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Genome-edited crops and 21st century food system challenges

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ABSTRACT

The international food system is facing important challenges and must become more sustainable. Plant breeding can contribute to this, for instance, by developing crop varieties that require fewer inputs.

Recently, genome editing has been added to the plant breeders' toolbox. Genome editing enables the targeted alteration of a few DNA letters within the existing genetic blueprint of an organism. The most widely used genome-editing tool is CRISPR Cas, because it is easy to use, affordable and versatile.

The types of alteration introduced using CRISPR-Cas do not differ from the types of alteration (natural or induced) selected by conventional breeding, apart from when used to integrate genetic material that is foreign to the plant's gene pool.

In many countries worldwide, specific types of genome-edited crops are not subject to GMO legislation. In the EU, however, organisms developed with new genomic techniques are not exempt from those regulations. The worldwide adoption of genome editing in plant breeding requires the EU to determine which type of regulatory framework is warranted for genome-edited crops.

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Executive summary

Our food and agricultural system is facing major challenges, and not least among them is climate change. Crops are at the centre of the food system and, through plant breeding, it is possible to develop crops with increased resistance to environmental pressures, such as disease and climate stress. The EU's farm to fork strategy sets specific goals for the production of safe and healthy food and aims to reduce the use of chemical pesticides and fertilisers.

Crops can be improved by using various breeding technologies that have been developed over time. Plant breeding is about the introduction of genetic variation and the ability to develop and select plants with desired characteristics in an efficient manner. One such tool, recently added to the plant breeders' toolbox is genome editing. Genome editing is the targeted and deliberate addition, deletion, substitution and translocation of DNA letters within the existing genetic blueprint of an organism. The technique builds upon knowledge and understanding of the role and function of specific genes in a crop. When such knowledge is available and a desired trait can be achieved by a targeted change, genome editing is a more efficient way to introduce that change, compared with other breeding technologies. CRISPR-Cas is currently the most widely used genome-editing tool and has been adopted in crop research and development worldwide.

CRISPR-Cas genome-editing technology can be applied in different ways. The genetic changes that are introduced by means of the SDN1 and SDN2 types of CRISPR-Cas technology do not differ from changes that can occur naturally or result from conventional breeding. This also means that, without prior knowledge, it is not possible to determine whether the genetic change is the result of genome editing. This means that once genome-edited products are released from the lab it is challenging to trace them through internal markets or across external borders.

The genome editing that is used to introduce changes that can also occur naturally differs from transgenesis, in which foreign DNA is inserted into the genome of an organism. Transgenic organisms are easily detected because transgenesis creates a unique genetic signature.

CRISPR-Cas technology is highly precise, but 'off-target mutations' do occur rarely. The application of appropriate molecular characterisation of the genetic changes allows the selection of only those plants that have the desired genetic changes.

Genome editing is used for a diverse range of crops and characteristics, and the first genome-edited crops have already been introduced onto the market in the US and in Japan. Currently, genome-edited crops are regulated differently around the world. In North and South American countries, and also in countries such as Australia, India and Japan, specific applications of genome-edited crops are not subject to legislation on genetically modified organisms (GMO). In the EU, however, organisms developed with new genomic techniques (NGT) are not exempt from those regulations. In the EU, it is difficult to obtain authorisation for the cultivation of a GM crop. Currently, only multinational companies can afford to market GM crops and deal with the regulatory hurdles.

In the EU, plants – not varieties – can be protected by a patent if the plant has resulted from a patented invention. Plant characteristics resulting from the application of genome editing can also be protected by a patent, even when the edit could also have occurred naturally. However, in such cases there must be a clear disclaimer in the patent application that the characteristic has been obtained using a method that is not 'essentially natural'.

There is virtually no threshold for the application of genome editing in research. However, the landscape of patents and patent applications on different components of genome-editing

technology, derived tools and their applications is complex. Getting access to intellectual property to use genome editing in agricultural crops for commercialisation may create a certain threshold.

The EU has among the highest standards in the world when it comes to protecting human health and the environment. Whether a genome-edited crop presents a safety risk is predominantly determined by the genetic and phenotypic characteristics of the resulting crop. In order to present a risk, the crop must have a concrete functional property that has the potential to result in harm. Whether that property was introduced by a conventional technology or modern genetic technology does not make a difference for the resulting property and its potential to result in harm.

In the EU, when a crop is not subject to GMO legislation, there is no authorisation procedure that would require a pre-market safety assessment, unless a 'non-traditional propagating practice has resulted in significant changes in the composition or structure of the food affecting its nutritional value, metabolism or level of undesirable substances'. In that case, the crop would fall within the scope of Regulation (EU) 2015/2283 on novel foods, which includes an authorisation procedure subject to a food safety assessment.

Irrespective of the regulatory approach taken, all actors that introduce a genome-edited crop into the EU territory are liable under the Environmental Liability Directive (Directive 2004/35/EC) should that introduction result in damage to protected species and natural habitats, to water or to land.

Views on the benefits and risks associated with different applications of genome editing in crops diverge. There are questions regarding the status of new genomic techniques such as CRISPR-Cas in comparison with genetically-modified crops. Furthermore, there are concerns that the precise and targeted nature of the changes made with CRISPR-Cas will lead to difficulties in detectability and traceability once genome-edited plant varieties enter the market. This could also lead to difficulties in the practical implementation of co-existence policies, in establishing potential patent infringements, and in international trade with countries that have different regulatory frameworks. For legislation to be enforceable, there is a need for robust detection methods that provide certainty as to the origin of genetic alterations. However, this is problematic for specific types of genome-edited crops.

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1. Introduction

We are facing important global challenges as we move further into the 21st century. Climate change being a pressing one. The growing world population and changing consumption patterns weigh on the sustainability of our food systems. Technologies have played a role in bringing the food systems to where they are today. But what can and will emerging technologies bring to address challenges of the future?

This paper aims to provide state-of-the-art information about genome-editing technology. Moreover, given the current regulatory developments the EU, risks and other relevant aspects for policy-making are also considered.

Plants are at the centre of our food systems. It all starts with a crop in a field, in a greenhouse, on a rooftop or in a backyard. Agricultural crops have evolved over many millennia to become what they are now and over time breeding technologies have played an increasing role. Recently, 'gene editing' or 'genome editing' has been added to the plant breeders' toolbox.

Humans have been domesticating plants since the dawn of civilisation. They assessed the value of plants and their products by how they looked, smelled, tasted. They learned which plants are nutritious, safe to eat, and which plants to avoid. Plant domestication started thousands of years ago by farmers who selected plants with desired characteristics in a field. Unknowingly, they selected plants in which genetic changes had occurred that resulted in desirable characteristics such as increased yield or resistance to diseases. Genetic changes or alterations - also referred to as mutations - are changes in the heritable material of an organism. Spontaneous genetic changes occur in each generation of every living organism, including humans, and drive evolution.

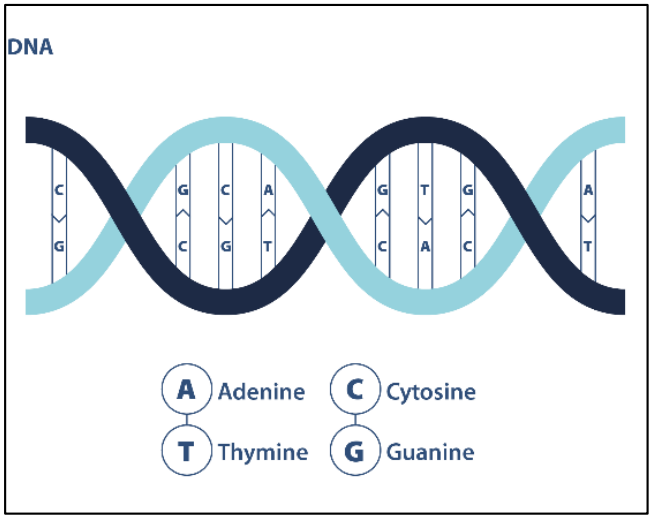
The selection process is intrinsic to plant breeding, which is the activity that results in the development of new plant varieties with specific desired characteristics. It was not until the discovery of Mendel's laws of heredity in the 19th century that plant breeding became an expertise. Since then, plant breeders have been improving breeding methods to facilitate two major steps in plant breeding: increasing the number of genetic changes and selecting the best performing plants in a more targeted and efficient manner. Plant breeding has contributed to the more than 42 000 varieties that are available for farmers in the EU today (EU Plant Variety Database). These varieties are mostly used to produce food, feed or ornamentals. There is much more genetic variation available than present in the crop varieties that are grown in the field today. The majority of plant diversity is stored in seed banks.

2. What is genome editing?

2.1. Defining and clarifying the concept of genome editing

The terms 'gene editing' and 'genome editing' are used interchangeably and generally the same is meant by both. Literally, 'gene editing' refers to the editing of 'genes', which are the units within heritable material that are translated into functional components such as proteins. The word 'genome' refers to the hereditary material of an organism. A gene can be compared to a sentence in a text, and the genome to the whole text. Genes determine heritable characteristics, but it is the genome that determines the organism. Spontaneous changes to the sentences are the basis of evolution.

Figure 1 – The DNA helix



Editing is the activity of deliberately altering the sentences in a text with the goal of adding meaning, changing meaning, removing errors or increasing readability. It is about changing, adding or deleting letters and words, and sometimes even deleting or adding complete sentences. This is also what genome editing is about. It is about deliberately substituting, deleting or adding a few DNA letters (Figure 1) within the existing genetic blueprint of an organism, with the goal to alter its genetic properties. Genome editing is a targeted approach in which one or more predetermined sites in the genome are edited, to achieve a predetermined characteristic. Over the last decades, our knowledge of plant biology and genes has grown exponentially, and genome editing enables researchers to employ that knowledge in a more efficient manner.

Heritable material

Heritable material is built of DNA, which makes use of four DNA letters: adenine, cytosine, thymine and guanine, commonly referred to as A, C, T and G. Where our language alphabet makes use of 26 letters, our DNA-alphabet makes use of just these four letters. Just as in words and in sentences, it is the order of the DNA letters that determines their meaning.

The genomes of plants are large in size, ranging from about 100 million to more than 100 billion DNA letters. A crop like wheat has a genome that is at the large side of the spectrum. For comparison: the human genome is 3 billion letters in size.

