# GMOs in 2018

## TIME FOR A THOROUGH REVISION?

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# GMOs in 2018
# Time for a thorough revision

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# Executive Summary

Finding a sustainable way to feed the ever-growing global population is one of humanity's greatest challenges in the 21st century. GMO technology – GMO meaning 'genetically modified organism' – is often mentioned as part of the solution, but also as an obstacle to the solution. The cause of this contradiction is because the debate on GMOs often focuses on aspects not inherent to GMOs, such as multinationals, monoculture, the use of pesticides or the negative consequences of GMO legislation in Europe. The GMO technology is often reduced to specific examples and current GMO practices. The European GMO legislation was created in the late '80s and was revised at the turn of the century. Plants that are identified as GMOs are considered a distinct category. They are subject to more thorough monitoring than other plants. This was warranted thirty years ago, due to lack of knowledge and experience, but today it leads to a two-track policy without scientific backing.

In the past decennia it has become clear that GMO technology does not create specific risks for health or environment in comparison to current food production. For all living organisms it is possible for the DNA to break and recombine, similar to the recombination technique in GMOs. Recombinations and rearrangements can occur spontaneously in the DNA of living organisms. Both in wild plants and in crops, examples have been discovered of the incorporation of DNA from *Agrobacterium*, the bacterium that is frequently used to create genetically modified plants. This emphasises the occurrence of natural genetic engineering, and hence indicates that there is too great a contrast between the GMO legislation and the legislation applicable to other plants. Furthermore, several disadvantages attributed to GMOs are a consequence of the stricter regulation and are not due to the GMO technology itself. As a consequence of the current legislation, GMOs also remain in the hands of multinational companies and the genetic varieties on the agricultural market are often limited.

A lot of scientific progress has been made since the introduction of GMO legislation: new techniques such as genome editing or precision breeding have been added to the portfolio of plant breeders. These techniques put a strain on the current legislation. Should crops resulting from these new techniques be subject to the more stringent legislation? Are there any reasons to strictly regulate the products of these techniques? In many cases the use of these modern methods results in plants that could also have originated from classical breeding. Since plants obtained by either classical breeding or precision breeding are indistinguishable, it is difficult to find scientific arguments for applying strict regulation to crops obtained by precision breeding.

The focal point is sustainability and how we best serve this purpose. If we want a greater diversity in seed companies and robust varieties to make agriculture more

sustainable, we urgently need to incorporate the acquired scientific knowledge on GMO technology into European legislation. This legislation needs to secure access to new breeding techniques for smaller companies. Policymakers should feel supported by scientific research proving that European citizens are not questioning the technology but rather specific implementations of this technology.

# Foreword

**The Standpunten series**

The Academy's Standpunten (Position Papers) series contributes to a scientifically validated debate on current social and artistic topics. The authors, members and workgroups of the Academy write under their own name, independently and with complete intellectual freedom. The approval for publication by one or more Klassen of the Academy is an assurance of quality. This Standpunt was approved for publication by the Class of Natural Sciences on 1 December 2017.

**Justification**

Agriculture is a major supplier of food, energy and raw materials for a global population that is growing and experiencing a rise in living standards. One of the main challenges for the coming decades is therefore to make agricultural production both more efficient and more sustainable. Many of the seventeen *Sustainable Development Goals* (SDGs) [1] established by the United Nations in 2015 are directly or indirectly related to agriculture and food. Better agriculture is necessary for reducing poverty and starvation; it requires a responsible approach to production and consumption, can contribute to energy provision and economic growth, and leads to better water quality and health. However, current agricultural practices fall short of satisfying many of these aspects. Small-scale, local and ecologically responsible agriculture is losing the competition in a globalised market and the diversity of the agricultural crops used is in gradual decline. Multinational companies are increasingly taking control of seed and food production and the low cost of foodstuffs in industrialised countries is encouraging food waste. Intensive farming may well be efficient in terms of high output, but it often focuses more on the economic side and not enough on the ecological and social aspects of food production. Is technology the answer, or is it holding back sustainable agriculture?

During the last century technology contributed to an increasingly efficient agricultural sector; unfortunately, this often resulted in environmental problems or soil exhaustion. In the 21st century we should be in a position to do better: there is sufficient scientific and technical knowledge to grow crops that have less of an impact on the environment. It also seems logical to consider any knowledge and technology that could contribute to sustainable solutions and not to block certain technologies from the start. Applications that use genetically modified organisms (GMOs) can also contribute to sustainable farming.

For decades we have been promised that GMOs would help solve the food problem, but in practice the most important applications are still restricted to a handful of commercially interesting crops and the technology is chiefly related to herbicide tolerance, whether or not in combination with insect resistance. Many promising

applications, such as virus-resistant plum trees or vines never get further than field trials. In Europe in particular, this is largely to do with GMO legislation and how this is translated politically. SMEs do not have the finances to invest in the prolonged and expensive tests required before a GMO can be marketed. Large companies only invest in crops and traits, such as soy and herbicide tolerance, that guarantee a sufficient return.
In 1990 the European directive concerning the targeted introduction of genetically modified organisms in the environment was published. This EU document [2] laid down the rules for the responsible use of GMOs in cultivation and food. More than 25 years later, there has been an impressive rise in molecular biological knowledge about plants, technological progress has been significant and much useful experience has also been built up in relation to the use of GMOs. The legislation has been adapted on several occasions (for example, in 2001 [3]), but seems to be heading in the direction of an ever stricter framework, without taking into consideration the scientific knowledge and technological progress. This Standpunt would like to review the current state of affairs in order to provide clarity in this complex and often controversial matter. The focus is on the technology, safety and legislation.

It goes without saying that a technology must be seen in the context of its applications and the society in which it is used. A technology can influence that society. In this way the discussion on GMOs has shaped the debate on sustainable and ethically responsible agriculture [4]. On the other hand, the debate about GMOs is all too often *not* about GMOs but about other issues, such as herbicide use in agriculture [5]. In this *Standpunt* we focus on the GMO technology itself and the associated legislation. The use of a technology in a society must also be checked against ethical values. Readers who wish to immerse themselves in an analysis of the social context of current and future GMO applications are recommended to read the more extensive Metaforum report on GMOs [6].

# 1. Setting

**Breeding and safety**

Since the emergence of agriculture approximately 10,000 years ago, mankind has gone about selecting plants that are better adapted to his needs. This process of artificial selection is different from natural selection. A nice example is that grains selected by humans, such as wheat and maize, retain their seeds so that nothing is lost during harvesting. This property is a disadvantage for wild plants, whose chief desire is to spread their seeds as much as possible. The aim of adapting plants for breeding is to create better plants: with equally high or higher yields and less input (pesticides, fertilisers, water …) and/or of better quality. Over the course of the last century the techniques for breeding and selection have increased dramatically in number, and in recent decades they have also become increasingly efficient (see 2).

Since we want sufficient and tasty as well as safe food, legislation has been drafted with this in mind. The aim is to protect humans and nature against possible harm. In 2000, following several incidents concerning food safety in the 1990s (mad cow's disease, the dioxin crisis, …) and so as to respond faster to microbial and chemical contamination in food, the Federal Agency for the Safety of the Food Chain (FASFC) was set up in Belgium. At the European level, the European Food Safety Authority (EFSA) was established in 2002. On the basis of scientific data, authorities monitor possible risks and apply suitable measures where necessary. The priority must of course go to the highest risks with the most serious consequences. In 2011, for example, more than fifty people died and 4000 fell ill after eating plants infected with the toxic bacterial strain *Escherichia coli* O104:H4 [7] due to the use of liquid manure. Fast tracking and withdrawal of the source of an infection is vital in order to limit the number of casualties.

**Suppressing a revolution**

Flanders is a pioneer in the research and development of plant biotechnology. Since the 1980s the techniques for improving plants have become increasingly sophisticated and the results are often indistinguishable from plants obtained by breeding. In order to distinguish the most recent innovations from already established methods, the term 'new breeding techniques' is used.

The fast and targeted alteration of plant genetic material has caused a revolution in crop enhancement, resulting in good yields with fewer pesticides. However, the strict legislation for GMOs prevents applications in crops, such as numerous vegetables and fruit varieties and local crops in developing countries, that are less financially attractive to big companies. Europe has completely halted new permits for the cultivation of GMOs. Is this a missed chance to use the technology for more sustainable agriculture with high-quality food products? After twenty years of (an almost) outright blockage of innovative applications of plant biotechnology and irrational reactions to new breeding techniques, it is time to return to the basics: we all want sustainably produced food that is safe for humans and environment.

