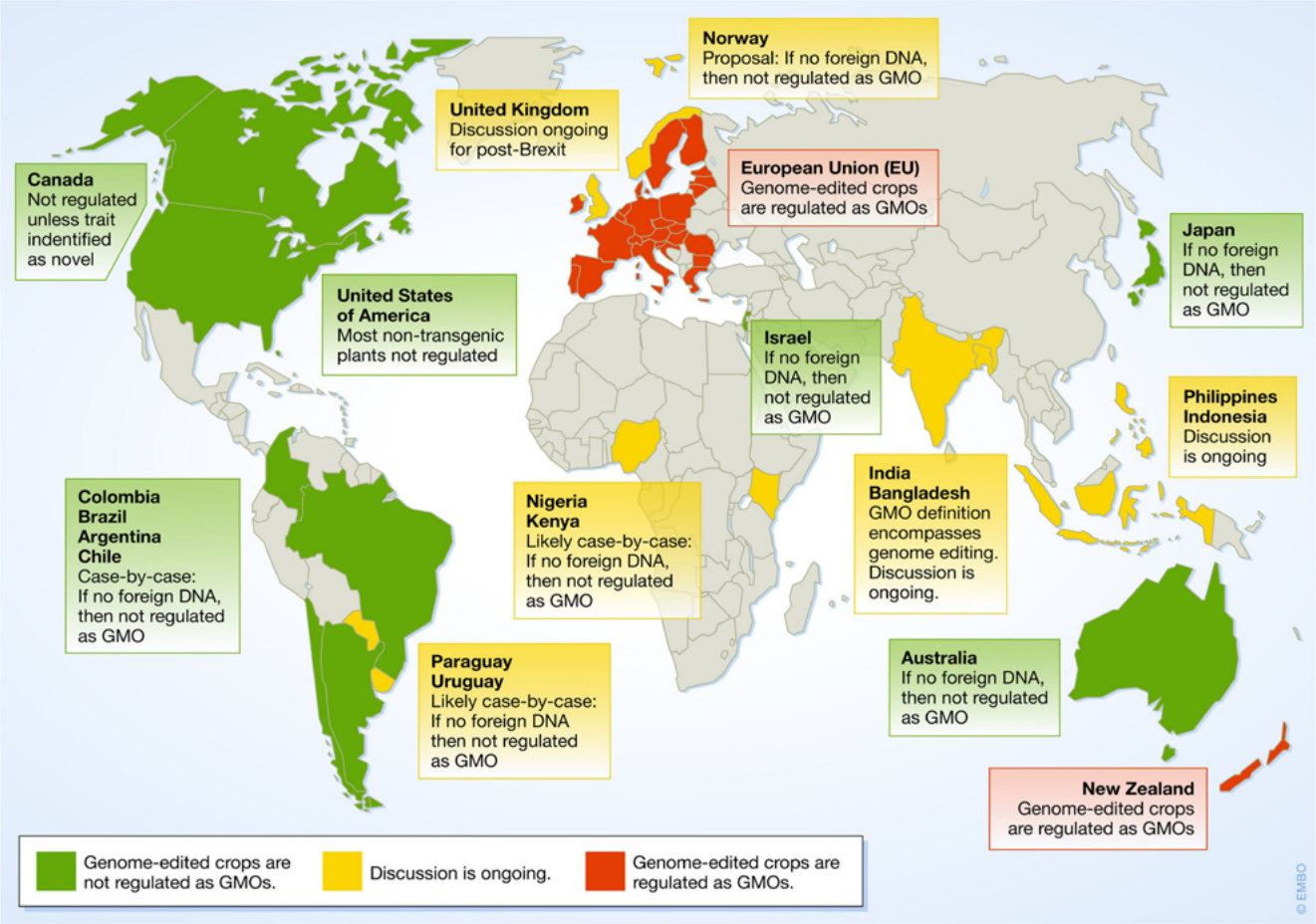

Figure 9. Global overview of regulatory approaches implemented or discussed in different countries for genome-edited crops (status May 2020). *In green: countries that do not regulate genome-edited crops as GMOs; in yellow: countries that started discussions but did not take decisions yet on their policy approaches for plants resulting from genome editing; red: countries in which court rulings interpreted that established GMO regulations also apply to all plants resulting from genome editing, even if these plants are indistinguishable from conventionally bred plants. Figure adopted from Schmidt et al., 2020*[125].

6. Ethical considerations of agricultural applications with genome editing

Decision making in the regulation of the use of new technologies depends on numerous factors, such as technical availability, education of professionals, public perception, adaptation of existing organisations and corporations, and ethical considerations.

Whereas the research contexts are constantly evolving and intrinsically bound to novelty, applications often go through an evidentiary time lag. Between the moment the technology is ready and in principle applicable and the moment that data on issues, actually encountered through its application in reality, are available, there is a time period with lots of uncertainty on how to optimally implement the technology.