2.2. The genome-editing toolbox

The genome-editing toolbox consists of different methods that can be employed to introduce specific, targeted changes into the genome of an organism (Songstad et al., 2017). Currently, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system is by far the most widely used genome-editing tool (Menz et al., 2021; Parisi and Rodríguez-Cerezo, 2021). It is based on a natural mechanism and was introduced to the scientific community as a genome-editing tool about a decade ago. CRISPR has swept aside most other, earlier developed genome-editing tools such as meganucleases, Zinc Finger Nuclease (ZFN) technology and transcription activator-like effector nucleases (TALENs). Similar to CRISPR, these editing tools make use of three biological mechanisms (Songstad et al., 2017):

1. the ability to **find a specific sequence** of DNA letters in the genome;
2. the ability to **cleave DNA** at that location, and
3. the activity of the innate DNA **repair** machinery.

All these tools make use of a nuclease, which is an enzyme that cleaves DNA. Next to these nuclease-based technologies, there is also oligo-directed mutagenesis (ODM), which can be used to change a DNA letter at a desired location (Sauer et al., 2016). CRISPR has become the dominant genome-editing method because it is much more versatile than the other systems and can be very easily programmed to direct the nuclease to the desired location in the genome (Menz et al., 2021).

2.3. How does genome editing compare to other breeding technologies?

Plant breeding has a long history of continuous innovation (Schlegel, 2017) (Figure 2). Since the discovery of Gregory Mendel's laws of inheritance in 1865, plant breeding underwent many technological breakthroughs ranging from deliberate cross breeding and hybrid breeding to mutation breeding. Another wave of innovations was introduced in the last quarter of the 20th century, through technological advancements in molecular biology.

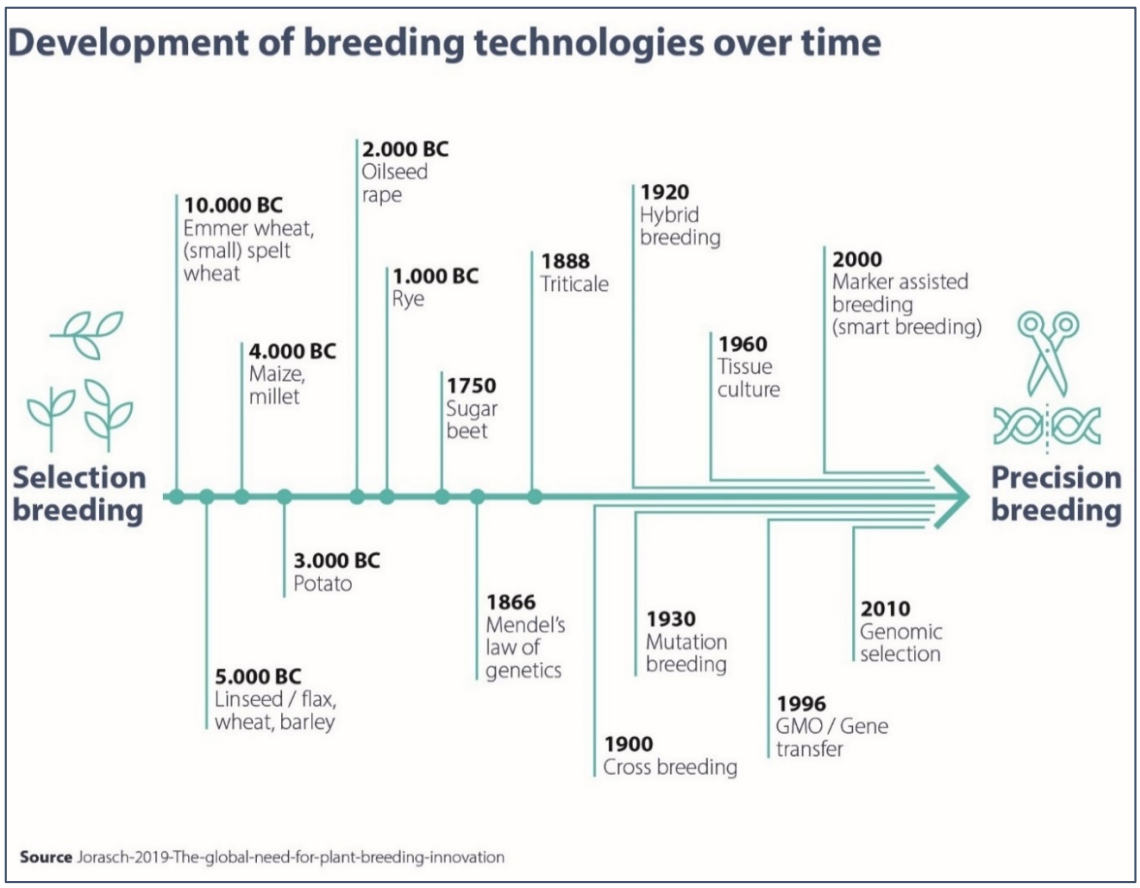
The basis of plant breeding is genetic variation: the availability of different gene variants. Those variants are the result of genetic changes in the genome of a plant. This variability has been utilised to develop and select new plant varieties with desirable characteristics. Spontaneous genetic changes occur in each generation of a plant and result from natural processes such as copying errors of the genetic information during cell division and external factors such as radiation from sunlight (Pedersen et al., 2014). It is estimated that in a single wheat plant approximately 238 spontaneous genetic alterations occur in each generation (extrapolated from Ossowski et al., 2010), implying that all individual plants in a field slightly differ genetically from each other.

The ability to select new plant varieties with desirable characteristics has been limited during most of our agricultural history by the rate of spontaneous genetic changes. The first attempts to increase genetic variation by technical means occurred in the middle of the 20th century.

Plant breeders started to use ionizing radiation (e.g. X-ray, gamma radiation) and chemicals (e.g. ethyl methane sulfonate) (Spencer-Lopes et al., 2018) to introduce thousands of random genetic changes in the hereditary material of a plant, a process known as mutation breeding. While this process is very efficient in generating additional genetic variation, intensive and time-consuming backcrossing and selection procedures are required afterwards to filter away hundreds of undesired mutations and identify offspring with the desired characteristics.

Mutation breeding has been a key contributor to the development of crop varieties with improved characteristics. The Joint FAO/IAEA Mutant Variety Database lists more than 3000 plant varieties that have been produced using ionizing radiation. Many of the plants that we consume today are derived from plant varieties in which mutation breeding is part of their pedigree. An example is the durum wheat varieties that are used for making bread and pasta.

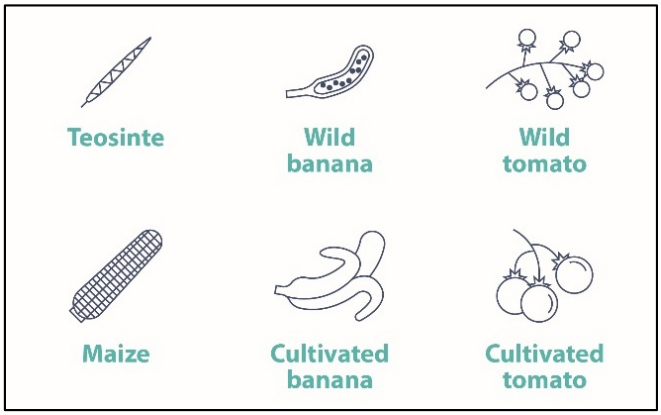
Figure 2 – The development of breeding technologies over time



At the end of the 20th century, advancements in genetic analysis methods such as DNA sequencing made it possible to identify genetic changes that are (cor)related with a certain plant characteristic e.g. resistance to a pest. Genetic signatures of specific characteristics allowed the identification of plants with desired properties in a breeding population (Figure 3). These advancements have accelerated the breeding process.

All of the technological advancements described above are part of what we today call **conventional breeding methods.**

Figure 3 – Three cultivated crops and their wild ancestors



In the beginning of the 1980s, genetic modification of plants was introduced, leading to the distinction between conventional crops and genetically modified (GM) crops, and the subsequent introduction of GM crops into agriculture from 1994 onwards (De Block et al., 1984; Ramkumar et al., 2020). Plant breeders are continuously searching for ways to increase genetic variation and to improve and broaden their repertoire of breeding methods. Over the past 20 years, additional breeding methods have been developed for which the general term 'new breeding techniques' (NBT), also called 'new genomic techniques' (NGT) is now widely used. Genome editing has become the most

widely used new plant breeding technology and CRISPR-Cas (CRISPR-associated protein) is the dominant genome-editing tool. It enables the introduction of desired genetic variation in plants in a very targeted and efficient manner. When CRISPR-Cas is not used to introduce foreign genetic material, the nature of introduced genetic changes, including possible off-target changes, does not differ from genetic changes selected in conventional breeding (spontaneous or induced). Genome editing is often referred to as precision breeding.

Wild plants versus cultivated crops

Crops have evolved over thousands of years to become what they are today, resulting in significant differences from their ancestors (Purugganan et al., 2009). This is the result of what is called domestication: the sustained human intervention by which plants with desirable characteristics are selected.

The appearance and the characteristics of many crops have drastically changed during domestication. An important domestication property of cereals, for instance, is that they no longer spontaneously disperse their seeds, like wild grasses do. This ensures that only few seeds are lost before and during the harvest.

Some of these characteristics are caused by small changes in the DNA. However, over time more drastic changes to the DNA of crops have occurred. Segments of DNA have been lost (deletions), substituted, duplicated, and rearranged (moved to another place in the genome, inversed…), etc; even whole chromosomes and genomes have been duplicated. In other words, there has been already substantial genetic variation through natural selection and breeding – without genome editing – and this has resulted in crop genomes that would have never been developed without those interventions.

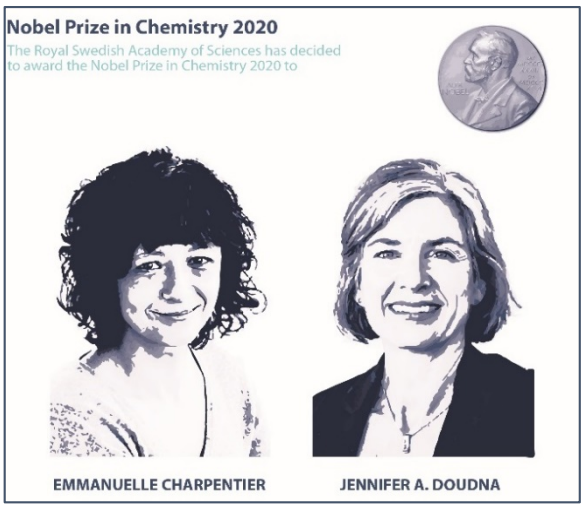
The development of new plant breeding methods has not led to a complete replacement of the older ones. Depending on the challenge that plant breeders aim to address, they choose the breeding method that enables them to reach their breeding goals in the most efficient manner. Conventional crossbreeding is still indispensable for generating different crop varieties that are adapted to specific regions and specific climatic conditions. Regardless of the breeding method, plant breeders need to do field testing and analyse crop characteristics in detail over multiple years and in several geographical locations to select the varieties that will meet consumer and grower expectations and show reliable performance under different environmental conditions. New crop varieties are only allowed to be introduced on the market when they show improvements compared to existing varieties during legally required variety testing trials.

