



Società Italiana di Genetica Agraria
Italian Society of Agricultural Genetics

Società Italiana di Biologia Vegetale
Italian Society of Plant Biology



Position document on genome editing techniques applied to agriculture

Summary and Recommendations

- Spontaneous, random genetic mutations have been essential for the evolution of cultivated plants and are thus at the basis of our own existence. All plants cultivated today are the results of a long process of selection of mutated plants. Starting thousands of years ago from wild species, this has led to plants that are suited to cultivation and now supply nutritious and safe food at affordable costs.
- Since mid-nineteenth century, new mutations have been artificially introduced, using technologies that produce random mutations at high rates. These plants are not subject to specific regulations. New, more accurate technologies have been developed during the last thirty years, allowing to introduce specific characteristics: genetic modification using recombinant DNA (these plants are generically termed GMO) and, very recently, *genome editing*. The latter can introduce defined mutations indistinguishable from spontaneous ones. These plants are either severely regulated (GMOs) or are at present in a legal limbo (genome editing).
- During more than twenty years of cultivation, no danger for health or the environment has been specifically associated to the GMO technology. Conversely, the environmental and economic effects have often been positive. Accurate monitoring must continue for these cultivations, as well as for any agricultural practice and cultivation, traditional or innovative. However, the stringency of GMO regulations imposes an economic burden that practically favors large multinational companies at the expense of public research and small enterprises. Time is ready to implement regulations that modulate severity according to both the technology and the genetic trait. Evaluation of a new plant should be mainly based on the specific characteristics that have been introduced, rather than only on the technology used.
- Within this perspective, **we recommend a revision of the European Union Directive 2001/18/EC, which regulates GMO approval and cultivation. The regulatory system must be based on the predictable risk of the product, which depends on the combination of genes used and the species receiving them.**
- A decision to include among GMOs the products of genome editing, when these have mutations indistinguishable from spontaneous ones, would be a serious mistake under many aspects: science, regulatory matters, logics and economics. **Until Directive 2001/18/EC is revised, we recommend that genome editing products be excluded from it, when they do not contain gene combinations different from those potentially resulting from random natural mutations or conventional breeding.**

We are living in an era of great changes in society, economics and the natural environment, some of which are particularly rapid and profound. One of the effects not immediately perceived by most people, but probably very important for our future, is the strong pressure endured by agriculture to provide sufficient, safe and nutritious food to a fast-growing global population, and at the same time reduce the environmental impact of food production. Building on the advancements of scientific research, all agricultural practices are under continuous revision and improvement to face these challenges. Genetic improvement has historically been, and continues to be, the main option to obtain more nutritious and productive plants better suited to a changing world and to reduce the environmental footprint of agriculture.

Scientific research produces new knowledge and technology that induce society to revise its opinions and regulations to avoid the existing rules becoming equivocal, outdated or inapplicable. This position document provides scientific information and proposes recommendations regarding plants obtained using some of the novel technologies called *New Plant Breeding Techniques* (NPBT), especially those involving *genome editing*. This will be discussed within the larger framework of plants produced using recombinant DNA, commonly and collectively called Genetically Modified plants (GM plants) or, using a more generic term, Genetically Modified Organisms (GMO). Because plant genetic improvement has actually existed since agriculture was born, we will also relate these techniques to the technologies that have been used historically to produce and select novel plant varieties.

Documents and position papers on this topic have recently been published by other European scientific organizations and societies¹. There is substantial agreement on the recommendations that are considered necessary and urgent.

1) The importance of mutations in cultivated plants

Cultivated plants are the products of human selection through millennia. Their properties derive from random mutations that make them different from their wild relatives. These properties are advantageous for us – more productivity, better quality – but often negative for the survival of plants in the wild: the characters associated to plant domestication, collectively named “domestication syndrome”, make plants less likely to survive in a natural environment. A similar phenomenon occurs in domestic animals: consider that most breeds of dogs have been artificially selected for characteristics that make them unable to survive without human care.