When the first genetically modified plants were developed, GMO legislation was drafted in Europe on the basis of the precautionary principle, which must ensure that the new plants are safe for humans and environment. In contrast to new plants that are obtained by means of conventional or mutation breeding (see 2), GMOs are only allowed on the market after a safety analysis by EFSA and after a vote in a committee of representatives from the European member states. It is therefore primarily the technique used that determines whether or not a crop is subject to strict legislation, rather than the characteristic that was introduced in the crop using that technique. Therefore, very similar plants – for example, plants with the same herbicide tolerance (see appendix) that have been obtained using a different method - can either be put directly on the market, or they are investigated via a much stricter procedure and usually blocked. This is an extremely illogical situation: both plants are equally safe, but one is put on the market and the other not. It is more logical to have the risk analysis focused primarily on the plant and its characteristics.

Let’s take the example of a potato with a modified starch composition used for industrial applications, such as paper and glue production. BASF had such a potato, Amflora [8], developed by genetic modification (RNAi, see section 2). Because it was a GMO, the potato underwent a very thorough analysis as regards its safety for humans and environment. The dossier was submitted to EFSA in 2003 and the cultivation of Amflora was finally permitted in 2010. However, the discussion dragged on, and in 2012 BASF decided to cease cultivation in Europe. In the meantime, in its place a mutant potato with the same traits has been brought on the market without any discussion. The mutant was obtained by means of irradiation [9] and is not subject to GMO legislation.

## 2. Definitions and techniques: an overview

**Plant breeding: from the first farmers to the genetic revolution**

One of the most important steps in the history of mankind was undoubtedly the transition from hunter-gatherer to farmer: the moment that humans intentionally started planting seeds in a field. That is when the domestication of plants began. Humans gathered seeds with favourable traits and high yields to be sown the next season. As a result, over the course of a few millennia the traits of our cultivated crops have become increasingly distant from the wild plants from which they were derived. At a certain point, humans intentionally began crossing plants, in the hope of combining favourable traits in the 'progeny'. Mendel's discovery of the rules of heredity accelerated this process. A few new traits proved very important for agriculture. So, for example, shorter stalks in wheat and rice laid the basis for the green revolution. [10] More compact plants don't lodge as much and use the nutrients they absorb for seed production rather than vertical growth. Many cultivated crops now look nothing like their wild parents. Just take a look at the innumerable variants of cabbage (Figure 1).

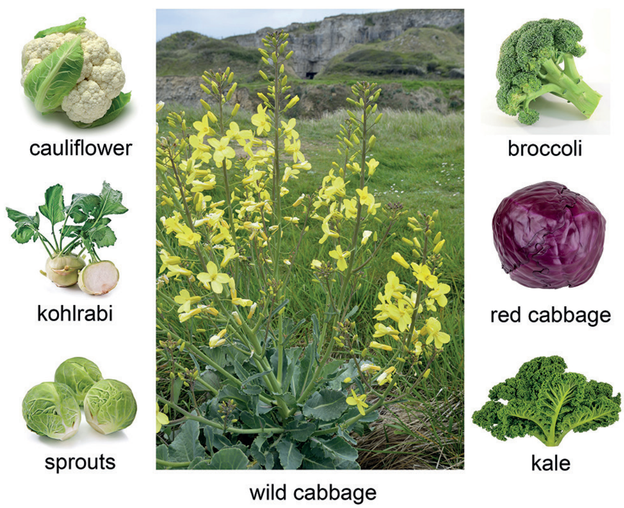


Fig. 1. Many different cabbages have been developed by breeding from the wild cabbage (central photo). They all belong to the same plant species *Brassica oleracea*.

The 20$^{th}$ century witnessed a dramatic acceleration in plant breeding. The first major innovation was the development of F1 hybrids, a cross between two inbred parents. The crossing of inbred lines creates a homogeneous and very vigorous next generation. Other important innovations were mutation breeding and embryo rescue.

In mutation breeding chemical substances or ionising radiation randomly induce small changes in the DNA. Some of these changes lead to desirable traits, which are then selected. The number of changes generated using this blind method vary from a few dozen to thousands per treatment.

Embryo rescue is a technique whereby newly formed embryos are removed from the seeds of the plant and placed on an artificial sterile medium containing all the necessary nutrients. This technique enables crossing between species that would never have happened spontaneously in nature. The modern-day wheat varieties for pasta or bread are the result of such interspecific crossings whereby embryo rescue and irradiation were used to transfer disease resistance, amongst other things, from other grains and wild grass species. [11] [12]

With the discovery of the double helix structure of DNA in 1953 came the genetics revolution. All living organisms on Earth have DNA as their genetic material and also use the same genetic code to produce proteins from that DNA (Figure 2). Technologies to cut DNA into specific fragments using enzymes and then stick them back together provided a new dimension. This process is known as recombinant DNA technology. In the 1970s this technique was used to make the first GMOs in microorganisms, a significant example being the production of human insulin in a bacterium. Compare it with a fragment of text (a recipe for a protein) from the DNA book of humans that is inserted into the DNA text of the bacterium, enabling the bacterium to make human insulin.

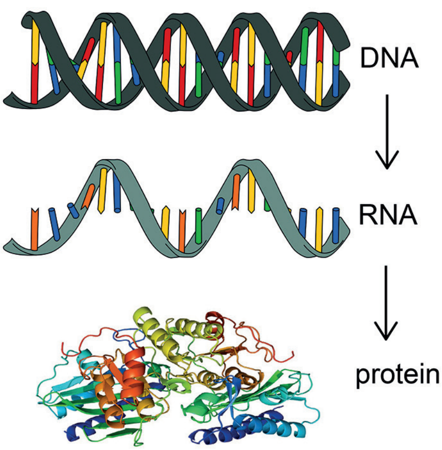


Fig. 2. The information transfer of DNA, the genetic material, via an intermediary messenger (RNA) and ultimately translated into the amino acids of a protein.
DNA and RNA consist of four letters (nitrogen-based organic bases) that form the code language on which all life is based. Proteins are the building blocks of the cell, either as structural proteins or as enzymes that carry out reactions to make other building blocks, such as fats and sugars.

Plants had to wait until the early 1980s and the important discovery that when infecting plants, the bacterium *Agrobacterium tumefaciens* inserts a fragment of its own DNA into the genome of the plant host. This knowledge, combined with recombinant DNA technology, enabled the development of the first genetically modified plant in 1983. Pioneering work took place in the United States, and concurrently, in Belgium, in the laboratories of Jeff Schell and Marc Van Montagu at Ghent University. This technology has made it possible to introduce specific hereditary traits in crops in a more targeted way. Instead of combining all the genes of both parents by crossing and then searching for the progeny with the desired traits, recombinant DNA can be used to transfer the desired gene from one plant to the other. Moreover, the technology allows genes to be utilised from other organisms without the problem of species barriers. One of the first applications was the use of a bacterial gene (Bt from *Bacillus thuringiensis*) that codes for a protein that is selectively poisonous for certain insects to make crops, such as cotton and corn, insect-resistant (see also Bt aubergine in the appendix).

Techniques, such as RNAi, have also been developed to suppress the expression of undesirable genes in plants. [13] The method is based on the natural defence mechanism that plants use to defend themselves against plant viruses. Plants recognise double-stranded RNA (dsRNA) that comes from viruses, whereby the dsRNA as well as the corresponding mRNA is broken down. Similarly, the introduction of a modified gene that produces dsRNA against an allergenic protein will ensure that that protein either cannot be made or only in minor quantities (see examples in the appendix 'Virus-resistant papaya', 'Improved potato' and 'Hypoallergenic apple').

In parallel with these developments, a technology was also appearing in the 1970s to determine DNA sequences. In combination with recombinant DNA technology to study the effect of deactivated genes, this laid the foundations for the further unravelling of genes and the whole genome. As a result, plant breeding has become increasingly knowledge-driven and targeted. Marker-assisted breeding uses DNA analysis to make a fast and efficient selection of the best progeny for further selection in a breeding programme.

### New breeding techniques

In discussions about green biotechnology, classical breeding and GMOs are often pitted against one another. In reality, there is a continuum of innovations in the breeding of agricultural and food crops (see Figure 3). The term 'classical breeding' covers an array of methods and techniques that have emerged over a period of many decades. Also, the term 'genetic modification' groups different techniques that have been used to make changes in genetic material that could not occur naturally. The terms 'genetic modification' and 'GMOs' are mainly used in a legal context to determine which types of organisms fall under a special law.
Note: not every targeted modification of the genetic material of an organism falls under the legal term 'genetic modification'. We will come back to this later in the document (see section 5).