2.4. CRISPR-Cas

CRISPR-Cas has its origin in a bacterial immune system. CRISPR systems are widely distributed in bacteria and have an important role in the defence against viral pathogens that attack the bacteria. Fundamental research on this system paved the way to adapt this system into an efficient genome-editing tool.

In October 2020, Jennifer Doudna and Emmanuele Charpentier were awarded the Nobel Prize in Chemistry for the development of the CRISPR-Cas[1] genome-editing tool (Figure 4).

Figure 4 – The Nobel Prize 2020

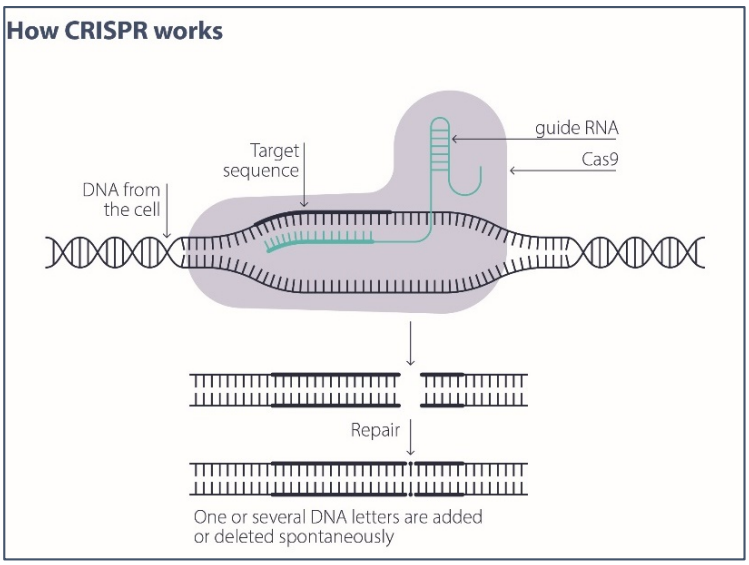


2.5. How does CRISPR-Cas work?

The CRISPR-Cas genome-editing tool is a system made up of a guide RNA molecule and an enzyme that cleaves DNA which is called the Cas protein (Figure 5). In essence, the tool works as follows:

The guide RNA molecule directs the Cas protein to the desired location in the plant genome. The guide RNA/Cas complex is able to recognize and bind to the predetermined location, corresponding to a stretch of 20 DNA letters in the genome and thus acts like the 'FIND' function of a word processor (CTRL-F). Subsequently, the Cas protein cleaves the DNA at that location resulting in a break in the DNA (Jinek et al., 2021). This break is repaired by the natural DNA repair machinery that is present in the cell.

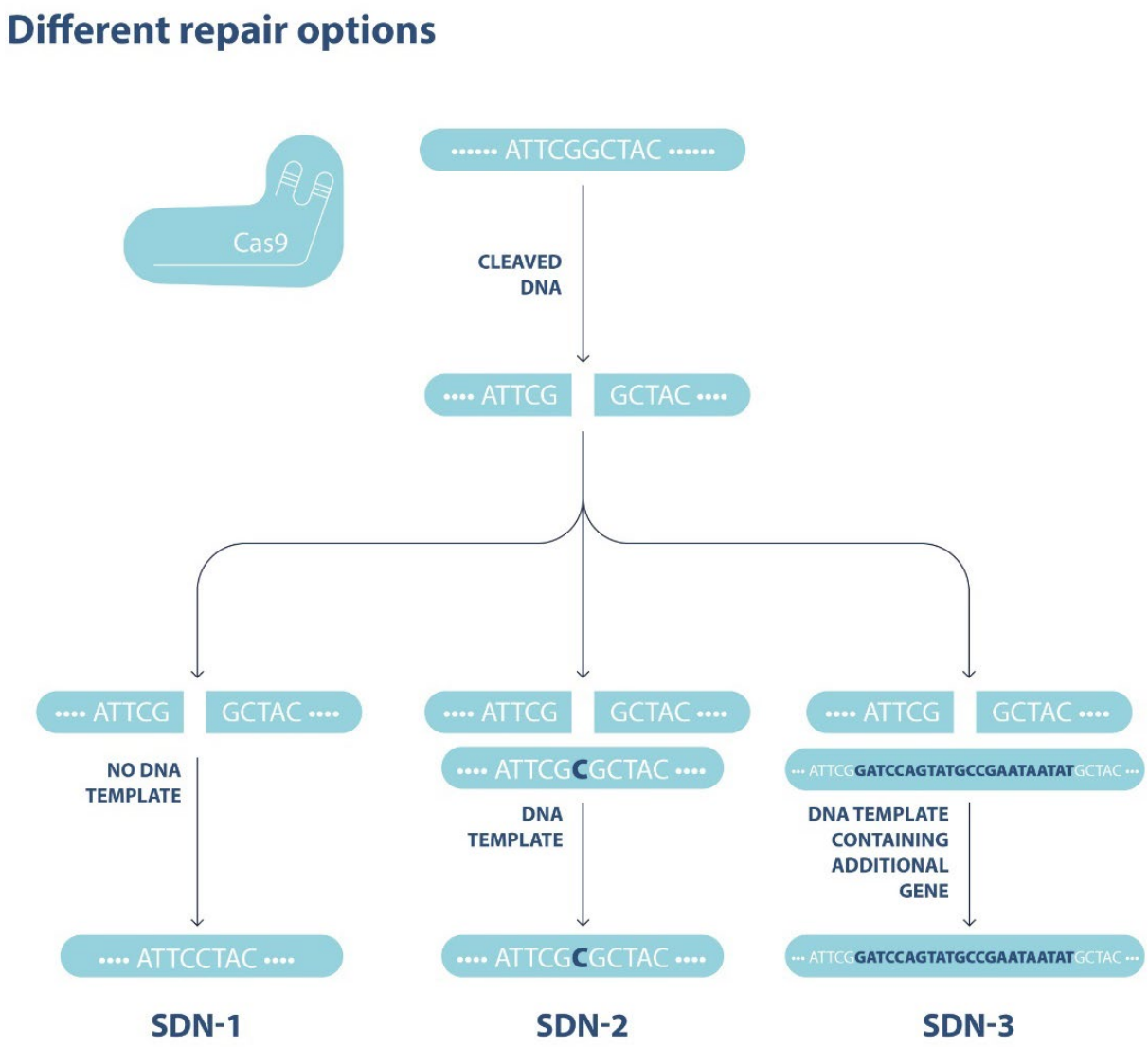
Figure 5 – Overview of the different steps of CRISPR-Cas genome editing



DNA repair mechanisms are present in the cells of all living organisms and repairs damage to DNA to protect the integrity of the genome (Bair et al., 2005). Without this mechanism, living organisms would for instance not be able to survive the UV radiation that is emitted by the sun. The goal of this mechanism is to repair the DNA to its original state. Similar to gluing two pieces of porcelain together, the repair is not always 100 % correct. This is how CRISPR-Cas activity can result in small changes in the DNA by deleting or adding a few DNA letters. It is the same occasional imperfect repair of DNA that produces spontaneous mutations that drive evolution.

[1] **CRISPR** stands for **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats and **Cas** for **C**RISPR-**as**sociated protein (Wiedenheft et al, 2012).

Figure 6 – Three categories of genome editing based on the repair option



In practice, there are different options of how the DNA can be repaired (Figure 6), and these options have led to dividing CRISPR-Cas and similar genome-editing applications into three '**SDN**' categories, which stands for **S**ite-**D**irected **N**uclease (Podevin et al., 2013):

SDN-1: DNA edits consisting of the addition or deletion of a few DNA letters resulting from imperfect repair. The scientific term for this mechanism is 'non-homologous end joining (NHEJ)'. This repair mechanism is most frequently used by plants.

SDN-2: DNA edits consisting of changes in a few DNA letters (adding, deleting or altering a few letters), as a result of repair that makes use of a template which directs the repair. The scientific term for this mechanism is 'homology-directed DNA repair (HDR)'.

SDN-3: This type of edit is based on the same mechanism as SDN-2, but the used template results in the insertion of a larger piece of DNA that constitutes an additional gene. These genes can originate from the organisms' gene pool, or be foreign to the organism as is the case with transgenic plants.

It is important to note that this 'SDN' typology was developed when only CRISPR-Cas editing technology was available that results in both strands of the DNA being cleaved. Over the past years

additional variants of the CRISPR-Cas editing tool have been developed that result in only one of the two strands of DNA being cleaved. Also in these variants, the sometimes imperfect repair of the DNA can result in small changes being introduced.

Two specific variations of the CRISPR-Cas editing tool that are relevant to mention, are **base editing** and **prime editing** (Mishra et al., 2020; Hao et al., 2021). With the first one it is possible to specifically replace one letter in the DNA by another one, whilst with the second variant a short piece of DNA can be rewritten using a template.

All the different new variants of the CRISPR-Cas editing tool have made it more difficult to classify the applications specifically into one SDN category or the other. What one can achieve with the CRISPR-Cas editing tool now forms more of a continuum from very small to more profound changes to the DNA.

To employ CRISPR-Cas as a plant genome-editing tool, one must go through the following successive steps:

1. Genome study. Genome editing starts from the functional understanding of the genes in the plant genome. The desired plant characteristic must first be analysed at the genetic and molecular level. This study will reveal how changing a few DNA letters in a specific gene, results in a certain plant characteristic.

2. Guide RNA molecule design. Based on knowledge from the genome study, a guide RNA molecule is designed so that it recognizes a specific stretch of 20 DNA letters at the predetermined location in the plant's genome where one aims to introduce the DNA alteration.

3. Import of the guide RNA molecule and the Cas protein into the plant cell. The CRISPR-Cas system has to be introduced into the plant cell. This can be done by introducing a genetic construct into the cells that will produce the guide RNA and Cas protein, or by introducing the guide RNA/Cas complex directly.

4. Screen for the desired DNA edit. The plant cells, plant tissues, plants or seeds in which CRISPR-Cas performed the desired edit need to be identified. This is often done using DNA sequencing techniques to verify whether the edit at the predetermined location in the genome has been successful. Some plants will not contain the desired DNA edit and/or may contain additional undesired changes and will be disposed of.