To cover this time gap, questions need to be addressed without evidence-based answers. Examples of such questions are: What are good practices? What is the actual advantage of the new technology? What are the most realistic guidelines? What is the best method of sharing experiences? These questions make deciding on a framework to apply novel technologies in practice difficult.

Currently, recommendations and decisions for regulations in domains where genome editing can be applied are not yet stabilised throughout the world. In addition, besides the binding instruments of legal framework, such as national laws and treaties, there are also non-binding instruments to be taken into account, such as professional guidelines, international rules and ethics.

In 2016, the European Group on Ethics in Science and New Technologies (EGE) published a statement on genome editing of human germline and somatic cells[126]. EGE is an independent, multi-disciplinary body which advises on all aspects of European Commission policies where ethical, societal and fundamental rights issues intersect with the development of science and new technologies. In summer 2018, the European Commission requested the EGE to work on an opinion on the ethics of genome editing, which is now in preparation and will cover humans, animals and plants together with policy recommendations.

EGE's domains of concern regarding plant genome editing are traceability, impact on agricultural biodiversity and environment, as well as industrialisation of agriculture. EGE recommends an open, honest dialogue with all stakeholders, including the public, in the decision-making process for introducing genome-edited products into the market, ensuring the veracity of the information is provided to the public.

EGE also acknowledges the statement of the group of Chief Scientific Advisors of the European Commission, recommending revising the current GMO Directive in order to reflect the current knowledge and scientific evidence, in particular on genome editing and established techniques of genetic modification[4]. This should be done in accordance with other relevant legislation covering food safety and environmental protection.

The ethics of genome editing draws considerable attention in EU member states. The opinion published by the Ethics Committee of INRAE (the French National Institute for Research on Agriculture and Environment) provided a perspective on the link between agricultural and environmental considerations[127]. In the statement released by the Danish Council on Ethics, a large majority of the Council members stated that "It is ethically problematic to reject GMO varieties if they can help alleviate or solve significant problems and there are no good arguments for rejecting them"[128]. Another valuable point was raised by the Max Planck Society Ethics Council calling upon politicians "to pursue new and amended legislation that takes into account the differences between conventional genetic modification using recombinant DNA technology and transgene-free genome editing"[129]. In summary, it remains essential to consider the collection of public viewpoints while taking into account a background of knowledge.

In regard to the ongoing discussion on genome editing, it is important to clarify what aspect of the technology is being discussed. When decisions are taken based on claims different from scientific evidence, then it should be clearly communicated for transparency reasons. For this purpose, it is important to disentangle the facts and the values, although it can be difficult. Often scientific decisions can be interpreted as a kind of 'proxy' for other, non-scientific concerns. Because of this, it is even more crucial today to get the scientific facts straight.

There are concerns for example about the safety of genome editing, in particular about the potential of off-target changes that might occur in the genetic blueprint of a plant. This is partially based on misunderstandings and partially on the lack of knowledge. Scientific evidence demonstrated that genome editing is specific and more precise compared to random mutagenesis techniques, and yet it remains important to address this concern about the safety of genome editing. Until now, many articles have been published, which reported on off-target changes of genome editing in mammalian and human cell lines and these raised a lot of questions. These studies are still often referred to in the context of genome-edited applications in plants but is this relevant in the agricultural context? Many studies in plants show that there are no or very little off-target mutations in genome-edited crops[72,130]. Furthermore, breeding by itself inevitably creates a mixing of the genetic blueprints of the parents, causing much more elaborated changes than the small modifications introduced by genome editing.

From a scientific point of view, it is important to highlight that scientists aim to further improve the predictability of genome editing, although this can be wrongly interpreted and perceived by the public as unsafe.

Another concern is for example that genome-edited plants are perceived as unnatural. Humans have made use of the natural genetic variation since the beginnings of agriculture and they always have selected for the traits that were beneficial and suitable for them. In this regard, all food products we consume today can be considered unnatural. On the other hand – from a scientific point of view – mutations can occur spontaneously in nature, so they would have to be considered natural. This disagreement can be explained because 'naturalness' is related to values as well: 'natural' is often associated with something positive, whereas 'unnatural' is associated with something negative.

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These examples illustrate that it remains important to disentangle the facts and the values of a certain technology. Public participation should be incorporated into the policy-making process for genome editing and should include ongoing monitoring of public attitudes, informational deficits, and addressing concerns about certain applications of genome editing.

7. Misconceptions about genome editing

In order to translate advancements of scientific knowledge into innovations, four fundamental ingredients are needed: high-quality research, an innovation system that enables a seamless transition from research to the production system, a regulatory corpus that allows innovations to arrive on the market without unnecessary restrictions and/or constraints, and finally the consumers' acceptance and uptake of the innovations proposed by the production system.