In the last decade a third category of breeding has been named: the 'new breeding techniques', although not all of these techniques are in fact new. All of them, however, have emerged since the development of recombinant DNA technology and the implementation of the GMO legislation. A clear and comprehensive overview

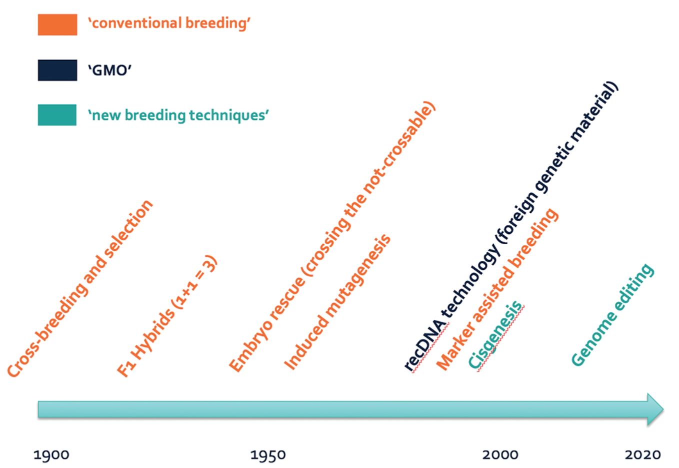


Fig. 3. Innovations in plant breeding over the years.

of the new breeding techniques can be found in the brochure *Opportunities of New Plant Breeding Techniques* from Wageningen University. [14] We will limit ourselves in this article to the two most relevant examples.

1. ***Cisgenesis and intragenesis*** make use of the same recombinant DNA techniques as applied in genetic modification. Cisgenesis means the introduction of species-specific genes (one or more cis-genes) with their natural regulatory signals. These genes can also end up in a crop through interbreeding. The only difference between a classically cultivated crop and a cisgenic crop is the location of the gene in the genome. An advantage of cisgenesis is the ability to avoid undesirable genes that may come along in a standard crossing. In intragenesis all sequences that are introduced in the crop come from the natural gene pool, but new genes with different components can be combined, for example to express a root gene in fruit. The chance of such an intragenic crop emerging in the traditional way is very slight. Both cisgenic and intragenic gene constructs can be inserted in a specific place in the genome with the help of gene editing.

2. Gene editing or genome editing or precision breeding. ***Gene editing*** is a targeted and very precise method for making minor modifications to the genetic material of an organism. Various techniques are used to make the *edits*, the most well-known being the CRISPR/Cas9 system (CRISPR = clustered regularly interspaced short palindromic repeats). Table 1 offers an overview of a number of typical modifications that can be made using this system. Essentially, the method involves cutting at a specific location in the genome, thereby generating additional genetic variation. This is different to what is generally understood by the legal term 'genetic modification'. There, in the main, genetic material is introduced across the species barrier (transgenesis). Since very specific mutations or alleles can be generated using gene editing, the term precision breeding is very appropriate.

Table 1: Examples of precision breeding

| Type | Voorbeeld | technologie | mechanisme |
|---|---|---|---|
| Changes to one base in DNA | ...ATA... → ...A**C**A... | CRISPR combined with a fragment of DNA that contains the desired modifications | Creation of a double-standed cut in the DNA followed by repair on basis of DNA homology |
| Removal of several bases | ...A**ATAG**C... → ...AC... | CRISPR | Creation of a double-standed cut in the DNA followed by repair of the cut |
| Change of allele | ...Allele-1... → ...Allele-2... | CRISPR combined with a fragment of DNA that contains a different allele | Creation of a double-standed cut in the DNA followed by repair on basis of DNA homology |

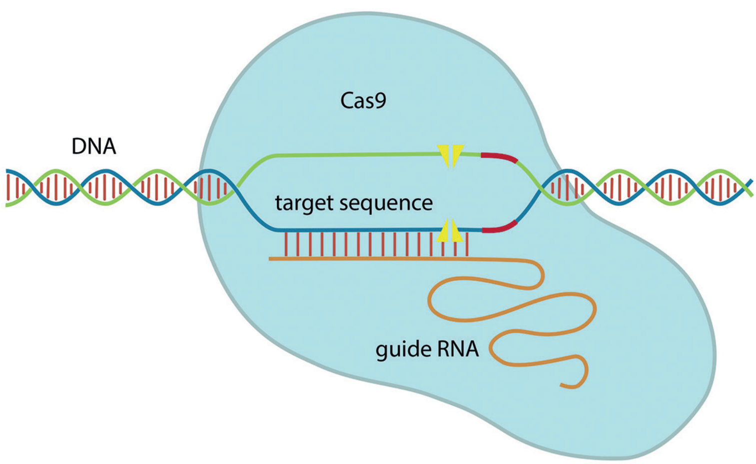


Fig. 4. CRISPR-Cas system

CRISPR-Cas is a bacterial complex consisting of the Cas enzyme and RNA molecules. In nature that complex can recognise virus-DNA, then bind and cut it at that place. Scientists can modify the RNA to cut a desired DNA target sequence in the cell. A cut DNA sequence is repaired in the cell by enzymes that stick the loose ends back together, but small mutations often occur in that place during the process. [target sequence – guide RNA].

## 3. Safety of the technology

When the first genetically modified plants were developed, more than thirty years ago now, there was very little known about the possible unexpected effects of the technique on the DNA and properties of those plants. As a result, specific legislation was introduced to examine such plants before they were allowed onto the market (see section 4). The legislation therefore starts from the assumption that the process of genetic modification may incur certain risks. Research from the last thirty years proves, however, that there is no scientific basis for presupposing such risks. For a detailed overview see also [15-16].

In every population of living beings, there are individuals with different genetic make-ups. The DNA differences occur spontaneously due to errors (mutations) when the DNA multiplies, or due to external factors, such as ultraviolet radiation. Most changes are small (a few DNA letters), but major rearrangements can also take place (breaking and moving chromosome segments). The new DNA sequencing techniques now allow the genome of many individuals of one species to be analysed quickly and relatively cheaply. This has shown that the process of genetic modification causes fewer changes than those which occur as natural variation or caused by breeding. [17-20]

Thanks to gene technology we can exchange DNA between non-related species, for example from a bacterium to a plant. This seems unnatural because the species barriers are crossed. It is called horizontal gene transfer (HGT) because it differs from the vertical gene transfer that occurs between parents and progeny. Genome analyses in recent decades have shown that horizontal gene transfer also frequently occurs in nature and that it was important for evolution. While it has long been known that HGT occurs all the time between bacteria, it now appears that plants and animals also use DNA that they get from bacteria or moulds. Aphids use fungal genes to acquire a colour that protects them against predators [21]; mites use bacterial genes to produce enzymes that break down plant products [22]; and plant-parasitic worms have an entire arsenal of proteins to better infect the plant; virtually all are produced with genes that come from bacteria or fungi. [23] Fish of non-related species exchange DNA, probably via the sperm that is released into the water to fertilise eggs [24], and more than 10,000 years ago both tobacco [25] and sweet potato [26] acquired DNA fragments of *Agrobacterium*, the bacterium used in the lab to make GMOs.

Conventional breeding can also exchange DNA between different species, at least if they are related in some way and if various laboratory techniques are applied (see section 2).

Quite a lot of improved plant varieties have resulted from mutagenesis. In mutagenesis the DNA variations that occur naturally are increased, by irradiation for example. While the chance of a natural error is approximately one in 100 million DNA letters, that can rise to one in 1000 with irradiation. This increases the chance that amongst those many thousand mutations in the genome, there is one that leads to the desired trait. Examples are the pink grapefruit and the durum wheat used to make pasta. Most other mutations that occur during irradiation cause no visible effect but are still present in the plant that is obtained by means of mutation breeding. You can compare it to a hail of bullets: a few reach their target and the rest go elsewhere. Gene technology has recently developed a method whereby mutagenesis acts like a sniper. With CRISPR-cas9 (see section 2) one well-aimed shot causes a mutation in the desired place, and the sniper can disappear without trace. The technology is not failsafe, but it is possible to identify the desired mutants. Because gene technology is used during this process, there have been discussions in Europe for the last decade about whether the strict GMO legislation does or does not apply to such mutants. Nevertheless, mutants that have resulted from the 'hail of bullets' technique do not have to go through the safety procedure and can go directly to market. In any case, the debate about whether a CRISPR-generated mutant is a GMO or not is completely irrelevant in terms of whether or not that mutant is safe.

So on the basis of the DNA modifications applied, there is no scientific justification for subjecting plants that have been created by gene technology to more stringent safety checks than plants resulting from breeding. That has been confirmed by very thorough analyses of what has changed in GMO plants at the level of proteins or metabolites. [27-28] All kinds of feeder studies [29-30] with animals (rats and mice, salmon, goats, cows, pigs …) show no difference in feeder quality between GMO and non-GMO plants. The animals suffer no adverse effects from the GMO food, either in long-term trials (longer than two years) or over different generations (tested in rats). Extensive analyses of possible environmental effects also show no difference compared to cultivated plants. [31] Specific applications then again appear to come with environmental advantages, such as reduced insecticide use, resulting in a greater diversity of useful insects in the fields. [32-33]

The cultivation of GMOs began in 1996 and has been going on for years on approx. 180 million hectares around the world. In all those years, not a single piece of evidence showing environmental damage has been reported. Between 2000 and 2011 almost 100 billion chickens and more than 300 million cattle in the US were fed with GMOs. The statistics kept since 1983 show not a single negative effect on the health of the animals. [34] The scientific consensus is clear: gene technology incurs no new risks compared to those with breeding. And we have centuries of experience with breeding to show that the process is sufficiently safe.