2.6. What can genome editing do?

With genome editing one can introduce targeted DNA changes into the genomes of crops (Figure 7). At the genetic level this means that one can:

- delete one or more DNA letters, or larger pieces of DNA,
- insert one or a few DNA letters,
- change one or a few DNA letters, or rewrite a larger piece of DNA,
- replace an existing gene by another version of the same gene,
- insert a larger piece of DNA (even complete genes) at a desired location.

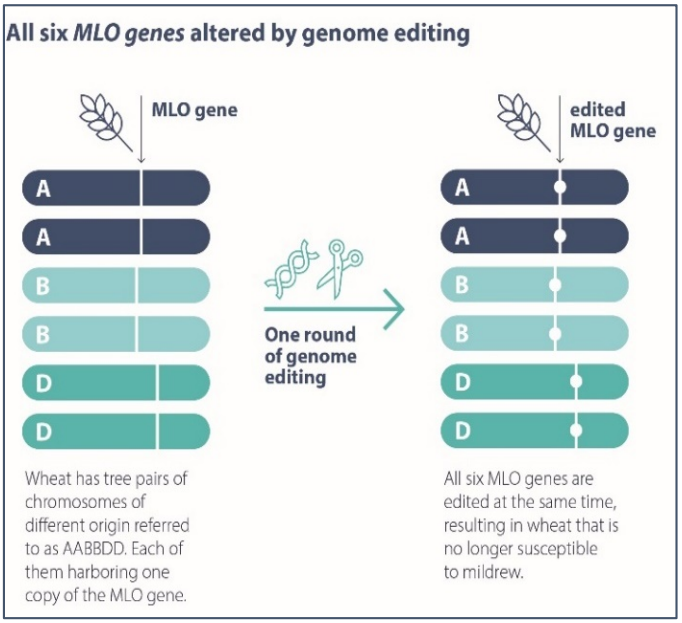
Figure 7 – The types of changes that CRISPR and similar genome editing tools can introduce in the DNA

The unedited sequence	**CGTAGTCCGTGGATCGGATCGTTGACAACTCGAA**
Delete a few letters of DNA	**CGTAGTCCGTGGAT . . . . TCGTTGACAACTCGAA**
Insert a few DNA letters	**CGTAGTCCGTGGATCGGTGATCGTTGACAACTCGAA**
Change one or a few letters	**CGTAGTCCGTGGATTGGATCGTTGACAACTCGAA**
Replace one gene with other version	**CTTAGTCCGTCGATCGGATCGAAGACAACTCGAA**
Insert a complete gene at desired location	**CGTAGTCCGTGG – gene X – ATCGGATCGTTGACAACTCGAA**

Genome editing enables the introduction of desired alterations in a targeted manner at a predetermined location. In addition, it is possible to introduce multiple changes at the same time in two different ways.

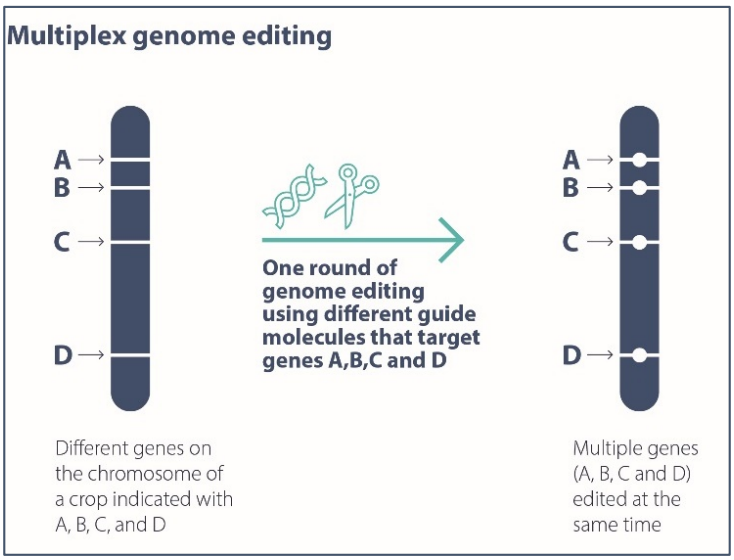
If multiple copies of a gene are present in an organism, one can **change all these copies at the same time**. Wheat, for instance, has six copies of the so-called *MLO* gene (Figure 8). Using genome editing, all six of them were edited in one research study, resulting in wheat that is resistant to mildew (Wang et al., 2014, Li et al., 2022). This is very difficult to achieve with conventional methods.

Figure 8 – Genome editing enables targeting multiple copies of a gene at the same time



One can also **target different genes at the same time**. This is achieved by introducing different guide RNAs simultaneously, resulting in different locations being targeted at the same time. This type of genome editing is called multiplex genome editing (Armario-Najera et al., 2019). An illustrative example is the simultaneous targeting of six genes in wild tomato resulting in new domestication of this tomato (Zsogon et al., 2018). Compared to the wild tomato, the genome-edited tomato has an altered morphology, including alterations in the size (times three), number (times ten) and nutritional value of the fruit (increased levels of lycopene and vitamin C), while maintaining important characteristics of the wild plants.

Figure 9 – Genome editing enables targeting genes at multiple locations at the same time



At the genetic level, edits can vary from very simple to more elaborate and complex (Figure 9). But what this means for the characteristics of the crop depends very much on which gene or genes are being targeted and what their biological function in the plant is. Some characteristics can be changed by editing a single gene. This means that editing that specific gene has a direct effect on that specific characteristic. An example: targeting the *GBSS* gene in potato directly results in the production of only one type of starch in the potato tuber, instead of two (Andersson et al., 2018).

Other characteristics are determined by a combination of different genes. In properties such as plant height, or seed weight, the action of several genes determines the final characteristic. As several of the most important crop properties are of this nature, many research programs aim to identify the genes controlling them. Finally, properties such as drought tolerance are determined by a complex interplay of genes that are highly connected in regulatory networks (Ali et al., 2017). Those networks are characterised by robustness and changing such properties requires the editing of several genes in the network simultaneously.

2.7. Which DNA alterations can occur naturally and which ones cannot?

With CRISPR technology, one can introduce different types of genetic changes. Small changes like the alteration of one DNA letter (a point mutation) happen frequently in nature, as do changes like the deletion of one, a few or even larger segments of DNA. These changes occur in nature and in cultivated crop species, but specific desired changes may not be present in the crop variety of interest. Genome editing enables the introduction of those changes in the variety in a direct manner without introducing additional changes, so that, for instance, the Chardonnay characteristics remain fully intact when a characteristic like fungal resistance is added to the grapevine.

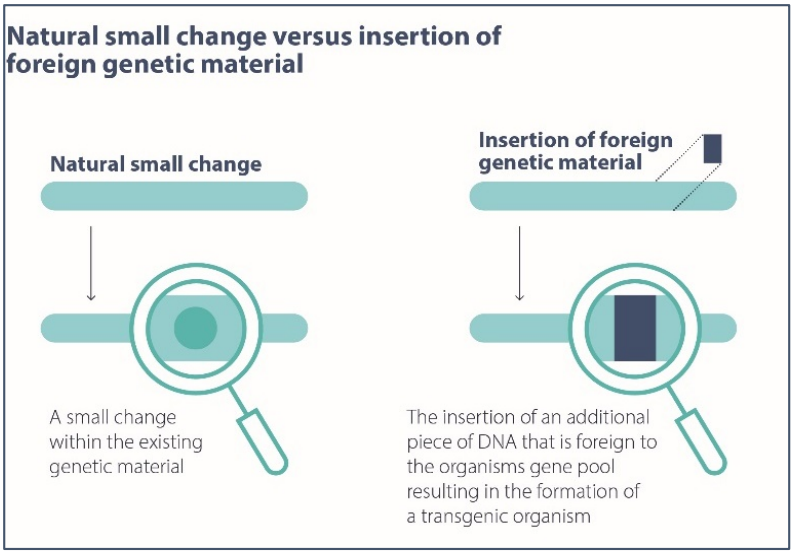
However, when one uses CRISPR to introduce an additional gene into the genome of a crop at a predetermined location (*cf.* SDN-3), this goes beyond what can happen in nature, especially when that additional gene is from another species (a 'transgene').

In other words: one can employ CRISPR and other genome-editing technologies to introduce changes that (can) occur spontaneously in nature and be used in conventional breeding, as well as changes that go beyond what can occur in nature. A significant number of stakeholder organisations and countries outside of the EU make a distinction regarding the regulatory oversight between those two types of genome-edited crops (Schiemann et al., 2020).

2.8. There are crucial differences between genome editing and transgenesis

Transgenesis refers to the introduction of a piece of 'foreign' DNA, meaning a piece of DNA that is not present in the natural gene pool of the plant species, for instance from a bacterium. The additional DNA is stably integrated into the genetically modified plant's genome. A classic example are the insect resistant crops that express one or more bacterial genes (Castagnola & Jurat-Fuentes, 2012).

Figure 10 – The difference between a natural small change and the insertion of foreign material



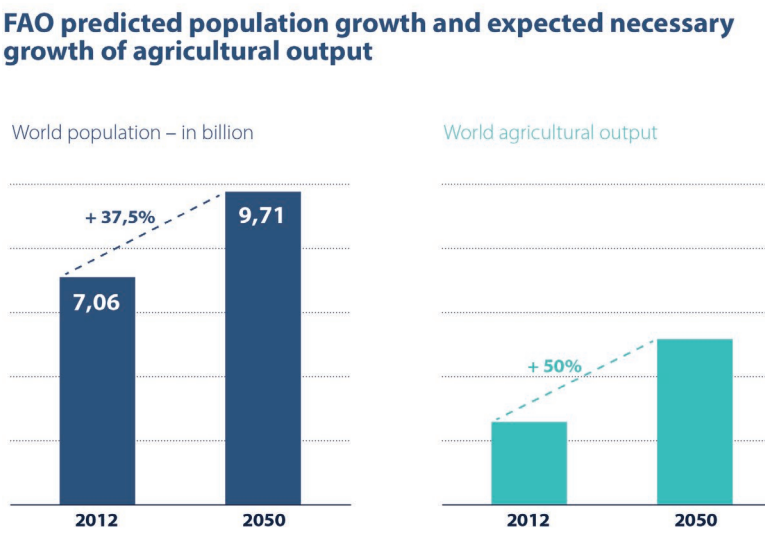
A crucial difference between genome editing and transgenesis is that most applications of genome editing in plants do not result in the insertion of foreign DNA in their genome (Figure 10), and only introduce changes that can also arise spontaneously in nature or result from conventional breeding activities. This is also why, without prior knowledge, it is not possible to determine whether such genetic change is the result of genome editing or conventional breeding methods (ENGL, 2019). Transgenesis can result in the addition of a new function to the plant that could not have occurred otherwise. When genome editing is used to generate a new function, this generally is a function that had the potential to be there and could have arisen without human intervention.