Today, in European agriculture, genetic innovation reaches the market only with great difficulties both due to regulatory constraints and a lack of acceptance by proponents of bio-agriculture. The most commonly accepted method of crop improvement is currently based on crossing of existing varieties, including crosses with wild relatives and crosses with plants that have been obtained through mutation breeding. The negative perception of genetic innovation in agriculture is mainly based on two simple aspects both founded on logical fallacies: the first is "old equals good, new equals bad", and the second is "natural equals good, artificial equals bad".

In order to change parts of the public's negative perceptions of food produced from genome-edited crops, it is necessary to increase the global understanding of the complexity of the food production systems. Technological improvements in agriculture over the last centuries have led to increasing productivity, reasonable prices of food products and safeguarding of high-quality food standards. Agriculture is intrinsically linked to scientific progress and access to innovative technologies that facilitate transferring the progress from laboratories to the dinner table. In order to solve the problems of environmental, economic and social sustainability of agriculture, it is essential to embrace the path of scientific innovation.

A large part of the public is generally not aware of the role of technological innovations in agriculture to contribute to economic and social wellbeing and that progress in agriculture will help us to better cope with climate adversities.

As fewer and fewer people work in the primary sector and are thus further removed from the production of their food, a romanticised vision of agriculture is growing in many European countries as a result of a distorted understanding of the agricultural system. The agricultural system is a fundamentally man-made and artificial system, not a natural ecosystem and as such does not follow the laws of natural evolution but those of artificial selection. The agricultural environment changes much faster than a natural environment would and the cultivated varieties must continually adapt to new growth conditions and new threats. This makes it necessary to continuously select new varieties.

To make consumers aware, it is important to communicate the role of technological innovations in agriculture through evocative narratives instead of explaining the technicalities and possibilities of the technology itself.

To make consumers aware, it is important to communicate the role of technological innovations in agriculture through evocative narratives instead of explaining the technicalities and possibilities of the technology itself. For example, genome editing has the potential to protect regional food traditions and to favour diversification. This can be illustrated with an example on wine production with traditional varieties in Italy. Wine industry is the most profitable area of agriculture in Italy[131]. However, it suffers from a major sustainability problem, *i.e.* it requires large amounts of chemicals to safeguard the yield. An example of such a traditional variety is Sangiovese, a type of grape used for the production of red wines such as Chianti and Brunello in Tuscany. Classical breeding for disease-resistant Sangiovese is hardly feasible without losing the characteristics of this traditional variety. However, genome editing could enable to maintain the traditional Sangiovese variety and proof it against fungal diseases such as powdery mildew.

Genome editing is easy to use, cost effective and time efficient. Many researchers in Europe have the expertise and infrastructure to explore the potential of this technology. In the case of the Sangiovese variety, genome editing can be employed to inactivate susceptibility genes, resulting in resistance to powdery mildew. The main advantages are that all characteristics of the Sangiovese variety will be preserved, while for example the need to apply chemicals will be reduced. Inactivating susceptibility genes by genome editing has been successfully used for powdery mildew resistance in wheat and resistance to the devastating bacterial blight disease in rice[79, 98, 132].

The resistance to embracing novel methods in plant breeding leads us where we have arrived today in viticulture: centuries-old grapevine varieties are no longer able to defend themselves from fungal pathogens, requiring the use of large quantities of chemicals to protect them. By continuing to use these outdated practices, agriculture cannot become more sustainable. To achieve better sustainability and to reduce the usage of chemicals, access is needed to the most advanced technologies enabling the improvement of existing varietal heritage and increasing the ability to respond to new challenges of changing environments. At the same time, these new technologies may contribute to a reduction of the environmental footprint of agriculture.

8. Questions of intellectual protection related to genome editing

Genome editing is a promising technology both for medical and plant related applications. Under European patent law, plant varieties and essential biological processes for the production of plants such as crossing or selection are not patentable[133]. The rationale is to exclude from patentability the plant breeding processes, which contain conventional methods of plant breeding, in particular those based on the sexual crossing of plants and the subsequent selection of the plants having the desired trait(s)[134]. Since 1961, an International Union for the Protection of New Varieties of Plants (UPOV) provides a *sui generis* form of protecting the intellectual property rights of plant breeders alternative to patents that is widely used in many agricultural sectors[135].

Inventions relating to plants are however patentable to the extent that the technical feasibility of the invention is not limited to a specific plant variety. Moreover, plants that are obtained by an essentially biological process are not necessarily excluded from patentability on the ground of the following reasoning: if a process of sexual crossing and selection includes within it an additional step of a technical nature, which itself introduces a trait into the genome or modifies a trait in the genome of the plant produced, then that process is not excluded from patentability. This is with the prerequisite that the introduction or modification of that trait is not the result of mixing of the genes of the plants chosen for sexual crossing[134].

Research has shown that Europe is no longer a frontrunner when it comes to innovation in plants, and hence also not in patent applications related to genome editing.