Among plants, the most striking example is the loss of seed dispersal ability in cultivated cereals and legumes (Figure 1), a feature that obviously helps seed collection by farmers, but makes the plant almost unable to reproduce spontaneously. Had these random mutations - like many others beneficial for agriculture but detrimental for plants' survival - not been selected by ancient farmers, they would have been quickly eliminated by natural selection.

¹ European Academies' Science Advisory Council (EASAC): <http://www.easac.eu/home/reports-and-statements/detail-view/article/easac-statem-2.html>

European Plant Science Organization (EPSO): <http://www.epsoweb.org/file/2147>

Biotechnology and Biological Sciences Research Council (BBSRC), United Kingdom:

<http://www.bbsrc.ac.uk/news/policy/2014/141028-pr-position-statement-on-crop-breeding-techniques/>

The domestication syndrome consists therefore in the stacking of DNA mutations that alter the expression of specific genes or the function of their products. **It follows that all cultivated plants are genetically modified organisms** when compared to their wild relatives, and that these modifications are inherent to agriculture. It does not make sense to judge genetic modifications as dangerous or inherently negative *per se*: instead, the characteristics of the resulting plant should be considered.



Fig. 1. Cultivated and wild rice

2) Genetic improvement by crossing and selection

Genetic improvement usually relies on the genetic variability of plants obtained from crosses within a species or within sexually compatible species, among which those with the desired trait are selected by breeders.

Cells contain at least two copies, also called “alleles”, of each gene. The two alleles can be identical, but they often have small differences due to random mutations. For each gene, every individual receives one allele from both parents. Therefore, sexual reproduction generates new combinations of alleles and consequently, new genetic variants (Figure 2). Depending on the species, every plant has between 25,000 and 80,000 genes. Therefore, the number of possible allele combinations available for selection is so large that it is practically unlimited. To improve plants, breeders choose the individuals to cross and then select the progeny with the best traits.

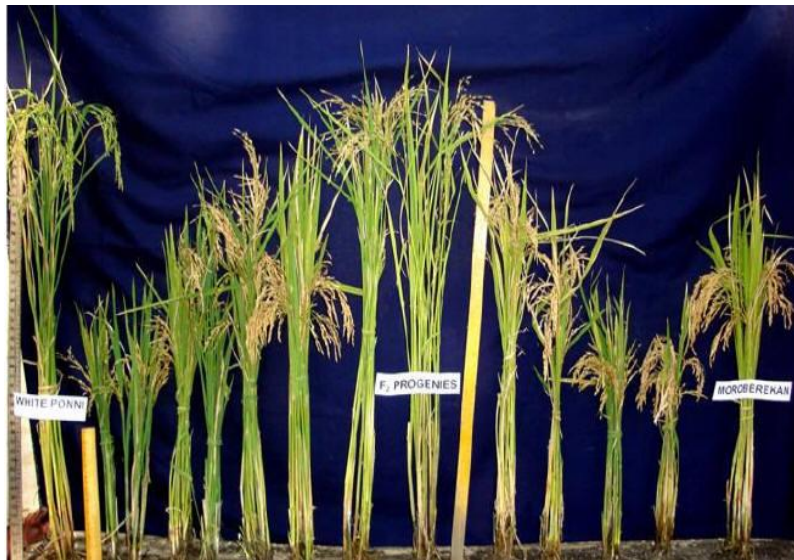


Fig. 2. Variability in the progeny of a cross between two rice varieties. The two parents are at left and right. *Image by I. Selvaraj, Vellore University (India).*

Crossing between different but sexually compatible species is mainly used to transfer one or more favourable traits from the donor species, usually a wild relative, to a cultivated one. However, crossing not only transfers the alleles of interest, but it combines all parental genes. To have a stably improved variety for the desired traits, breeders must therefore “clean” the new plants, by removing all the unwanted alleles that have been inherited together with the desired ones, especially when the donor plant is a wild relative. To this purpose, a series of consecutive backcrosses with the original cultivated parent is performed. The final result will be a plant almost identical to the original cultivated parent, but with tens or even hundreds of genes from the wild donor. These will include those responsible for the desired new traits, which will be acquired by the new plant while retaining all or most of the original beneficial traits of the cultivated parent. This process, known as “introgression by backcrossing”, is widely used in breeding to introduce, for example, disease resistance from wild relatives.