It is sometimes stated that not all scientists are of the same opinion on this subject. And that is true: there are scientists who are critical about the use of GMO technology, just as there are scientists who deny that climate change is at least partly caused by humans, or scientists who doubt the use of vaccines or the existence of evolution. According to a study in the U.S. [35] the difference in opinion between scientists and the general public is however biggest when it comes to GMOs. The research showed that 88% of scientists think that GMO food is safe, versus 37% of the general public. For the climate discussion that was 87% versus 50%, and for vaccines 86% versus 68%.

We can conclude the following: there is no evidence at all that the GMO technology in itself entails specific risks compared to breeding. Any potential risks depend mainly on the nature of the modification that is carried out, as well as the genetic background of the organism itself. For instance, after classical breeding a potato may produce more toxic glycoalkaloids. [36]

# 4. Legislation

**GMOs: definition and legislation in Europe**

There is legislation on GMOs all over the world. Their shared foundation is that GMOs must first undergo safety checks – in relation to food safety and possible environmental risks – before they are allowed to be used. Although all this legislation is based on global consultation that was laid down in 2000 in the Cartagena protocol for biosafety [37], the definition of a GMO and the precise legislation differs everywhere. Here we focus on the European definition and legislation, starting with the 90/220/EEC directive in 1990. [2-3]

This is the definition of a genetically modified organism (GMO) according to 90/220/EEC: 'genetically modified organism (GMO)' means an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.

This Directive shall not apply to organisms obtained through the techniques of genetic modification listed in Annex 1 B, (1) mutagenesis (2) cell fusion of plant cells or organisms that can exchange genetic material via classical breeding methods.

The European legislation requires a thorough toxicity analysis and environmental risk assessment before a GMO can be marketed. For this purpose, a dossier must be submitted to EFSA containing the following information:
- a detailed molecular characterisation: what is changed in the DNA?
- the detailed chemical composition compared to a non-GMO;
- an analysis of the nutritional value;
- an analysis of the toxicity and allergens, via animal trials amongst other things;
- an analysis of possible environmental effects.

The information in the dossier must be gathered on the basis of several field trials that are conducted over a number of years.

If EFSA finds a GMO to be safe, a political procedure follows whereby the EU countries debate and ultimately vote. At the end of 2017 more than sixty GMOs (mainly soy and maize) had been granted permission for import in Europe using this procedure. Only one GMO was approved for cultivation: an insect-resistant maize that is grown primarily in Spain. The approval concerns not only the original variety mentioned in the dossier but also all varieties derived from it via cross-breeding. The permit is valid for ten years and during that period monitoring is also required, to instantly flag up any problems. For all GMO products or their derivatives, this must be stated clearly on the label (see, for example soy oil, Figure 5).

The European GMO legislation is one of the strictest in the world. The large number of investigations required before a dossier can be approved and the subsequent political impediments to a product being allowed onto the market mean that the costs to the company submitting the file soon add up. As a result, only large multinationals have the capital necessary for this process to succeed, resulting in monopolisation and limits on the number of GMO varieties on the market. A relatively unique aspect of the legislation in the European Union is that a new dossier is also required for combinations (through crossing) of already approved GMOs. So a hybrid between two approved insect-resistant GMOs must go through the whole process again for market approval, albeit with a more limited risk analysis. In other countries such GMO hybrids are automatically covered by the original approval.

The biggest problem for European agriculture is the political obstruction to all requests for GMO cultivation permission, while at the same time many GMO products enter Europe via authorised imports.


Fig. 5. Label (in Dutch) with reference to genetically modified soybean.

## Legislation in America and Canada

In contrast to EFSA in Europe, there is more than one institution assessing the safety of GMOs in the US. Plants resulting from *Agrobacterium* transformation are assessed before their cultivation by the United States Department of Agriculture, Animal and Plant Health Inspection Service. Their food safety is investigated by the Food and Drug Agency and if there is a plant with a 'plant-incorporated protectant' the Environmental Protection Agency also gets involved. The requisite investigations into food and environmental safety are very similar to those in the EU procedure, but after approval from the government bodies the GMOs are treated the same way as conventional crops and can be brought to market without further monitoring. There are also less cumbersome procedures in the U.S. for GMOs that are the equivalent of already approved GMOs (the same characteristic in the same crop). It is therefore far easier to produce, for example, several commercial, disease-resistant potato varieties using GMO technology in the U.S. than in Europe, where the same lengthy procedure applies unchanged to every new GMO.

In Canada there is no GMO-specific legislation. There the government looks at the traits of the end product to decide whether a new product is *novel* – and must then undergo additional safety tests – not to the method used for developing the product (e.g. breeding or gene technology).

## New breeding techniques and legislation

In the light of the new breeding techniques it is useful to think about the ideas underlying the current legislation. Traditionally cultivated crops are not subject to special safety legislation. The reason for this is that classical breeding is regarded as a process that produces crops that are generally judged as safe. And in practice they are, with some exceptions that prove the rule. [36]

The classical breeding process is based on the following steps:

1. the generation of genetic variation – many different individuals –,
2. the selection of individuals with the desired traits,
3. the further characterisation and testing of those individuals,
4. the selection of an individual for variety development,
5. the carrying out of variety trials and registration on the varieties list, and
6. putting the variety on the market.

Although there is no safety legislation that applies directly to traditionally cultivated crops, there is indirect legislation that should make breeders think twice before putting a potentially unsafe crop on the market. For instance, there is the general food safety legislation which requires, amongst other things, that food which constitutes health risks should be removed from the shelves. There is also the general environmental liability legislation, which makes operators liable if they introduce substances or organisms into the environment that cause harm to protected habitats, species, soils and waters.

GMOs are subject to special safety legislation because of the new possibilities created by recombinant DNA technology and the lack of experience with this new technology in the 1980s. As a precautionary measure legislation was therefore set up to impose strict preliminary risk analyses. The GMO food safety legislation says that genetically modified food crops must be just as safe as comparable non-genetically modified – in other words: traditionally bred – food crops. And since traditionally bred food crops do not come with zero risk, no impossible demands can be included in the risk analysis for GMOs.

**Are products of the new breeding techniques subject to GMO legislation in the EU?**

It has long been debated whether products of new breeding techniques are subject to the EU GMO legislation. On 25 July 2018 the Court of Justice of the European Union (CJEU) ruled on a case that was brought before them by the French Council of State. This case concerned the regulatory status of organisms in which the genetic material has been altered using different forms of mutagenesis. Are organisms obtained by classical mutagenesis GMOs? And do genome edited organisms fall under the same exemption as classically induced mutants, to which the provisions of the EU GMO legislation do not apply?

The Court ruled that mutagenesis, including modern genome editing alters the genetic material of an organism in a way that does not occur naturally, and hence that such organisms constitute GMOs. It also ruled that the genome edited organisms do not fall under the same exemption as organisms that have been obtained by means of classical methods of mutagenesis. With this ruling the Court gave a very process-based interpretation to the EU GMO definition, and even though the case did not concern any of the other new breeding techniques, it is unlikely that the products of these other techniques would not be covered by the EU GMO legislation if the technique itself does not occur naturally.

**Should the products of new breeding techniques be subject to strict rules?**

The ruling of the CJEU creates a situation in which genome edited organisms are as strictly regulated as transgenic organisms, while classical mutants do not have to fulfil such requirements. From a scientific point of view and safety perspective this is problematic, as genome edited organisms are at least as safe as classical mutants. The mutations introduced by genome editing can also occur naturally resulting from traditional cross-breeding. The GMO legislation is therefore no longer in line with our current scientific understanding and what we know about safety. A thorough revision of the legislation imposes itself. But in what direction should that revision go?

In general, products should be subject to special legislation if there are legitimate grounds for this because of safety considerations. In principle a small change in the genetic material can have a major impact. Yet this does not automatically mean that every product with a small modification is subject to special legislation. That is not the case, for example, with the products of classical breeding. These are generally regarded as safe, even though a few exceptions have appeared in the past. Products that we regard as safe reach the market because of the experience we have with the whole process of breeding, up to and including conducting variety trials. This experience should also be considered when determining whether, and if so which, new breeding products should be subject to special legislation. When drafting the legislation in this area, the authorities should take the following principles into account. The legislation should

1. rely on sound scientific knowledge;
2. take into account existing experience with certain types of products and the way in which these are handled;
3. be proportionate (only be stricter for valid reasons);
4. offer legal certainty and be predictable (should lead directly to approval if a dossier meets the requirements);
5. be enforceable.
6. Finally, the legislation should not discriminate (in other words: the same products should not be treated differently).

The need for enforceability also means that authorities must be able to distinguish a product that falls under the legislation from a product that does not. However, with many products from the new breeding techniques it is impossible to tell whether their genetic composition has been made by humans or occurred spontaneously, for the simple reason that the modification is no different from what can occur naturally. This ultimately means that it is difficult to justify subjecting products of for example *gene editing* to strict legislation.