Genome-edited plants can in principle be patented to the extent that technical feasibility of the invention is not limited to a specific plant variety. The patent landscape relating to genome editing and plants in Europe comprises a plethora of patents and patent applications, which are normally not within the realm of conventional breeding and are equally not considered essentially biological processes. Most of those patents and patent applications, in particular the ones filed early, *i.e.* around 2013, cover multiple applications: human, animal and plant. Many of those patents and patent applications are currently still under opposition or appeal at the European Patent Office (EPO) and the legal outcome is not certain at this time. It is generally expected that a wide range of patents with applications to plants will eventually be granted. The applications of genome editing range from mutagenesis to gene correction.

It is plausible that for instance a genome-edited plant contains a DNA change that could conceivably also occur spontaneously. To that extent, it cannot be excluded that genome-editing patents may also cover plants that resulted from (random) mutagenesis. That could present evidentiary issues, as a genome-edited plant could be identical to a plant selected for a spontaneous mutation or as a result of mutation breeding.

Most of the objections, apart from the current technical legal objections against many of the patents and patent applications, will likely be in the area of human applications. In regard to plant applications, there are currently not many objections raised in the pending cases. However, in the near future, objections could be raised. The consequences for future generations in the long-term use of genome-edited plants cannot be assessed, as this largely depends on the future developments in the economic and legal framework.

The patent landscape relating to genome editing and plants in Europe also needs to be examined in the context of the current regulatory aspects of genome-edited products in Europe. The basic principle is that regulatory issues and patent law are entirely separate. Patents can be granted, but there may be issues with releasing commercial products to the market in Europe, as the ECJ ruling (case C-528/16) is interpreted by the European and EU member state authorities to mean that genome-edited crops are subject to the provisions of the GMO Directive[2].

What are the consequences of the judgment for the intellectual property landscape? Patent protection in the EU remains – at least for the moment – possible and open to applicants from across the world. Research has shown that Europe is no longer a frontrunner when it comes to innovation in plants, and hence also not in patent applications related to genome editing[136]. Legal uncertainty surrounding the patentability of plants in general combined with regulatory limitations relating to GMO plants reduces Europe's appeal to be an attractive place for plant-related innovation.

Guaranteeing broad access to the technology avoids that the technology would only benefit a handful of large, multinational corporations.

As a consequence, the number of patent applications in European countries related to genome editing is small compared to the US and China (**Figure 10**). It is rather unfortunate that the large majority of innovation in this area is in the hands of US universities who have licenced the technology exclusively to a selection of commercial enterprises[137]. This might cause more concentration of intellectual properties and accompanying price controls with a limited number of multinational players.

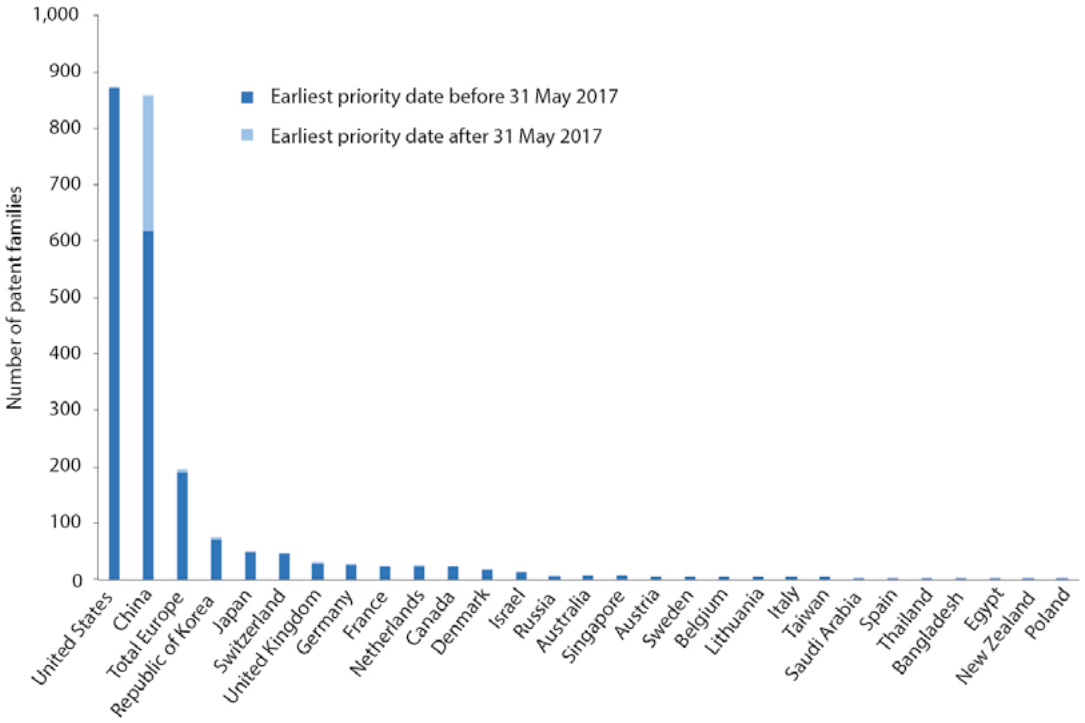

Figure 10. Number of patent families related to the CRISPR-Cas system per country. *Values correspond to the total amounts of patent families. 'Total Europe' comprises European countries. Dark blue indicates patents with a priority date up to 31 May 2017. Light blue indicates additional patents publicly available at the date of last update (priority date up to 31 December 2017). Figure adopted from Martin-Laffon et al., 2019[136].*