In those cases where genome editing is used to introduce an additional gene into the genome of a plant, another difference with transgenesis is that in the latter the insertion is random, while with genome editing the insertion of the additional gene is at a predetermined location of the genome (*cf.* SDN-3) (Begeman et al., 2017). Depending on where an insertion takes place, this may impact the function of genes present at that location and affect the plant's characteristics in unintended ways.

3. Genome editing and 21st century food systems challenges

3.1. 21st century challenges in agriculture, food and environment

Figure 11 – Predicted population growth and estimated necessary agricultural output growth



Food and agricultural systems are currently facing important global challenges. The world's population is expected to grow to 9.7 billion by 2050, boosting agricultural demand – in a scenario of modest economic growth – by approximately 50 % compared to 2012 (FAO, 2017a) (Figure 11). Different food requirements of young and old people, as well as different consumption patterns, jobs and living conditions of urban and rural populations, will affect the demand for and quality of food. Income growth in low- and middle-income countries will accelerate a dietary transition towards higher consumption of meat, fruits and vegetables, relative to that of cereals, requiring shifts in agricultural output and adding pressure on natural resources (FAO, 2017).

Although agricultural investments and technological innovations are boosting productivity, yield growth has slowed down over the past decades. Since the 1990s, average annual increases in global yields of staple crops have been slightly more than 1 per cent, much lower than in the 1960s (FAO, 2017). The required acceleration in productivity is furthermore hampered by climate change, the degradation of natural resources, the loss of biodiversity and the spread of plant pests and diseases (FAO, 2017).

It is expected that the extent and nature of the impacts of climate change will differ across regions, ecological zones and production systems (FAO, 2017). Increasing variability of precipitation and increases in the frequency of droughts and floods may create additional challenges to yields in general. Although higher temperatures can improve crop growth, studies have documented that crop yields decline significantly when daytime temperatures exceed a certain crop-specific level (FAO, 2016). In addition, climate change can make plants more vulnerable to pests and diseases because changes in temperature and moisture levels can stimulate the occurrence of pathogens, fungi and insects (FAO, 2017).

Food and agricultural systems are not only impacted by climate change, they are also among its main contributors. Although greenhouse gas (GHG) emissions resulting from agriculture, forestry and other land-use have almost stabilized worldwide over the past 25 years, the agricultural sector still produces close to 20 per cent of total global GHG emissions (FAO, 2018). However, mitigation options do exist. Climate change mitigation involves shifting to agricultural technologies and practices that increase food production in ways that are less 'GHG intensive'.

To address climate and sustainability challenges in agriculture and food, the European Commission has formulated the farm to fork strategy. Through this strategy the European Commission wants to ensure Europeans get healthy, affordable and sustainable food, tackle climate change, protect the environment and preserve biodiversity, guarantee fair economic return in the food chain and increase organic farming.

Specific goals of the farm to fork strategy are to:

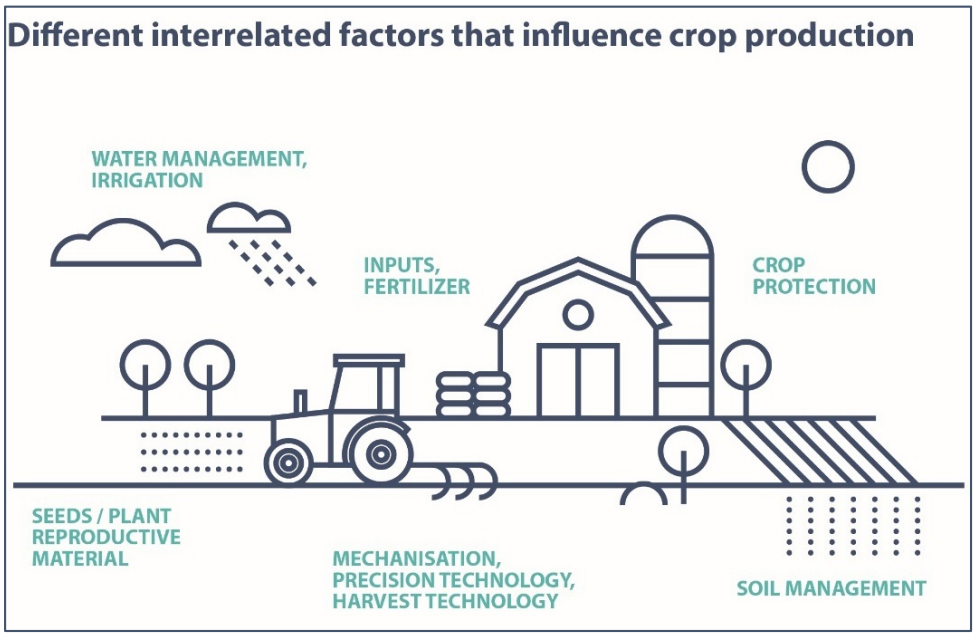
- Reduce by 50% the use and risk of chemical pesticides by 2030;
- Reduce by 50% the use of more hazardous pesticides by 2030;
- Reduce nutrient losses by at least 50%, while ensuring no deterioration of soil fertility;
- Reduce fertiliser use by at least 20% by 2030.

3.2. The role of crop genetics in food system challenges

To tackle the current food system challenges and reach the goals set out in the farm to fork strategy, a combination of approaches and measures will be necessary. The production of food starts with the cultivation of a crop in a field, in a greenhouse or in a backyard. The genetics of the crop is one factor that together with other factors will determine how the plant will grow and how it will respond to attacks by pests and diseases, to drought, heat or flooding and other environmental stressors (Figure 12). Historical data show that improvements in the genetics of crops have already led to significant improvements in yield and yield stability (Voss-Fels et al., 2019). More than 50% of all crop productivity gains are due to improved varieties resulting from plant breeding (Noleppa, 2016).

Plant breeding can be further exploited to improve the way a plant grows and interacts with its environment and to improve health-related properties and nutritional quality of food and feed products. This means that the introduction through breeding of properties like disease resistance, improved nutrient efficiency, drought tolerance or accumulation of health related compounds, could contribute to achieving some of the specific goals of the farm to fork strategy mentioned in the section above (European Commission, 2021).

Figure 12 – Crop production depends on interrelated factors



3.3. Genome editing in the context of food system challenges

Genome editing can contribute to the improvement of the genetics of crops in two different ways: (1) through use of tools such as CRISPR-Cas in research that aims to study gene function in complex biological processes, and (2) through use of these tools to introduce specific desired genetic changes into a plant.

Researchers use genome editing to better harness genetic diversity, to study the function of genes and to discover candidate genes and genetic variants governing desirable characteristics. The knowledge gained through the use of genome editing in research does not necessarily have to lead to the development of a genome-edited crop. It can also be used to direct conventional breeding approaches. Plant breeders will rely on the breeding tools they consider most adequate, depending on the problem they aim to resolve. Each tool has its advantages in terms of efficiency, speed and precision. Some breeding goals may be achieved with older breeding tools as well, but at the expense of speed and precision. Depending on the crop species, conventional breeding takes around nine to eleven years until a new variety can be released to the market (Kaiser et al., 2020). For fruit tree breeding, the time to produce new varieties via conventional breeding is even considerably longer. Genome editing can reduce this time to market considerably (Watson et al., 2018; Wolter et al., 2019).

Through genome editing, one can also introduce specific genetic changes into elite plant varieties without simultaneously transferring other undesired genetic changes (Wolter et al., 2019).

Whether or not a genome-edited crop will contribute to the improvement of the sustainability of the food system as a whole will also depend on other factors which are discussed in chapter 4.

3.4. The genome-edited crop pipeline

The amount and variety of genome-editing applications in crops has been analysed in different studies and proves to be substantial (Modrzejewski et al. 2019, Parisi and Rodríguez-Cerezo, 2021). These applications include, but are not limited to:

- Improved **resistance against diseases** to lower the need to use pesticides;
- Improved **resistance against abiotic stress** in order to mitigate climate change effects on our food production;
- Improved **agronomic traits** in order to boost crop yields, improve productivity, and avoid pre-harvest losses;
- Improved quality traits;
- Improved health related traits

The database on the EU-SAGE website shows that edits in 63 types of plant species have been published in scientific literature (Dima et al., 2022). Concrete examples of genome-edited crops include:

Banana, removal of banana streak virus	Early flowering rice
Camelina with altered oil composition	Carotenoid enriched rice
Fungal resistant grapevine	Soybean with increased oil and protein content
Waxy maize hybrid	Strawberries that flower multiple times
Maize with enhanced grain yield	Sugarcane adjusted saccharification behaviour
Maize with enhanced yield under drought stress	Tomato, self-pruning, early flowering
Non-browning mushroom	Tomato, improved shelf life

Mustard with improved flavour	High lycopene tomato
Amylopectin potato	High GABA tomato
Potato with no glycoalkaloids	Reduced allergens in wheat
Peanut with altered oil composition	High fibre wheat
Rice with enhanced grain size and number	Low gluten wheat
Rice with disease resistance	Fungal resistant wheat

The applications at market and pre-market stages are however still few (Parisi and Rodríguez-Cerezo, 2021). The reasons for this may relate to the fact that new genomic techniques (especially those based on CRISPR) are still a recent discovery and/or to regulatory uncertainty about these techniques in several countries. However, many more applications are expected to appear in the future and eventually to reach the market.

There are currently two genome-edited crops on the market. High-oleic soybean in the US and tomato with increased φ-aminobutyric acid (GABA) levels in Japan. In 2023, nutrient enriched mustard leaf will be introduced onto the US market. Many more are likely to be introduced onto the market in the years to come (Metje-Sprink et al., 2020). High oleic soybeans contain more oleic and less linolenic fatty acids resulting in higher heat and oxidative stability of the oil (Demorest et al., 2016). GABA is a natural substance that is reported to be effective at reducing blood pressure (Nonaka et al., 2017).