## 5. The general public and their opinion

The GMO legislation was originally introduced because of questions about safety. When European citizens became distrusting, at the time of mad cow's disease and the dioxin crisis, the legislation was tightened in the hope of reassuring them. Strict legislation can however cause distrusting citizens to ask more questions: "Something that has to be so strictly monitored must be dangerous after all." How widespread is that distrust? Supermarkets hesitate to offer GMO-labelled products for fear of losing customers. But is that reticence justified?

On the whole, European opinion is presented as being opposed to GMOs, especially by organisations with an anti-GMO philosophy, such as Greenpeace, Friends of the Earth, Bioforum, the European and national Green parties, etc. An analysis of the Eurobarometer survey of 2010 [38] does indeed show that in certain European countries more than half of the population is negative about GMOs. More recent surveys (e.g. [39]) reveal more positive than negative attitudes. For instance, only 10% of Belgians surveyed did not want to eat GMOs, 38% did and the majority requested more information before making a decision. The latter is also evident from the obviously higher percentage of positive reactions to questions about a specific GMO, such as a genetically modified apple or rice that is disease-resistant. For genetically modified plants that differ very little or not at all from cultivated plants, the support is generally greater than for transgenic crops. In the Eurobarometer 2010 55% of all Europeans surveyed were positive about cisgenic apples, compared to 33% in relation to transgenic apples.

A recent Dutch report concluded that there is a positive attitude to GMOs if they come with an obvious social benefit. [40] Most Europeans surveyed are enthusiastic about GMO applications with an environmental or health benefit and are even prepared to pay more for it. [41-42] The willingness to eat GMOs is also expressed in purchasing behaviour. In a Swiss study three clearly labelled types of bread were offered on a market stall in five cities. The only difference was the maize component, which came from either a conventional, GMO or organic plant. [43] GMO bread, which was the same price as bread with the organic label, was still bought by 20% of the customers. If it was cheaper, then that figure rose to one in four customers. This experiment nicely illustrates that consumers appreciate the freedom of choice.

## 6. Communication

Scientific and technological developments can and should lead to a social debate, to check what is acceptable and under what conditions. That is very pertinent, for example, in the case of the discussion on climate change. But as with the climate change debate, it is not up to society to judge which scientific conclusions are correct and which are not. The debate about social acceptance should be conducted on the basis of correct, fact-based information.

Extensive and independent scientific research has not been able to demonstrate any GMO-specific adverse effects in the last few decades. These facts must be clearly and widely communicated to the general public and policymakers. With the advent of new breeding techniques comes the opportunity to re-assess legislation that was based on a technology in development, i.e. the GMO legislation. Those responsible must design legislation that carries out a risk evaluation on the basis of the traits of a new plant variety, not on the way in which the new variety was developed: by breeding, by conventional genetic modification or by new breeding techniques.

## 7. Recommendations

— Whether we are talking about climate change or GMOs, scientific facts are important.
— The education sector must put more energy into teaching students how to think critically about information, especially the kind they see on social media.
— Scientific knowledge as well as twenty to thirty years' experience with genetically modified plants shows that GMO technology does not entail any risks that are not also inherent to breeding in all its forms. The EU legislation concerning GMOs is therefore backward and takes no account of the advances made in science and technology. A thorough revision is urgently required.
— The EU legislation constitutes a barrier to diversification in the range of GMO products and plays into the hands of monopolies. For this reason alone, a thorough revision is essential.
— Certain newer methods of genetic modification produce plants that are no different from plants that result from breeding. There is no scientific or legal justification for subjecting them to the current GMO legislation.
— Genetic modification is a safe technology. The vast financial and human resources invested in checking and monitoring GMOs would for the most part be better spent on more pressing food safety problems.
— The pursuit of a sustainable agriculture sector and food security is far too important to exclude methods that could be the solution on moral grounds.

**Box: Plant breeding and sustainability**

Sustainability is generally defined as a status or development in which there is a balance of focus on both environmental, social and economic aspects (*People, Planet, Profit*). New plant varieties can contribute to sustainable development if they can positively influence one or more of these aspects and on condition that this advantage is not undone by negative scores in other aspects.

There are various different visions about what is sustainable in agriculture and food. Some people believe that sustainability can only be achieved by organic farming and natural products. Others are convinced that high-tech innovations are also extremely important for making agriculture more sustainable. Organic farming scores well on important environmental and animal welfare aspects but generates significantly lower yields per hectare. Moreover, with their high price tag organic products are not affordable for people on a low income.

Plant breeding has contributed significantly to making agriculture more efficient. Innovative plant breeding can make agriculture even more sustainable by creating plants that are less dependent on pesticides, fertilisers, water, etc., and by increasing harvest security. The introduction of more forms of disease resistance, the more efficient use of nutrients, properties that result in less waste in food production, and an increase in general resistance of crops: all of these are concrete examples of traits that can increase the sustainability of agriculture. If these objectives are to be met, it is important not to exclude any breeding technology in advance. The extent to which a new crop contributes to more sustainable agriculture also depends on the way in which it is cultivated in practice. The genetics of a crop is after all just one of the factors in a much larger agricultural operation. Moreover, it is crucial for sustainable agriculture that there is sufficient variation in the crops and varieties being cultivated.

The socio-economic aspects are also important when striving for sustainability. In that regard there is a lot of debate about the consolidation in the breeding industry and what that means for the farmer. A farmer benefits not only from a sufficiently wide range of varieties, but also from a sufficient number of suppliers. Healthy competition must continue to exist. The development costs and the very high registration costs of GMOs have turned genetic modification as a breeding technology into a phenomenon that is only accessible to a limited number of large multinationals. The higher the regulatory bar, the less accessibility enjoyed by small and medium-sized enterprises (SMEs). If such a bar is introduced for new forms of precision breeding, then it must be proportional, non-discriminatory and based on science.

**Box: Plant breeding and intellectual property rights**

Businesses and institutions that invest in crop breeding are entitled to fair compensation for their efforts. In plant breeding there are two systems of intellectual property protection in this area: plant breeders' rights and patent law.

Plant breeders' rights protect new varieties, regardless of the way in which they are generated. The holder of plant breeders' rights has the right to prohibit others from reproducing that variety for seeds and selling it. Farmers are allowed to use part of the harvest as seed, provided that they pay compensation to the person holding the plant breeders' rights. This is known as the *farmers' privilege*. Other breeders may use the variety freely for further breeding activities: that is called *breeders' exemption*.

The patent law protects inventions that are not restricted to a variety. A patent gives the holder the right to deny others the use of the invention for a predefined period, in exchange for making the invention public. Most genetically modified crops are patented. There is no *farmers' privilege* and *breeders' exemption* under patent law, which makes it stronger. Keeping aside part of the crop for use as seed is usually prohibited in practice by the purchase agreement for seed.

Products from classical breeding can also be protected by a patent, provided that the invention is new, innovative and industry-applicable, and is also not restricted to one variety. There are only a few examples of patented classical breeding products, the best known of which is a broccoli that is easy to harvest with machines.

Of course, innovative precision breeding techniques and their products can also be protected by a patent if they comply with the aforementioned stipulations. In the meantime, CRISPR/Cas9 and its variants have spawned a mass of patent applications. A limited number of them have now been approved, but because of the opposition in many cases, it is not really clear which patents will play a dominant role in plant breeding. Any policy restricting the use of these techniques to the happy few would unnecessarily dissolve the trust in the companies concerned and in the products developed using these techniques. Responsible development, the use of innovative precision breeding and the achievement of broad sustainability objectives would suffer as a result.

# Appendix: Examples of GMO applications

The discussion in the media is often about herbicide-intolerant GMO crops, but there are countless other examples, which we will illustrate below. We have prioritised crops that are already in the field or are almost ready for market. For that reason, some interesting developments have not been included, such as nitrogen-efficient crops that generate a good yield with fewer fertilisers, or rice that emits less methane during cultivation or that can be grown in seawater.

Fig. 6. Farmer Hafizur Rahman from Bangladesh with his crop of Bt aubergines (BARI variety 3), which generates a good and healthy harvest with far fewer insecticides. Source: Arif Hossain Alliance for Science.

**Bt aubergine**

Aubergines or brinjals are one of the most popular vegetables in Bangladesh. For many farmers, therefore, this fruit is an important source of income. However, to protect their aubergine harvest they have to spray the plants several times a week with insecticides. [44] If they don't, the plants and fruit would be eaten by a caterpillar, the eggplant fruit and shoot borer (*Leucinodes orbonalis*) And despite the spraying, 20-40% of the harvest is still lost to the caterpillars. Moreover, the intensive use of insecticides leads to health problems among farmers, unhealthy levels of insecticide residue on the aubergines that are sold and environmental damage.