In summary, patent protection for genome-edited plants is possible, but it cannot be excluded that in the future some ethical objections may be raised. These are likely to be at least partly overlapping with those identified for transgenic plant (thus plants containing DNA foreign to the species) patents. It indicates that genome editing brings new and exciting developments in plant breeding, but may open the discussion around the patentability of plants all over again, even more so as we currently witness a rather volatile and uncertain statutory and case law framework for inventions related to plants.

In the meantime, most of the innovation in the area of genome editing takes place outside of Europe, and the current legal uncertainty disincentivises investment into genome-editing research. The current regulation makes cultivation and marketing of genome-edited plants very difficult in Europe, but it has no

effect on patentability. It is vital to ensure that SMEs are also able to benefit from the use and application of genome editing. The threshold for SMEs to introduce genome-edited crops on the market should remain as low as possible. Guaranteeing broad access to the technology avoids that the technology would only benefit a handful of large, multinational corporations.

9. Policy options for genome editing of crops

The ruling of the European Court of Justice (ECJ) in case C-528/16 is interpreted by the European and EU member state authorities to mean that genome-edited crops, whatever the DNA change or edit introduced, are subject to the provisions of Directive 2001/18/EC (the GMO Directive)[2,3]. Based upon two decades of experience with the market introduction of GMOs, one must conclude that regulating genome-edited organisms as GMOs *de facto* blocks the development and market introduction of such crops in Europe, in particular for cultivation in Europe.

There are different policy options for the European Union to address the current situation:

a) The null scenario: do nothing;

b) Exploit existing mechanisms in the EU GMO legislation;

c) Introduce a limited change to the EU GMO legislation;

d) Elaborate a more thorough revision of the EU GMO legislation.

a. The null scenario: do nothing

When the EU does not act, the current situation would extend infinitely. This would mean that the development and market introduction of any genome-edited crop would continue to be *de facto* impossible. It would also mean that the current enforcement problems – crops with small edits cannot be detected – would continue. The current situation is also likely to result in trade disruptions, because in other parts of the world genome-edited crops will be further developed and released on the market. Those products may become part of exports to the EU and may even enter EU territory undetected. Doing nothing has too many downsides and is therefore not a real option.

b. Exploit existing mechanism in the EU GMO legislation

The GMO Directive contains in Article 7 the possibility to introduce differentiated procedures for certain GMOs resulting in less elaborate dossier and risk assessment requirements for those GMOs. This article however can only be applied to GMOs for which sufficient experience of releases in certain ecosystems has been obtained and if the GMOs meet specific criteria. Differentiated procedures can therefore not be applied to genome-edited organisms from the beginning. The application of Article 7 would also imply that all genome-edited organisms remain considered as GMOs, for which labelling is required and maintain the current discriminatory situation in which an organism with a specific mutation generated using modern tools would be treated differently than an organism with that same mutation obtained through conventional methods. It would also not solve the enforcement problem. And differentiated procedures will only work when the EU member states want it to work and do not vote in the same politically inspired manner as they do for GMOs today.

c. Introduce a limited change to the EU GMO legislation

Another policy option is to introduce a limited change to the EU GMO legislation that would align the scope of the EU GMO legislation with the legislation in other major nations in the world. Such a limited change would impose organisms in which alterations have been introduced that can also spontaneously arise in nature or be the result of conventional breeding activities, outside the legislative scope. The scope of the EU GMO legislation would then conform to the scope of the Cartagena Protocol on Biosafety to the Convention of Biological Diversity. It is important to realise that when the EU implemented the Cartagena Protocol in

2003, it did not alter its GMO definition based on the judgement that the differences between the GMO and LMO definitions did not have operational consequences. Today, we must conclude that the differences do have important operational consequences, which cannot be ignored. A small alteration of the EU GMO Directive would also solve the detection and enforcement problem and prevent disruptions of international trade.

Technically there are different legal options to narrow the legislative scope:

1. Alter the EU GMO definition in Article 2 of the EU GMO Directive to align it with the LMO definition of the Cartagena Protocol on Biosafety.

2. Add to Annex 1A part 2 of the EU GMO Directive certain forms of genome editing that are considered not to result in genetic modification. Basically, it would be those forms of genome editing that introduce alterations that can also spontaneously arise in nature or be the result of conventional breeding activities.

3. Introduce into Article 2 of the EU GMO Directive a definition of mutagenesis that would also encompass the modern, targeted forms of mutagenesis.