4. Risk, regulation and other perspectives

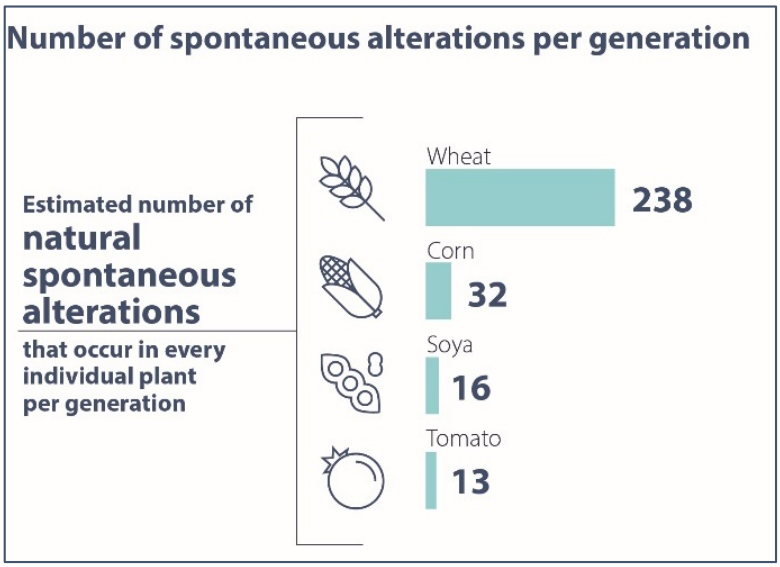
4.1. Risks and uncertainties related to genome editing and CRISPR in particular

Genome editing with CRISPR-Cas is highly accurate. But this does not mean that the desired edit is present in all cells and in each derived plant. In the majority of plants CRISPR-Cas leads to the desired alteration in the target site. However, additional alterations do rarely occur at the target site, but also at other locations in the genome (Modrzejewski et al., 2019; Modrzejewski et al., 2020, Biswas et al., 2020). At the target site additional changes can occur directly adjacent to the place where the DNA was cleaved, caused by an imperfect repair of the DNA break. In other occasions the Cas protein may cleave the DNA also at another location in the genome that has an almost similar DNA sequence as the target site. The latter are called **off-target alterations**.

These observations do not mean that CRISPR-Cas technology is inherently risky or that the genome-edited plants would present a risk. In conventional cross breeding, for instance, the genetic material undergoes many more changes resulting from crossing. However, a molecular characterisation of the genome-edited plants is required to determine the introduced change with the necessary precision and detail. The chances of **off-target alterations** increase when (1) there are multiple genes within a gene family with very similar sequences, and (2) the guide RNA that directs the Cas protein to the target site is not very specific. There are procedures in place that allow for a significant reduction of the chances of off-target alterations. This starts from in-depth knowledge about the target gene and the genome as a whole and making sure that the guide RNA and Cas protein are highly specific. The latter depends in large part on the design of the guide RNA, and the software tools currently used that enable the design of highly-specific guide RNAs (Gerashchenkov et al., 2020). With a correct design, the chances of off-targets become very low. In addition, appropriate screening can help to avoid the selection of plants that contain undesired off-targets. It is confirmed that off-target mutations

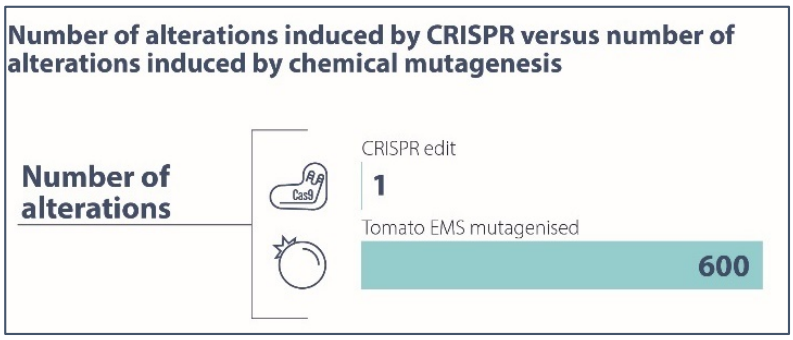
potentially induced by site-directed nucleases such as CRISPR-Cas are of the same type as those mutations induced in conventional breeding which have a history of safe use (EFSA, 2020).

Figure 13 – Comparison of the number of spontaneous alterations per generation



Spontaneous mutations occur often, also when using conventional breeding methods, and it is estimated that in a crop like maize there are on average 32 spontaneous mutations from one generation to the next (Ossowski et al., 2010) (Figure 13). In wheat, which has an eight times larger genome size than corn the number goes up to about 238 spontaneous mutations from one generation to the next (see Figure 13). In a significant amount of genome-edited plants one can find additional small changes which are neither in a target location, nor in an off-target location. These additional small changes are likely the result of such spontaneous mutations and/or of the process of *in vitro* regeneration. The latter is an intermediate step, which may be used in the process of generating a genome-edited plant, and is known to cause some changes to the genetic material (Jain, 2001). Because of its potential to generate changes to the genetic material, *in vitro* regeneration is deliberately used in some conventional breeding strategies.

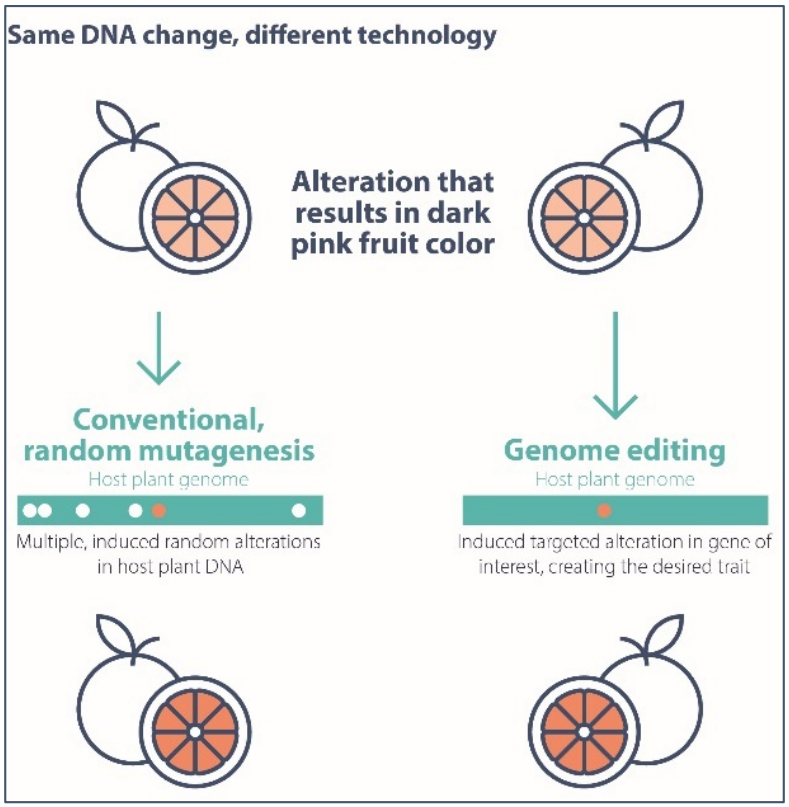
Figure 14 – Comparison of the number of changes introduced by CRISPR versus chemical mutagenesis



In relative terms, the risks and uncertainties of genome editing are lower than the risks and uncertainties of conventional random mutagenesis which makes use of radiation or chemicals to induce genetic changes. In this conventional process several thousands of changes are induced in a random manner, followed by a selection of plants which show new, desirable characteristics (Spencer-Lopes et al., 2018). Besides the desired characteristic, the plant will contain additional changes (Figure 14). Today, more than 3 000 varieties of crops are available that have been produced using radiation. Some examples include 'Golden Promise' Barley (high yield, improved malting), durum wheat (for bread and pasta), disease resistant Japanese pear, dark pink grapefruit, semi-dwarf rice, disease resistant bean, peanuts with tougher hulls, and varieties of peas, cotton, peppermint, sunflowers, peanuts, grapefruit, sesame, bananas, cassava and sorghum. More information can be found in the Mutant Variety Database.

To further illustrate the above: the risks related to the change that causes the dark pink flesh colour in pink grapefruit do not depend on how this genetic change was introduced (See Figure 15). It is the property itself – the pink flesh colour – that will determine whether there is a potential to cause harm. Additionally one will have to consider possible off-targets and their potential to create a property that will result in harm. In conventional random mutagenesis there will be a significant amount of off-target mutations, and their effects will largely be unknown. In genome-editing the number and frequency of off-target mutations is multitudes lower than in conventional random mutagenesis and during the process of generating a genome-edited crop one has the possibility to select plants that only possess the desired change.

Figure 15 – Different technology can result in the same alteration



Changes introduced into crops can have an impact on the way the crop interacts with the environment, and this holds true for genome-edited crops too. Any potential for harm to the environment will depend on the actual property that is introduced and whether or not such changes can disperse to wild plants depends on the ability of the crops to successfully exchange genetic material with wild plants.

In the applications of genome editing to insert an additional piece of DNA in the plant genome (*cf.* SDN-3), the risks are similar to any other technology that leads to genomic insertion of a transgene (EFSA GMO panel, 2012; EFSA GMO panel 2020). There is however an important distinction between genome editing and 'conventional' GM technology: in GM technology the insertion is random, while with genome editing the insertion is directed to a desired location in the genome (*cf.* Section 2.8).