Since 2014 farmers in Bangladesh have been able to grow four varieties of a genetically modified Bt aubergine that is resistant to the fruit borer. The varieties are developed and tested at BARI, the Bangladesh Agricultural Research Institute. The result: higher yields, less work, lower costs and more income, but most importantly of all, much lower levels of insecticide use (approx. five times less).

Bt refers to a family of proteins that are produced by the soil bacterium *Bacillus thuringiensis* (abbr. Bt). Bt proteins are typically toxic for specific kinds of insects: Cry1 is toxic for caterpillars, Cry3 for some types of beetles. They are used as a natural insecticide also in organic farming. Bt proteins are only toxic for insects that eat the protein and have the appropriate receptors in their digestive tract. When binding, the cells leak and digestion ceases to function. Bt is not toxic for other insects, other animals or humans, because they don't have the Bt receptors. Rather than spraying Bt on the aubergine, which would have to be done very frequently and would therefore be expensive and labour-intensive – and doesn't help if the caterpillar is already in the fruit – researchers have introduced the gene for the Bt protein into the aubergine, so that the plant itself produces the protein to protect itself against the caterpillar. The Bt aubergine was developed and tested in India and at BARI. Thereafter field trials and tests for food and environmental safety were repeated in Bangladesh. [45]

Bt cotton and Bt maize have been on the market for twenty years already and have resulted in significantly lower levels of insecticide use on these crops, leading to higher incomes, fewer hospitalisations among farmers in developing countries and more useful insects in their fields. [32, 33, 46-47]

### Herbicide-tolerant soy

Soy is the most traded agricultural product in the world, because it is a major source of protein in livestock feed. China is the largest customer, importing approx. 90 million tons in 2016. Europe imports approx. 35 million tons of soy every year. The annually increasing demand from China for soy can only be met by increasing yields per hectare or by expanding the cultivation area. This cultivation takes place primarily on the American continent, where more than 300 million tons of soybeans are produced annually. [48]

In 2016 78% of the soy crop (worldwide approx. 120 million ha) was herbicide-tolerant thanks to genetic modification, making GMO soy by far the most cultivated GMO crop. Herbicide-tolerant soy is an example of a GMO with a direct benefit for farmers. The resulting crop leads to efficient weed management, which significantly reduces the labour required and the cost of production. Furthermore, herbicide-tolerant crops require less tillage. This low or no-tillage farming comes with environmental benefits: less fuel consumption and lower $CO_2$ emissions, greater carbon capture in the soil and a better soil structure with more biodiversity. [49-50]

The other side of the coin is less rose-tinted: the unilateral large-scale use of glyphosate has led to a steep rise in glyphosate-tolerant weeds and residues, some of these in the groundwater. In Europe as well, the discussion on the use of glyphosate in farming rages on, even though glyphosate-tolerant GMO crops are not permitted in the EU. This is because glyphosate is also used to get rid of weeds between crops.

Glyphosate operates on an enzyme from the biosynthesis of aromatic amino acids in the plant. The glyphosate-tolerance of soy (and also of corn, maize, sugar beet …) came about by providing the plant with an enzyme variant that is not sensitive to glyphosate. [51] Other herbicide-tolerances are also available for herbicides such as glufosinate and imidazolinones, by means of mutation or production of an enzyme that deactivates the herbicide. [52] This is how for example the French winter wheat variety Fidel was converted by chemical mutagenesis into an imidazolinone-tolerant mutant. Such mutant herbicide-tolerant plants are not subject to GMO legislation.

**Virus-resistant papaya**

In many developing countries papaya is an important fruit for a healthy diet. A quarter or 100 g of papaya per day contains enough vitamin C and a quarter of the recommended amount of vitamin A. This also promotes the uptake of iron. Global production is however threatened by ring spot, a disease caused by the papaya 'ringspot' virus (PRSV). Because of this virus the papaya plants grow less vigorously, their leaves become malformed and the fruit are less abundant and of lower quality. The virus was discovered in 1945 on the Hawaiian island of Oahu and spread to other islands. Between 1992 and 1997 papaya production in Hawaii fell by more than 30% as a result of PRSV infection, which caused many farmers to stop growing the fruit.

In the 1980s Cornell University (U.S.) in collaboration with Hawaiian researchers developed a genetically modified papaya variety that is resistant to PRSV. [53] The resistance is based on the activation of the plant's natural resistance mechanism to the infection, whereby the papaya immediately breaks down the incoming viral RNA. [54] Following successful field trials between 1992 and 1995, the authorisation procedure for commercial cultivation of GMO papayas was started in 1996, and in 1998 the seeds were made available to Hawaiian farmers. With the massive adoption of the virus-resistant papaya, production was restored to pre-PRSV levels in less than four years.

Similar resistant papayas have since been developed in Brazil, China, Indonesia, Malaysia, Thailand, Venezuela, Australia and the Philippines, amongst other places. In 2015 in China the virus-resistant GMO papaya was grown on 7000 ha, approx. 90% of the total crop surface for papayas in that country.

Fig. 7. Researcher Dennis Gonsalvez with the virus-resistant papaya (Source D. Gonsalvez).

The strategy for developing genetically modified virus-resistant plants is fairly simple. On the basis of the genetic information of the virus a construct is developed that produces a fragment of double-stranded RNA after transformation in the plant. That activates the RNAi process that occurs naturally in the plant and thus protects the papaya from the viral attack, much like the way in which a vaccination works on humans. With this same process a virus-resistant plum and potato have also been approved for commercialisation in the U.S. and field trials are currently underway in Uganda with virus-resistant cassava, amongst other things.

**A better potato**

The potato is important in Belgium, both for our national love of French fries and for its inclusion in all kinds of products that are also sold abroad. However, the crop is still plagued by the potato blight, a very destructive disease caused by the water mould *Phytophthora infestans*. Around 1845 the potato blight caused the complete failure of the potato harvest and led to the greatest famine in Irish history. In an average year potato fields in Belgium are sprayed between ten and fifteen times with anti-fungal products; in wet summers this rises to twenty times. As a result, the potato is the number one recipient of fungicides in Belgian farming. In organic potato cultivation, farmers try to prevent blight by harvesting early and being content with a lower yield, or by spraying copper fungicides.

Tuberous Solanaceae from Latin America, wild relatives of our potato, are in many cases resistant to blight. These natural resistance genes can be introduced into commercially grown potato varieties by means of a complicated and lengthy crossing process. Thus, in the Netherlands in 1959 a breeding project was started that eventually brought the varieties Bionica and Toluca to the market in 2005. However, these two new, blight-resistant varieties are unpopular because they don't taste as good and are unsuitable for industrial processing.

Cisgenesis is an alternative method for developing blight-resistant potatoes. [55] In this process genetic modification is used to insert the same (wild) natural resistance genes into a commercial potato variety. This is faster and more targeted than breeding, with the added advantage that you can combine several resistances and that the good characteristics of the potato variety are retained. In Flanders a cisgenic Bintje is being developed that combines four blight-resistances.

In the U.S. scientists are already a step ahead. There, the firm Simplot is bringing the Innate® potato onto the market, in four varieties. Apart from being blight-resistant, this potato is less vulnerable to bruising, has a longer shelf-life, does not colour after peeling or cutting and produces less of the potentially carcinogenic substance acrylamide during deep-frying. [56] That last aspect recently popped

Fig. 8. Field trial in 2012 with genetically modified potatoes that are resistant to potato late blight (central bed), in comparison to control Bintje (the two rows with brown plants). Bintje potatoes are very susceptible to blight and didn't last long. In the field trial the crops were not sprayed for blight at all, while farmers elsewhere in that season conducted up to twenty fungicide treatments in order to keep the blight under control.

up in the media when the EU called into question the typical Belgian fries because of the high levels of acrylamide formation. All these improvements in Innate® potato are the result of cisgenesis or RNAi. So, for example, RNAi results in lower levels of the enzyme polyphenol oxidase, which causes bruising and other discolorations. Moreover, now in the U.S. you can buy genetically modified apples that do not change colour so quickly when cut open. [57]

### Fungal-resistant wheat

Mildew is a frequently occurring disease in wheat. It is caused by the fungus *Blumeria graminis,* which affects the leaves and is clearly visible as fungal spots that turn grey and then later much darker. The disease affects the photosynthetic capacity of the plant, which results in smaller grain kernels and a lower yield. If the fungus attacks early and the weather conditions promote fungal growth, the decline in yield can run to 40%.

The fungus in wheat happily makes use of the presence of the *MLO* gene which suppresses resistance to the infection. Scientific research has shown that when the equivalent of the *MLO* gene is no longer functional in thale cress, barley or tomato, these plants are resistant in the long term to the type of mildew that affects them. In wheat, however, spontaneous or induced changes in the *MLO* gene that make the plant resistant to the fungus have never been discovered. This is because the *MLO* gene is present in three variants and because wheat is hexaploid. This means there are six copies of the gene which all have to be deactivated in order to achieve functional resistance.