4. Add to Annex 1B of the EU GMO Directive modern, targeted forms of mutagenesis.

The advantage of options 1 and 2 is that these changes would be a full harmonising measure, whereas in options 3 and 4 EU member states would still be able to introduce national regulatory requirements for those organisms, up to the level of what applies to GMOs. In addition, any change to the scope of Directive 2001/18/EC would also have to be introduced to Directive (EU) 2009/41 on the contained use of genetically modified micro-organisms.

It is important to realise that a proposal to revise the EU GMO Directive to introduce a limited change, does not guarantee that such a limited change will be realised. Once such a regulatory proposal is launched by the European Commission, it is open for discussion and amendment by the European Parliament, and one cannot predict the outcome. The current international context should act as a guide to prevent the regulatory discussions going in all kinds of different directions and help make sure that the end-result is in harmony with the situation in other major nations in the world[124,125]. The scientific community and other important stakeholders, such as the seed sector, are very much in favour of introducing such a limited change to align the EU GMO legislation.

d. Elaborate a more thorough revision of the EU GMO legislation

There is a growing consensus among scientists that the current EU GMO Directive is no longer in line with our scientific understanding. Over the years, it has become apparent that it is not the use of a certain technique that will determine the safety of an organism, but the genetic and phenotypic characteristics of that organism. The current legislation is considered to be too much process based. A risk-based approach based on the product characteristics is considered more appropriate. The difficulty with trying to elaborate a more thorough revision of the GMO legislation is that it would expose the whole GMO Directive for a much more fundamental discussion. Such a discussion will be very difficult and lengthy, and it will be extremely hard to predict in what type of regulatory regime it would result. One should also realise that it is very difficult to come up with a regulatory system that would not risk bringing into scope the majority of conventionally bred organisms, a class of organisms considered to have a history of safe use. Another point with this option is that there is a genuine risk that it would end up in a regulatory system that again would not be in harmony with the regulatory systems in other parts of the world. Any discussion on a more elaborate revision of the GMO regulatory framework should therefore not be rushed but rather be a long-term endeavour. Europe should not undertake this endeavour alone. This must be discussed at a higher international level.

10. Concluding remarks

The symposium on 'Genome Editing for Crop Improvement' organised by KVAB and ALLEA gathered in Brussels a number of professionals from different disciplines interested in discussing possible ways that would allow Europe to go further with the applications of genome editing in crop plants. The scientific data that was presented during the symposium confirmed that the new methodologies based on the precise targeting of molecular editors to the genetic blueprint are useful to generate new traits in crop plants that may allow facing important challenges in agriculture. They are being widely adopted for research and in some non-European countries, genome-edited crop plants have entered breeding programs and field trials are being carried out. The central questions that were discussed in the symposium explored how Europe may be able to profit from the possibilities offered by these technologies in the framework of existing regulations and particularly after the ruling of the European Court of Justice (Case C-528/16). From the presentations and the discussions held during the symposium we may conclude:

1. The genome editing techniques allow precise targeting of specific DNA sequences in the genomes of a variety of crop plants. The methods are continuously being improved, and it has been shown that questions related to off-target changes or the presence of foreign DNA sequences can be solved by using specific methods of delivery of RNA and proteins or by eliminating them during the process of breeding of specific varieties.

2. The information available shows that the techniques allow accelerating the process of plant breeding and that it is being widely adopted in public and private research. It offers an increasing collection of genetic solutions for the problems faced by agriculture in Europe and worldwide.

3. The new technologies face societal and ethical questions similar to other new approaches related to food production. This is a very sensitive issue particularly in Europe. From this point of view, it appears that priorities for the applications of new technologies are those related to food security, food safety and sustainability of food production.

4. Influential sectors of European society are not aware of the value of innovation in agriculture, including the one needed for preserving traditional varieties. A narrative for European food production that includes the importance of innovative, more efficient approaches in the whole value chain could be necessary.

5. The present legal framework on GMOs, if applied to genome-edited plants in a strict manner, will face problematic outcomes related to the distinction of the mutations produced by editing from spontaneous mutations or other means of mutagenesis. Traceability and labelling of food products derived from genome-edited plants, as mandated by present European regulations, will also be very problematic.

6. European regulations, as they are presently enforced in the case of GMO plants, result in a heavy economic burden for those applying for the approval of new varieties. This cost will become disproportional if applied for the approval of genome-edited crop plants. It may create a significant competitive disadvantage for European public and private plant breeders.

7. The intellectual property issues related to genome-edited genes and plants containing edited genes have to be solved taking into account the present legislation and particularly international agreements that have been specifically devised to protect new plant varieties.

8. European research institutions and academies have repeatedly called for support towards the use of biotechnological approaches to solve questions raised by agriculture. An appropriate framework of risk assessment based on a scientific analysis of the products obtained may be required.

9. The European Union may find itself in an impasse after the ruling of the European Court of Justice regarding genome-edited plants. All possible alternatives have to be explored: seeking further clarification of the ruling; exploiting existing mechanisms in EU legislation; introducing minor changes in EU legislation or a complete revision of GMO legislation. To do nothing seems not an option for Europe in the present situation.