3) Random mutation breeding

The wider the genetic variability in a population, the higher the possibility of finding individuals with new, desirable traits. Besides exploiting natural variability, it is possible to increase markedly the frequency of mutations by exposing seeds or plants to high-energy radiation (X-rays, gamma-rays), chemicals (usually alkylating agents) and/or biological tools (transposons, which are DNA sequences that naturally change their position in the genome, thus randomly inactivating genes). In any case, the final goal of all these mutagenesis techniques is to generate new alleles in a population.

These treatments, in use since the mid-twentieth century, induce random mutations in hundreds or thousands of genes. Simple substitution of single nucleotides (the “letters” of the genetic code), deletions of thousands of letters, repositioning of entire “chapters” (millions of nucleotides), gene duplications or deletions are randomly generated. The range and effects of most of these mutations are never identified. As

already mentioned, mutations are natural events that occur in any living organism; random mutagenesis breeding just increases their frequency. A classic example of successful mutagenesis breeding through high energy radiation is the production of semi-dwarf durum wheats by Italian public researchers in the '60s-'70s of the last century (Figure 3), the most well-known being Creso, widely used to create new varieties of pasta wheat by crossing.

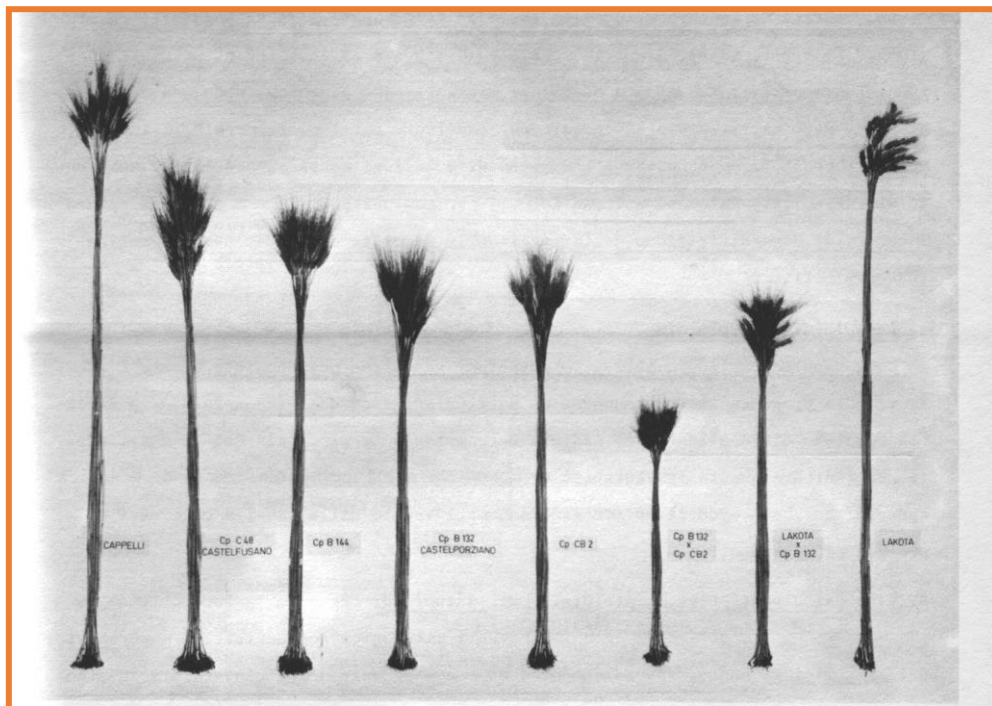


Fig. 3. Durum wheat mutated plants generated in Italy through high energy radiation. CP B144 has been used by breeders to produce the Creso variety.
Source: ENEA