Chinese scientists have now succeeded in deactivating the six *MLO* genes in one go thanks to recent precision breeding techniques. [58] The resulting wheat seems to be perfectly resistant to mildew. In this specific case the scientists did not use CRISPR, but the similar TALEN technology which is designed so that a cut is performed in the six *MLO* genes simultaneously. This is then repaired by the DNA repair machinery naturally present in cells, a process in which minor changes may occur spontaneously. During this kind of repair activity some DNA letters will often get lost, which results in a gene that is no longer functional. An additional benefit of this precision breeding technique compared to classical breeding is that the variety traits of the wheat used are retained.

### Drought-tolerant maize thanks to biotechnology

Every year, crops around the world are affected by drought, a phenomenon that leads to a considerable loss of yield. The extent of this loss depends on the duration of the drought and on the growth stage of the plant when the drought begins; in maize, losses can run to 30% or more. Climate change will only exacerbate this phenomenon because drought will become more common, mostly in combination with heat. The extremely high temperatures that we witness now especially in southern Europe are a taste of what is to come. For this reason, a lot of research is being targeted at drought tolerance and companies are searching for innovative solutions to reduce crop failure caused by a shortage of water. As an example, Syngenta and Pioneer used advanced breeding techniques and molecular biology to respectively bring Agrisure Artesian and Optimum AQUAmax maize hybrids to market. These do noticeably better in drought conditions than the traditional hybrids. Monsanto put the drought-resistant Genuity DroughtGard hybrids on the market. These were produced by means of biotechnology: a gene from a naturally-occurring soil bacterium was inserted to offer the plant protection under drought stress. [59] The DroughtGard plants give a 5 to 7% higher yield in drought and the technology has no effect on the yield in non-drought conditions. The plants use the available water more efficiently. It is generally expected that there will be more biotechnological solutions on the market in the coming years to offer greater protection to plants in drought conditions.

Fig. 9: In Africa the WEMA project (WEMA = Water Efficient Maize for Africa, https://wema.aatf-africa.org) tests new maize varieties that are more drought-resistant. The photo was taken in January 2017, in Tanzania's first GMO field trial (Dodoma). Source: Hannah Smith Walker, Alliance for Science.

## Biotech superfoods: purple tomatoes help in the fight against chronic diseases

Plants contain thousands of metabolites, many of which have a natural healing effect on humans. For instance, phenols in vegetables and fruit play a key role in preventing harmful oxidation reactions. They are the active components in so-called *superfoods* and prevent the development of chronic disorders, such as certain cancers, cardiovascular diseases, obesity and age-related degeneration. There are strong indications that a diet rich in antioxidants can help prevent chronic disorders. These active components include anthocyanins, which are responsible for the purple colour in blueberries and blackcurrants, amongst others. Many wild tomatoes also contain these anthocyanins, in contrast to cultivated varieties.

A team of British scientists affiliated to the John Innes Institute in Norwich succeeded in creating purple tomatoes by adding two genes from the snapdragon. [60] The levels of anthocyanins in these transgenic tomatoes are just as high as that in blackcurrants and blueberries. It was not possible to obtain such levels with classical breeding techniques. Tests showed a reduced risk of tumors in cancer-susceptible mice. An extension of life by 30% was established in mice who were given purple instead of red tomatoes in their diet. [60] Moreover, the shelf-life of the purple tomato is twice that of the red tomato and it is also more resistant to the fungus *Botrytis cinerea*, which causes fruit rot. [61] Another advantage is that the fruits can ripen for longer on the vine, making them tastier.

For the large-scale cultivation of purple tomatoes scientists had to relocate the plants to Canada, because European legislation still prohibits the cultivation of transgenic tomatoes. Clinical tests have begun on the juice of these tomatoes, with the aim of finding out if the fruit juice is effective in preventing cardiovascular diseases in humans.

Fig. 10. The purple tomatoes produce more anthocyanins, making them more resistant to fungal diseases in the process. Source: Hsi-Hua Wang (Cathie Martin’s lab).

**Biofortification: biotechnology offers a solution for the problem of ‘hidden hunger’**

In April 2016 the United Nations set itself a goal of banishing all forms of malnutrition from the world by 2030. Although acute hunger (calorific malnutrition) has declined, affecting about 800 million individuals, more than 2 billion people are currently suffering from 'hidden hunger' due to a shortage of vitamins and minerals, the result of an unbalanced diet. Micronutrients are very scarce in the main staple food crops, feeding more than half of the world's population: rice, maize, wheat, cassava and potato. They are a daily source of calories for the poor. While a varied diet is obviously an ideal way of preventing deficiencies, this is often difficult to achieve in practice. Alternative solutions are required.

'Hidden hunger' can be tackled with supplements in the form of pills or by adding nutrients to flour, but these solutions are not feasible in developing countries. Biofortification on the other hand is a strategy whereby the plant itself produces or acquires more micronutrients. The importance of this approach was recently recognised with the award of the World Food Prize 2016 in this area. [62] In some cases good results can be obtained with conventional breeding. This is the case with the enhancement of provitamin A (beta-carotene) in sweet potato. In other cases, the necessary increase in the micronutrient cannot be obtained by breeding because the level of the micronutrient in the harvested product, for example the seed or the fruit, is far too low, and the diversity in the content of that micronutrient is insufficient in wild varieties. In such cases the only solution is to add the necessary genes by means of genetic modification. Very good examples are the increase in vitamin A and vitamin B9 (folates) in rice.

Fig. 11. Golden rice (left) contains more provitamin A than ordinary rice. Provitamin A is the same pigment as that which occurs in carrots. Source: Ingo Potrykus.

Golden Rice, which is rice that contains increased levels of beta-carotene (provitamin A), was created by Swiss and German scientists [63] but will probably not be available on the market until 2019, and then mainly in Bangladesh. The overly complex legislation as well as the anti-GMO campaigns have held back the marketing for almost 20 years. Approx. 250 million children worldwide suffer from vitamin A deficiency because of poverty and an unvaried diet, and every year between 250,000 and 500,000 of them go blind as a result. Golden Rice can offer them a better life. The local rice varieties in which the GMO characteristic was bred contain sufficient beta-carotene, so that 200 g rice provides half of the daily recommended amount of vitamin A.

Achievements in the area of rice containing folates (vitamin B9) are also promising. In addition to anaemia, folate deficiency in pregnant women can lead to incomplete development of the embryonic neural tube. That can in turn cause spina bifida. In certain regions of China and India studies report at least ten times more cases of such abnormalities compared to the West. In order to stay healthy, adults need approx. 400 micrograms of folates per day, and pregnant women 600 micrograms. In order to get the daily recommended amount of folates in a 150 g portion of rice during pregnancy, a 120-fold increase in folates in the rice grain should be attained.

By means of genetic modification, researchers at UGent have developed rice lines with up to 150 times higher folate levels, which are also stable when kept for several months at high temperatures. [64] These lines can be crossbred with local varieties that are consumed in countries where rice is the staple diet for large populations. Furthermore, these prototypes and the Golden Rice can be used as a basis for multibiofortification, whereby due to a combination of genes involved in the biosynthesis of various vitamins and in the uptake of minerals, a more all-round solution can be offered for ‘hidden hunger’.

## Hypoallergenic apple

One to two percent of the adult population has a food allergy and that percentage continues to rise. In Europe eight categories of food are indicated as high risk for people with food allergies, the most well-known being nuts and soy. If there is a real chance that these products or their derivatives are present in food, this must be stated on the label. There are also products for which there is no obligation to do this but which can nevertheless cause allergies. The apple is one of them. As with many food products that can cause allergies, there are several proteins (allergens) in apples that can cause an allergic reaction. The main culprit is the Mald1 apple protein.

The apple has 31 *Mald1* genes, twenty of which are expressed in the fruit. [65] Such a large number makes it virtually impossible to get rid of the genes with classical breeding. A different approach is needed. One possibility is to suppress the expression of the *Mald1* genes with RNAi. Using this technique researchers in the Netherlands have already succeeded in reducing the expression of the Mald1 protein in Elstar apples to a large extent (0.1-16.4% of the original quantity). [66] When people suffering from the allergy ate the apples, the allergic reactions seemed to be drastically reduced. The researchers were thus able to show that the reduction or elimination of Mald1 has real clinical benefits.

Modern precision breeding that uses the CRISPR/Cas system now offers the possibility to make apples in which the *Mald1* genes are completely deactivated in one go. The complete elimination of so many genes in one go has not previously been reported in plants, but scientists have succeeded in inactivating all 62 copies of a retrovirus present in the genome of pigs with CRISPR. [67]

# Appendix: Popular GMO myths

One important discussion point is that most cultivated GMOs are marketed by multinational companies who have patents for those GMOs and who want to make a profit. This is correct and is partly due to the regulatory aspects that have become more complex and more expensive with the years. It is also because of the opposition, which makes it increasingly difficult to carry out field trials without spending a lot of extra money on security. The virus-resistant papaya in Hawaii was developed and brought to market by a university professor (see the case study on virus-resistant papayas above) and was approved for market just before opposition to GMOs reached that country. Later field trials in Thailand with local virus-resistant papayas were destroyed by Greenpeace.