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List of figures

List of tables

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ANNEX 1. Abbreviations

AFBV	Association française des biotechnologies végétales
APHIS	Animal and Plant Health Inspection Service
ALLEA	All European Academies
BE	base editing
CCC	Consumer Choice Center
CRISPR-Cas	clustered regularly interspaced short palindromic repeats-associated systems
DNA	deoxyribonucleic acid
EASAC	European Academies Science Advisory Council
ECJ	European Court of Justice
EFB	European Federation of Biotechnology
EFSA	European Food Safety Agency
EGE	European Group on Ethics in Science and New Technologies
EMS	ethyl methane sulfonate
ENGL	European Network of GMO Laboratories
EPO	European Patent Office
EPSO	European Plant Science Organisation
EU-SAGE	European Sustainable Agriculture through Genome Editing
EURL GMFF	European Reference Laboratories for GM Food and Feed
FAO	Food and Agriculture Organization of the United Nations
GCSA	Group of Chief Scientific Advisors
GM	genetically modified
GMO	genetically modified organism
HR	homologous recombination

IAEA	International Atomic Energy Agency
indels	insertions and/or deletions
INRAE	French National Institute for Research on Agriculture and Environment
JCR	Joint Research Centre of the European Commission
KVAB	Koninklijke Vlaamse Academie van België voor Wetenschappen en Kunsten
LMO	living modified organism
MLO	Mildew resistance Locus O
NHEJ	non-homologous end joining
NPBT	new plant breeding technique
ODM	oligonucleotide-directed mutagenesis
OGTR	Office of Gene Technology Regulator
PE	prime editing
PNT	plant with novel traits
PVY	Potato Virus Y
RNA	Ribonucleic acid
gRNA	guide RNA
SAM	Scientific Advice Mechanism
SDN	site-directed nuclease
SME	small and medium-sized enterprise
TALEN	transcription activator-like effector nuclease
USDA	United States Department of Agriculture
VIB	Vlaams Instituut voor Biotechnologie
VInv	vacuolar invertase gene
ZFN	zinc-finger nuclease

ANNEX 2. List of statements on genome editing

Statements on genome editing

Scientific community:

» Letter to the European Commission by **EU-SAGE**

"Europe cannot afford to miss out on the important opportunities that genome editing offers for sustainable agriculture and food production. Strong political signals of commitment to solve the current regulatory deadlock are necessary to prevent irreversible damage to our European economy and to the transition to a green economy"

» Statement by the **Group of Chief Scientific Advisors (GCSA)** - A Scientific Perspective on the Regulatory Status of Products Derived from Gene Editing and the Implications for the GMO Directive

"...in view of the Court's ruling, it becomes evident that new scientific knowledge and recent technical developments have made the GMO Directive no longer fit for purpose."

"...we recommend revising the existing GMO Directive to reflect current knowledge and scientific evidence, in particular on gene editing and established techniques of genetic modification. This should be done with reference to other legislation relevant to food safety and environmental protection.

» **European Plant Science Organisation** (EPSO) - Statement on the ECJ Ruling regarding mutagenesis and the Genetically Modified Organisms Directive

"The ruling of the ECJ presents a considerable drawback for the future of innovative plant science and its societal benefits in Europe."

"...EPSO supports a science-based revision of the present European

legislation establishing a more proportionate product-based risk assessment."

» **German National Academy of Sciences Leopoldina and the German Research Foundation** (DFG) - Towards a scientifically justified, differentiated regulation of genome edited plants in the EU

"...the science academies and the DFG see an urgent need to reassess the products of the much more precise and efficient methods of genome editing and to amend European genetic engineering law."

» **European Academies Science Advisory Council** (EASAC) - The regulation of genome edited plants in the European Union

EASAC reaffirms the importance of exploring radical reform and urges the EU Institutions to explore the options recommended by Leopoldina *et al.* (2019)[8] and others:

- First, to revise the GMO definition/exemptions to enable the EU to capitalize on the plant breeding opportunities afforded by genome editing.

- Secondly, to develop a new legal framework to focus on traits not processes."

» "Gene editing regulations: A position paper from the **European Federation of Biotechnology** (EFB)

"The European Federation of Biotechnology regrets this ruling because it ignores scientific arguments that the interpretations of the technologies are scientifically inaccurate."

European seed sector:

» **Euroseeds** position paper - Plant Breeding Innovation Applying the latest Plant Breeding Methods for the benefit of sustainable Agriculture, Consumers and Society,

"ESA (European Seed Association) considers that the consequences of this ruling present unacceptable socio-economic risks for European plant breeding, for the wider agri-food chain, for consumers and for our European environment."

"The ECJ ruling shows that the existing GMO legislation no longer reflects current knowledge and scientific evidence. ESA therefore encourages Commission to apply the above-mentioned criteria and update the EU's current regulatory framework accordingly."