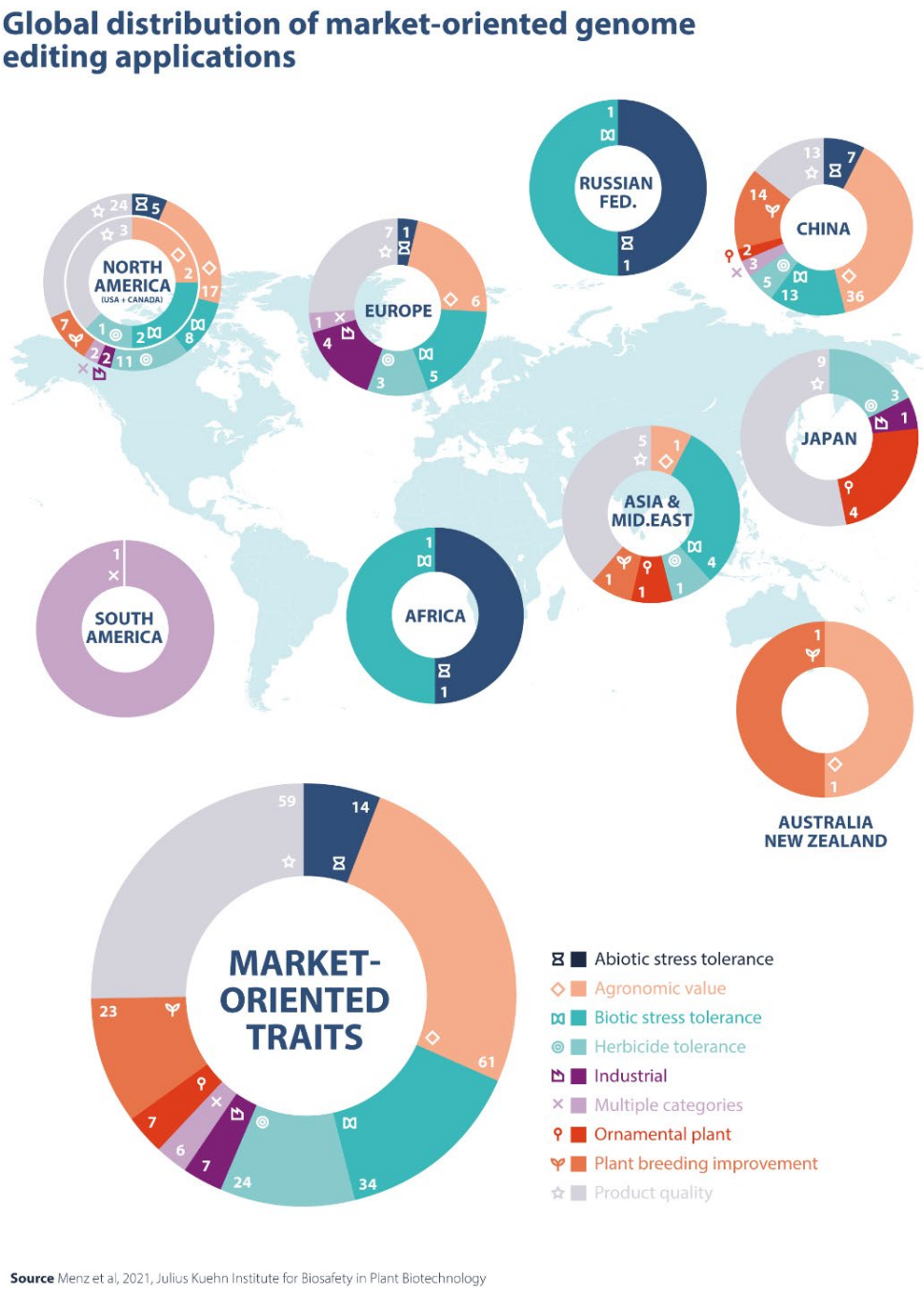
4.2. A global perspective

Genome editing, and in particular CRISPR-Cas, has been rapidly adopted in crop research activities worldwide. The majority of activities take place in China, followed by the US, the EU and Japan (Modrzejewski et al., 2019, Dima et al., 2022) (Figure 16). Moreover, there are activities in other parts of the world including Brazil, Argentina, Israel, Russia, Saudi-Arabia, India, Korea, the Philippines and Turkey. In Latin-American countries such as Chile, Colombia and Costa-Rica, genome editing is explored in crops such as rice, beans, cacao and banana. Furthermore, in South Africa and sub-Saharan African countries like Kenya and Ethiopia (Tripathi et al., 2020; Numan et al., 2021), modern biotechnological methods including CRISPR-Cas are employed for instance in CGIAR-related research institutes. In the US, the number of market-oriented genome-edited crops under development is the highest, followed by China and Europe (Menz et al., 2021; Parisi & Rodriguez-Cerezo, 2021).

In the US, genome-edited high-oleic soybeans are on the market, and in Japan genome-edited tomatoes that have significantly higher levels of φ-aminobutyric acid (GABA) compared to currently available tomatoes.

Genome editing is applied in a much broader variety of crops compared to transgenesis and the scope of introduced characteristics is much broader as well. Many of these crops are globally traded, such as soybean, rice, maize, wheat, banana and oilseed rape. This implies that genome-edited crops may also be traded and move from one part of the world to the other.

Figure 16 – The global distribution of market-oriented genome edited crops



Source Menz et al, 2021, Julius Kuehn Institute for Biosafety in Plant Biotechnology

4.3. The actors that apply genome editing

Genome editing is widely used in both the public and the private sector. In the public sector, genome editing is used to perform basic crop research, helping to expand the knowledge on how genes and genomes function and which factors play a role in the interaction between crops and the environment (Modrzejewski et al., 2019, Wessberg et al., 2021). Genome-editing functions as an accelerator. Researchers from public institutes are also involved in the introduction and evaluation of market-oriented characteristics through genome editing.

In the private sector, many EU-based companies that have intensive crop research and development (R&D) and breeding activities use genome editing as an additional tool for breeding (Jorash, 2020). Large companies use genome editing, but also a fair number of small and medium-sized enterprises (SMEs) are active in the field. Many breeding companies, especially the larger ones, have diversified approaches in different crops tailored to specific markets based on the regulatory situation and the consumer acceptance of genome-edited crops. Most small and medium-sized European plant breeding companies are active at international level and for them, compared to large companies, it is more difficult to cope with differences in legislation (Jorash, 2020).

Plant breeding is an intercontinental activity. Plant breeding companies develop their crops, often making use of plant nurseries in both the Northern and Southern hemisphere, enabling them to continue their breeding efforts during winter times. Different European plant-breeding companies have winter nurseries in, for instance, Chile or Argentina.

Genome editing, and especially CRISPR-Cas, resulted in the emergence of a series of new start-up companies that specifically use genome-editing technology to improve specific crops in a targeted manner. Examples of companies include Benson Hill, Calyxt, Inari, Pairwise, Plantedit, SolEdits and Tropic Biosciences. The number of start-ups that use genome-editing technology for crop improvement is lower in the EU compared to the US. This could be the result of them being regulated as GMOs in the EU.

4.4. Access to technology, ownership and control

There are virtually no thresholds to the application of CRISPR-Cas in research. However, for the commercialisation of genome-edited crops in most cases it will be necessary to have a licence on CRISPR-related intellectual property (IP). There is a complex landscape of patents and patent applications on different components of the CRISPR-Cas machinery, derived tools and their applications, amounting to more than 250 published patent families related to the use of CRISPR-Cas in plants (Jefferson et al., 2021). Universities and research institutes are a major contributor in these patent filings. Large players in the agricultural sector such as Bayer, Syngenta, BASF and Corteva have secured exclusive and non-exclusive licences on the IP related to the original CRISPR-Cas inventions from the University of Berkely and/or the Broad Institute of MIT and Harvard. Corteva has a right to sublicense to international research organisations and other companies (Jefferson et al., 2021).

Getting a licence on the necessary IP to use CRISPR-Cas in agricultural crops creates a threshold in the access to the technique. But the number of CRISPR-related genome-editing tools that are not patented or can be used free of licence is growing (van der Oost & Fresco, 2021).

Two systems of IP protection may apply to agricultural crops: plant variety rights, and patents. New crop varieties can be protected under a plant variety right, which ensures that others are not allowed to sell seeds of that variety without the permission of the breeder that has developed the variety. In the EU, plants - not varieties - can be protected by a patent if the plant has been the result of a

patented invention. A variety is a precisely defined group of plants within a plant species, with a common set of characteristics. For instance, Chardonnay is a grapevine variety. Plant characteristics resulting from the application of genome editing can also be protected by a patent, even when the edit could also have occurred naturally. But there must be a clear disclaimer in the patent application that the characteristic has been obtained using a method that is not 'essentially natural'.

The use of genome-editing tools in agricultural crops on a wider scale might result in a larger number of agricultural crops that are patent-protected. When breeders want to access those genome-edited crops and use them for further breeding, they will need to obtain a licence and cannot make use of the so-called 'breeders' exemption' that exists under the plant variety rights system. Genome editing enables breeders to introduce desirable characteristics in a much more targeted way. However, when patented technology was used, it provides more control over plants with these characteristics, compared to plants that are protected under a plant variety right.

4.5. The regulatory status of genome-edited crops under EU law

The status of genome-edited organisms under EU law has long been unclear. In 2018 the Court of Justice of the EU (CJEU) confirmed in case C-528/16 that organisms obtained by means of techniques of mutagenesis constitute GMOs within the meaning of Article 2(2) of the EU GMO Directive 2001/18/EC. The Court also ruled that only organisms obtained by means of techniques of mutagenesis which have been conventionally used and have a long safety record are excluded from the scope of that directive. The European Commission concludes from that ruling that organisms resulting from new genomic techniques (NGTs, including genome editing) fall within the scope of the EU GMO legislation. As a consequence, a crop with an alteration produced by a conventional mutagenesis technique is exempted from the provisions of the GMO Directive, whereas a crop with the same DNA alteration obtained with genome editing is not (Figure 15).

Since the introduction of the EU GMO legislation in 1990, only two genetically modified crops have been authorised for cultivation in the EU: MON810 maize and the Amflora potato. The latter has been withdrawn soon after its market launch. The EU GMO legislation has strict data and risk assessment requirements. Currently, only multinational companies can afford the marketing of GM crops and the regulatory hurdles.

Argentina was the first country that chose to apply a case-by-case approach to determine whether a genome-edited crop is subject to the GMO legislation (Lema et al., 2019). This approach is based on the definition of 'Living Modified Organism' (LMO) under the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. Under this definition, a genome-edited organism is only an LMO when the genetic alteration has resulted in the formation of a 'novel combination of genetic material'. When the edit has resulted in a genetic alteration that could also occur spontaneously or is the result of conventional breeding, the Argentinian authorities conclude that the organism is not subject to their GMO legislation. The implications of this regulatory approach are that the spectrum of genome-edited crops and characteristics that are being introduced are much broader compared to GMOs. In Argentina, SMEs and public institutions are also submitting dossiers for genome-edited crops (Whelan et al., 2020). The example of Argentina shows that the applied regulatory approach has a significant impact on the development and market introduction of genome-edited crops (Lema et al., 2019).