Many of the discussions about GMOs are based on myths not facts. Here we will explain a few of them.

**Myth 1: GMOs are unnatural compared to breeding.**

The bacterium *Agrobacterium* is responsible for naturally occurring transformations that are not subject to GMO legislation because they were not induced by humans (e.g. sweet potato, tobacco, common toadflax). If you look at the end result of a GMO compared to a plant resulting from certain forms of breeding, the bred plant has undergone far more dramatic changes. In breeding there is also crossing between species and/or radiation to induce mutations or to cause chromosomal breakages. Virtually every modern wheat variety contains DNA of grasses that was introduced via crossing and detached from the grass chromosomes by irradiation. Thereafter it was attached to a wheat chromosome by the plant's repair enzymes.

**Myth 2: GMOs are not tested and are unsafe.**

Nearly all studies and publications support the view that crops resulting from genetic modification are safe. The majority of analyses are not conducted by companies. The European Union has invested a lot of money over the last twenty years in GMO safety research with projects at universities and research institutes. Unfortunately, some publications rely on inferior research or on incorrect interpretations of data that claim the opposite and have reached the press. They are eagerly distributed on the internet to give the impression that GMOs are unsafe. Nothing could be further from the truth: GMOs are the most tested foodstuff ever because of the thorough biosafety analyses that have to be undertaken before a GMO can be approved. Recommended literature: [15-16]

**Myth 3: GMOs are sterile because of the terminator.**

This completely false claim is spread far and wide by organisations (and political parties) that are opposed to GMOs. Terminator technology consists of expressing a cell-killing gene in the new seed of a planted crop, so that this seed cannot germinate in the following generation. The strategy is patented as a means of preventing farmers from reproducing their own GMO seed and to stop the spread of GMOs. However, the principle has never been used in commercial applications.

**Myth 4: a GMO consists of just one variety and creates a big monoculture.**

An approved GMO can be used to make new varieties and hybrids by means of crossing; this automatically falls under the same approval dossier. So, on the basis of a GMO, varieties can be generated that are adapted to a different climate, soil, etc. Thus, in Spain alone there are already 200 varieties of Bt maize. In Argentina, more than 500 new roundup-tolerant soy varieties have been registered since the beginning of this century. [68-69]

**Myth 5: Bt cotton is the cause of the dramatic rise in the number of suicides in India.**

In India many poor farmers have huge debts and some of them become desperate and commit suicide. However, this phenomenon is not directly related to the cultivation of Bt cotton. In fact, quite the opposite is true: farmers that grow Bt cotton usually see an improvement in their economic circumstances. [32, 70-71]

**Myth 6: the patents of multinational companies are partly the reason why there are so few GMOs that are specifically beneficial to developing countries.**

All patent problems concerning Golden Rice were dealt with in six months, which meant that the rice could be distributed free to small local farmers. Moreover, most patents on GMOs are not applicable in many developing countries, because the applicant has only paid for an application in certain other countries. It would indeed be useful to make more publicly financed efforts to donate GMOs with benefits for poor farmers in developing countries, in the form of foreign aid, or to distribute them at a minimal cost.

# Appendix: Glossary

***Agrobacterium***: a soil bacterium that in nature transfers a fragment of its own DNA to the plant, whereby it is inserted into the genome. In nature the bacterium thus stimulates the formation of a gall or fast-growing roots that can be used by the bacterium as a food source. In the lab this bacterium is adapted so that each desired fragment of DNA can be transferred to the plant without the formation of a gall or roots.

**Allele**: variant of a gene. Every higher (diploid) organism has two alleles from each gene that lie on the homologous chromosomes. One of those alleles is passed on by each parent via the reproduction cells to the progeny. In plants there is often a higher ploidy, which means more alleles. Wheat is hexaploid and has six alleles for every gene.

**Biofortification**: adaptation of a plant's metabolism in order to obtain higher concentrations of vitamins (e.g. vitamin A), minerals (e.g. iron) or other essential nutrients. Biofortification can often be achieved by breeding, but in other cases only by genetic engineering.

**Bt**: *Bacillus thuringiensis*, a bacterium that in nature kills insects by producing proteins that are specifically toxic for a certain group of insects. The bacterium and its endospores are used in organic farming as a natural insecticide. The genes that code for the Bt proteins are introduced into crops, e.g. Bt maize, Bt aubergine, Bt cotton, via genetic modification to protect them against insect damage.

**Cisgenesis**: a form of genetic transformation that only uses genes that can also be introduced via crossing.

**CRISPR-Cas9**: a combination of CRISPR (*clustered regularly interspaced short palindromic repeats*) and the Cas9 enzyme that can be adapted to cut a specific sequence via an RNA that recognises the DNA and the Cas nuclease that cuts at that position.

**DNA** or Deoxyribonucleic Acid: the molecular carrier of the genetic information is a double helix in which every strand consists of a sugar phosphate backbone and is connected by nitrogen-containing base pairs. The four possible bases are the letter codes of the genetic information that is fixed in the specific sequence of the bases. The human genome contains approx. three billion base pairs.

**Phenotype**: a characteristic or combination of characteristics of an organism resulting from the genotype and the effect of the environment.

**Gene**: the basic unit of genetic characteristics is a fragment of DNA that codes for a protein and thus contributes to the phenotype. (This is a simplified definition, because certain genes code for an RNA that is not converted into protein. There are other exceptions too.)

**Gene editing**: targeted modification of a gene, in situ in the genome.

**GMO (genetically modified organism)** (legal definition in the EU Directive 2001/18/EC (http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32001L0018): 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. In principle therefore applicable to every kind of organism except humans, in practice usually referring to plants.

**Genetic modification**: can refer to all possible methods that alter the hereditary characteristics of an organism (including breeding) or more specifically to methods that lead to a GMO (legal definition). The term *genetic engineering* is also used and perhaps better describes the process of combining recombinant DNA and transformation in order to achieve a genetic modification.

**Genome**: the complete set of DNA in an organism.

**Genotype**: the specific DNA sequence of the genome or part of it.

**Hybrid**: the progeny of a crossing between two inbred strains (parents who have the same alleles for all characteristics). Hybrid progeny are all identical and usually have increased resistance and yield compared to their parents.

**Intragenesis**: a form of genetic transformation that only uses DNA that could also be inserted via crossing. Intragenesis differs from cisgenesis because it recombines this DNA to create a new gene.

**Inbred strain**: plants obtained by repeated self-pollination, so that after a few generations the two alleles of every gene are the same. As a result all progeny are like each other and their parents.

**Marker-assisted selection (MAS) or breeding**: the most suitable progenies were previously selected by comparing their characteristics. However, certain characteristics are strongly influenced by environment, and others, such as the fruit quality of trees, can only be analysed by means of time-consuming experiments. Nowadays, there is a quick and cheap way of finding out which DNA fragments are responsible for a certain characteristic. The absence or presence of that DNA fragment (the marker) can be used in the progeny to make the selection.

**mRNA**: *messenger RNA*. An RNA molecule that is transcribed from DNA and is used to translate the genetic information into protein.

**Recombinant DNA technology**: the enzymatic cutting and pasting of DNA to make new gene combinations, followed by the transformation of this DNA in an organism.

**RNA**: ribonucleic acid. Consists of a single strand with a sugar phosphate backbone and nitrogen-containing bases. RNA is transcribed from DNA and it can be mRNA or it can function as such, e.g. in controlling cellular processes.

**RNAi**: a natural phenomenon whereby cells recognise double-stranded RNA as something foreign (e.g. originating from a virus) and cut it into little fragments (siRNA). These fragments can in turn recognise the associated RNA and break it down. The process can be modified to inactivate a specific mRNA.

**Synthetic biology**: the design and construction (by chemical DNA synthesis) of new artificial biological pathways or organisms, or the redesign of existing biological systems. (This is one of the many possible and widely differing definitions. See annex III of the document on the website http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_044.pdf.)

**TALEN** (*Transcription Activator Like Endonuclease*): a protein that is a combination of a DNA-recognising protein (Transcription Activator Like Endonuclease) and an enzyme that cuts DNA. The combination can be applied to cut specific sequences in a genome.

**Transgenesis**: the process whereby DNA (if necessary adapted by recombinant DNA technology) is introduced into an organism by means of transformation. The new DNA can come from a non-related species, e.g. a bacterium that contains the human insulin gene.

**Transformation or genetic transformation**: technique for introducing DNA into an organism, at the cellular level. In the case of a plant cell, a complete plant is regenerated from the transformed cell.

**Breeding**: the improvement of plants by humans, by for example targeted crossing and then selection of the best progeny.

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