European farmers and agri-cooperatives:

» **Copa Cogeca** - NBTs are not a luxury but an urgent necessity for the vitality of the whole EU farming model

"...last year's ruling by the European Court of Justice is already having serious repercussions on the strategy of European breeders."

"New Breeding Techniques (NBTs) should be a priority within the Work Programme of the new Commission when it comes to agriculture. For Copa and Cogeca, it is now a matter of urgency that a real European strategy regarding these highly promising techniques is put in place, as they would ensure that our farming model is able to adapt to both the early effects of climate change and fierce international competition."

European Advisory Committees on Biosafety:

» **Advice of European Advisory Committees on Biosafety**

"It was agreed that an improved regulation is needed which focuses more on the result of the genetic modification than on the way this modification has been achieved. An adaptation should take into account the decades-long national and international experience with genetic engineering gained so far, the similarity of products derived from natural, classical and targeted mutagenesis, and the practical availability of tools for law enforcement and control."

Consumers:

» **Consumer Choice Center** (CCC) - Letter to Commissioner Kyriakides

"The European Union has traditionally objected most innovations in food science and prevented European consumers from accessing biologically-enhanced food. This can be seen in the very limited number of genetically modified crops authorized for cultivation in the EU, and a very cumbersome and expensive process of importing genetically modified food and a recent European Court of Justice ruling on treating gene editing as restrictive as GMOs."

Ethical perspective:

» The Danish Council of Ethics - GMO and ethics in the new era

The Council provides recommendations on the question of whether it would be ethically problematic to reject GMOs with beneficial traits provided they are not assessed as posing a higher risk to humans or the environment than similar varieties developed by conventional methods. The Council's opinion moreover implicates recommendations for a change of the EU's authorization system for GMOs and other plants with new traits."

ANNEX 3. Programme of the ALLEA-KVAB symposium 'Genome editing for crop improvement'

7 November

Welcome and Introduction:

Professor Karel Velle, President KVAB

Professor Hubert Bocken, Vice-President ALLEA

Professor Pere Puigdomènech, ALLEA/CRAG

Keynote Speech: Genome editing in different domains: same or different issues? How science and ethics interplay

Professor Anne Cambon-Thomsen, CNRS

Session 1: Genome Editing in Science and Agriculture

The science behind genome editing
Professor Sjef Smeekens, Utrecht University

What can genome editing deliver for agriculture?
Professor Stefan Jansson, Umea Plant Science Centre

Session 2: Genome Editing in International Trade and Society

Traceability issues
Dr Guy Van den Eede, European Commission Joint Research Center

Societal considerations related to agricultural applications of genome editing
Professor Michele Morgante, Laboratory of Plant Genomics, University of Udine

Closing Address

Hilde Crevits, Viceminister-president of the Flemish Government and Flemish minister for Economy, Innovation, Labor, Social Economy and Agriculture

8 November

Session 3: Legal and Regulatory Aspects

Intellectual Property law and genome editing of crops
Professor Sven Bostyn, Centre for Advanced Studies in Biomedical Innovation Law, University of Copenhagen

Risk assessment and regulation of genome-edited crops
Dr Fabien Nogué, INRA Center of Versailles

Breaking the Impasse: a Governance Framework for Gene Editing with Plants
Dr Michelle Habets, Rathenau Instituut

Session 4: Round Table Policy Options for the Legislator

Discussants:

René Custers, VIB

Georges Van Keerberghen, Boerenbond

Wouter Vanhove, Groen

Alain Deshayes, AFBV

David Hamburger, University of Passau

Summary and Conclusion

Closing Reception

For more information, please visit: https://allea.org/genome-editing-for-crop-improvement-symposium/

About ALLEA

ALLEA is the European Federation of Academies of Sciences and Humanities, representing more than 50 academies from over 40 EU and non-EU countries. Since its foundation in 1994, ALLEA speaks out on behalf of its members on the European and international stages, promotes science as a global public good, and facilitates scientific collaboration across borders and disciplines.

Academies are self-governing bodies of distinguished scientists drawn from all fields of scholarly inquiry. They contain a unique human resource of intellectual excellence, experience and multidisciplinary knowledge dedicated to the advancement of science and scholarship in Europe and the world.

Jointly with its members, ALLEA seeks to improve the conditions for research, to provide the best independent and interdisciplinary science advice available, and to strengthen the role of science in society. In doing so, ALLEA channels the expertise of European academies for the benefit of the research community, decision-makers and the public. Outputs include science-based advice in response to societally relevant topics, as well as activities to encourage scientific cooperation, scientific reasoning and values through public engagement.

ALLEA is constituted as a non-for-profit association and remains fully independent from political, religious, commercial or ideological interests.

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CONTACT US

ALLEA | All European Academies
Jägerstraße 22/23
10117 Berlin
Germany

+49 (0)30-3259873-72
secretariat@allea.org
www.allea.org
@ALLEA_academies