In the EU, when a crop is not subject to the GMO legislation, there is no authorisation procedure that would require a pre-market safety assessment, unless a 'non-traditional propagating practice has resulted in significant changes in the composition or structure of the food affecting its nutritional value, metabolism or level of undesirable substances.' In that case, the crop would fall within the

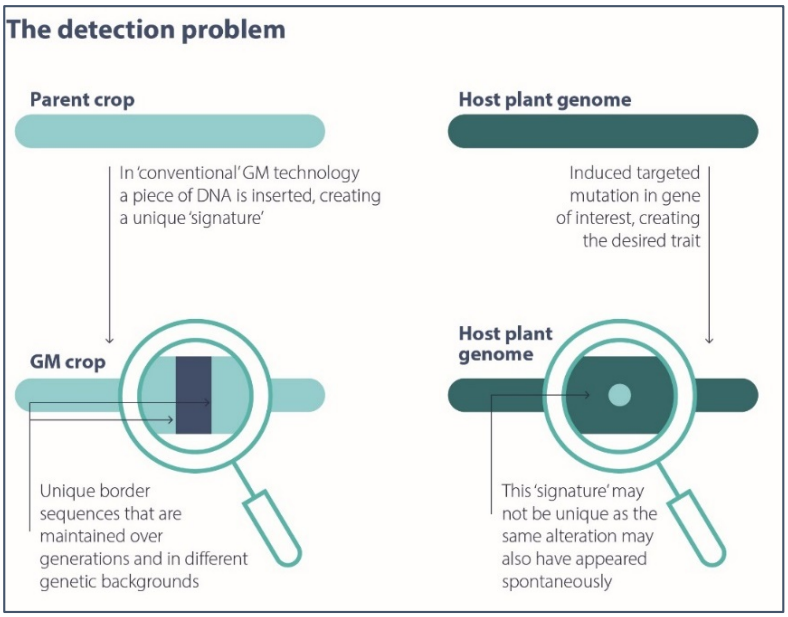
scope of Regulation (EU) 2015/2283 on novel foods, which includes an authorisation procedure subject to a food safety assessment.

Irrespective of the regulatory approach, all actors that introduce a genome-edited crop into the EU territory are liable under Directive 2004/35/EC in case that the introduction would result in damage to protected species and natural habitats, to water or to land.

4.6. The detection of genome-edited crops is problematic

An important difference between 'traditional' GM crops and most of the genome-edited crops is that the first contain an additional piece of DNA that is inserted into the genome (*cf.* Section 2.8). This creates a unique genetic signature that can be easily detected (Figure 17). Detecting that genetic signature in a bulk load or in a specific food item means that there is material present derived from the GM crop. It is different in the case of genome-edited crops: detecting a certain alteration does not automatically mean that this alteration has been introduced with genome editing. It could have also occurred spontaneously, or it could have resulted from conventional breeding. A substantial amount of additional information is necessary to enable the determination of the probability that the presence of the alteration is due to the presence of genome-edited plant material (Grohmann et al., 2019; ENGL, 2019). For example, information on the presence of additional specific (genetic) characteristics can help determine the origin of the mutation. Only information that is robust over different generations and within different genetic backgrounds is useful.

Figure 17 – The detection of insertions of foreign genetic material versus small alterations



However, this information may not be available, and when it is, it may not suffice for detection and identification in complex food matrices with traces of genome-edited plant material (ENGL, 2019). Detection methods must also be efficient, reliable and fast. Moreover, it would be difficult or even impossible to provide court proof evidence that the genetic change originated from genome editing (EU, ENGL). Enforcing the EU GMO legislation on genome-edited crops with DNA changes that could have occurred spontaneously or as a result of conventional breeding is therefore considered difficult.

This difficulty means that, once released from the lab, tracing genome-edited products through both internal markets and across external borders would be challenging, and they could unintentionally end up in certified product ranges that do not allow the presence of GMOs, without being detected. This may affect the consumers' freedom of choice and societal acceptance of the technology. Additionally, non-detectability of the use of genome-editing technology may make it more difficult to establish possible patent infringements.

4.7. Regulatory challenges

The regulatory approach to genome-edited organisms is not the same across the globe (Turnbull et al., 2021) (Figure 18). In South America, countries like Brazil, Chile, Paraguay, and Uruguay follow the Argentinian example, where crops with alterations that could also occur spontaneously or result from conventional breeding are not subjected to the GMO legislation. In the US, the US Department of Agriculture is following a similar approach in which it is no longer required to deregulate certain genome-edited crops under the Plant Pest Act (Wolf & Wolf, 2018). The food safety approach has not changed. The US Food and Drug Administration (FDA) maintains a voluntary food safety consultation process combined with strict food safety liability legislation, and that applies to genome-edited crops too. In Japan and Australia, genome-edited organisms with small genetic changes (*cf.* SDN-1) are not subject to the GMO legislation. In the UK, a bill called the Genetic Technology (Precision Breeding) bill was announced that will alter the definition of GMOs to exclude certain organisms (plants, including algae) created by genetic technologies in ways which could have occurred naturally or produced by traditional breeding. The goal is to establish a new science-based authorisation process for food and feed products developed using precision bred organisms and introduce two notification systems; one for precision bred organisms used for research purposes and the other for marketing purposes. In India, the government has created a differentiated procedure for plants resulting from genome editing in which no foreign genetic material is introduced.

Differences in regulatory approaches have international consequences. Plant breeders in the EU who conduct research and breeding activities in different regions of the world look into the possibilities of using genome editing in those regions, and may even consider relocating certain research and breeding activities outside of the EU. Differences in the regulatory oversight have consequences for international trade. If certain genome-edited crops are not regulated in parts of the world, then it may be difficult to prevent them from ending up in other parts of the world unnoticed.

In the EU, the European Commission has initiated process to develop a regulatory proposal for plants resulting from the application of targeted mutagenesis and cisgenesis. It is expected that the Commission will present a regulatory proposal in the second quarter of 2023. Stakeholder input, collected through questionnaires and targeted consultations will be used as a basis for an impact assessment and the development of the proposal.

4.8. Divergence in views and public debate

There is divergence in views on the implementation, usefulness and safety of genome-edited crops between different stakeholder groups in Europe, which also determines the positions that these stakeholders take in the regulatory debate on genome-edited crops (EGE, 2021).

The ruling of the CJEU has made clear that, from a legal point of view, organisms resulting from modern, targeted forms of mutagenesis constitute GMOs under the current EU legislation. Whether these GMOs should fall under the GMO legislation, or be out of scope or exempt is the core issue where consensus is lacking among different stakeholder groups. The European Group on Ethics in Science and New Technologies (EGE) recommends that regulation should be proportional to risk: light-touch regulation should be used where the modification could have been achieved naturally or the edit involves the introduction of genetic material from sexually compatible plants (EGE, 2021).

In its recent study, the European Commission states that several of the plant products obtained from NGTs (New Genomic Techniques, which includes genome-editing) have the potential to contribute to the objectives of the EU's Green Deal and in particular to the farm to fork and biodiversity strategies and the United Nations sustainable development goals (SDGs) for a more resilient and sustainable

agri-food system. However, some stakeholders consider that these benefits are hypothetical and achievable by means other than biotechnology.

Stakeholder groups that have voiced strong criticism against GMOs, such as IFOAM EU Group, TestBiotech, Confédération Paysanne, generally refer to genome-edited organisms as new GMOs, whereas the European Plant Science Organisation (EPSO), EU-SAGE, Leopoldina and other academy organisations are inclined to make a distinction between transgenic crops and genome-edited crops in which no foreign genetic material was introduced (Leopoldina, 2019; TestBiotech, 2021).

The European Non-GMO Industry Association (ENGA) has voiced concerns over the traceability and possible admixture of genome-edited material into product ranges that do not allow the presence of GMOs (ENGA, 2021). The United Nations has expressed concern over the impact of genetically-modified organisms on biological diversity and sustainability (EGE, 2021). Discussions are ongoing on how policies should be adapted to enable the potential of modern (breeding) technologies towards sustainability (EGE, 2021).

Another factor that fuels the debate is the level of corporate control over the food chain. GMOs are considered a risk factor that may contribute to this as GM crops can only be marketed by a limited number of international corporations. SMEs are not active in this market because they cannot afford the costs and complexities associated with the application of the GMO legislation. It is unlikely that the current GMO legislation will enable SMEs to enter the market of genome-edited crops and contribute to maintain or even expand diversity in the seed market. The EGE recommends developing measures to support small actors (EGE, 2021).

5. Concluding messages

- Genome editing is about the targeted and deliberate introduction of small changes to the heritable material of an organism. The CRISPR-Cas genome editing tool, introduced about a decade ago, has become the most widely used genome editing tool. It is applied in public research, but also by commercial plant breeding companies, to introduce desired changes in the DNA of plants.
- Food and agricultural systems are facing important challenges in terms of sustainability, food supply and security linked to global conflicts, demographic pressures, and climate change, not to mention consumer demand for healthier and safer products.
- Several genome-edited plants have the potential to contribute to a more resilient and sustainable agrifood system. Some stakeholders consider these benefits to be hypothetical. The number of examples of genome-edited crops is substantial, and growing. There are currently two genome-edited crops on the market outside the EU.
- Following a CJEU ruling, the European Commission has concluded that genome-edited crops are subject to EU GMO legislation, which aims to guarantee safety for consumers, animals and the environment from GMOs by means of an elaborate pre-market risk assessment that requires GMO products to be labelled and traceable on the market.
- In several countries outside the EU, genome-edited crops in which the change is similar to changes that can occur naturally and be obtained and selected in conventional breeding, are not regulated in the same way as GMOs.
- The European Commission is currently preparing a proposal for an update to this legislative package, which takes into account recent advances in genome editing technology, to be adopted in the second quarter of 2023.
- CRISPR-Cas technology offers a very high level of accuracy, but alterations beyond the desired alterations (off-targets) do occur. A good molecular characterisation of the edit followed by a selection of plants with the desired edit helps to mitigate the potential risks associated with the market introduction of plants with undesired off-targets.
- While new genomic techniques (NGTs) can offer faster and more precise edits, a few concerns remain. Off-target effects are discussed in the context of risks associated with genome editing. Recent studies have identified their frequency to be similar or lower than traditional types of breeding.
- There are concerns over the detectability of changes made by CRISPR-Cas, as this could lead to challenges regarding the traceability of genome-edited crops and products. These difficulties affect the enforceability of legislation and demand the development of robust detection methods. The development of such methods is however problematic, which may affect consumer trust and societal acceptance.
- There is a divergence of views among stakeholders as to which route to follow, indicating the need for debate in the EU on what type of regulatory governance is warranted.

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Genome editing is the targeted alteration of a few DNA letters within the existing genetic blueprint of an organism. By far the most widely used genome-editing tool is CRISPR-Cas.

CRISPR-Cas genome-editing technology can be applied in a number of different ways. The genetic changes that are introduced by means of the SDN1 and SDN2 types of CRISPR-Cas technology do not differ from changes that can occur naturally or result from conventional breeding.

While CRISPR-Cas technology is highly accurate, off-targets can occur. However, molecular characterisation of the genetic changes, combined with selection, can prevent plants with undesired changes from being introduced onto the market.

Views on this new technology differ widely, but there is a clear need to discuss which type of regulatory governance is warranted for genome-edited crops.

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