More than 3,000 varieties of different crop plants generated through this technique are cultivated globally, representing over 200 species². Because the mutations are generated randomly, their effects are unpredictable. Most importantly, due to the high number of mutations often obtained by a single treatment, it is practically impossible to completely eliminate the other unknown changes, even after many backcrosses performed to select the desired traits. These improved plants thus contain also an undefined number of unknown mutations. In spite of this, the experience accumulated in more than half a century and based on millions of mutagenised plants allows us to conclude that random mutation breeding has never caused any specific health or environmental problems. Accordingly, Directive 2001/18/EC, which regulates the cultivation of GMOs within the EU, correctly defines this mutagenesis technique as a “genetic modification”, but at the same time explicitly states that the Directive shall not apply to mutagenised plants.

² Mutant Varieties Database -MVD: <http://mvd.iaea.org>

4) Genetic improvement through horizontal gene transfer

Horizontal gene transfer is the introduction into a genome of a DNA sequence derived from an organism that can be of the same or any other species, while not being one of the parents. This is to distinguish this process from the “vertical” transfer of genes from parents to their progeny. Horizontal gene transfer is a particular type of genetic modification that also occurs in nature, where it plays an important role in the evolution of bacteria. Sequencing of genomes has shown that horizontal gene transfer also occurs in plants and animals, albeit rarely. One example is sweet potato: genes from a bacterium are naturally present in the genome of this tuber³, an important staple food for hundreds of millions of people, without causing problems or requiring specific regulation.

Transgenesis is when horizontal gene transfer occurs artificially in the laboratory using genetic engineering based on recombinant DNA techniques. Transgenic organisms produced in this way are commonly called **Genetically Modified Organisms (GMO)**. To produce **GM plants**, scientists often take advantage of a natural process through which the bacterium *Agrobacterium tumefaciens* transfers a number of genes to the plants that host it.

Any gene of any origin can be used to produce a GM plant. This allows the introduction of traits that are naturally very rare or absent in a given species or in sexually compatible species. The technology has therefore shown to be extremely powerful for introducing very useful traits into plants: resistance to diseases, pests, or environmental stresses, quality improvement, and even the use of plants to produce protein pharmaceuticals and vaccines. Twenty years after their first introduction into agriculture, GM plants are currently being grown on 180 million hectares, corresponding to 15 times the Italian cropland and representing more than 12% of the global cultivated area. It does not make sense to set transgenesis apart from the other techniques of genetic improvement that have been used for thousands of years. Conversely, it complements them and, like them, it is used to create plants with novel traits that would not have been possible without human intervention.

The more distant in evolution the donor and the recipient plant are, the more different the outcome is going to be from that of sexual crossing. The organisms resulting from horizontal gene transfer through genetic engineering can thus be divided into three groups, depending on the origin of the transferred DNA:

- **transgenic:** the inserted DNA fragment originates from donor organism(s) not sexually compatible with the recipient organism;
- **intra-genic:** the inserted DNA fragment originates from donor organism(s) of the same or a sexually compatible species as the recipient, but has been modified in the laboratory, or the sequence(s) that regulate its natural expression - the so-called regulatory sequences, which “decide” when, how much and in which organs a gene is expressed - have been altered or substituted with those of other genes.
- **cis-genic:** the inserted DNA fragment originates from the same species or a sexually compatible species as the recipient organism; the sequence has not been modified and it retains its own regulatory sequence(s). Regarding the desired trait, the product is equivalent to the one that could be obtained through sexual crossing and introgression by backcrossing, the advantage being that only the gene responsible for the desired trait is introduced, without other unwanted and unidentified sequences from the donor parent (see section 2).

³ Kyndt et al. (2015). The genome of cultivated sweet potato contains *Agrobacterium* T-DNAs with expressed genes: an example of a naturally transgenic food crop. Proc. Natl. Acad. Sci. USA 112:5844-9.

Since the end of 1990s, a number of new plant breeding technologies based on recombinant DNA have been developed. These have been named **New Plant Breeding Techniques, NPBT**, and are characterised by the fact that the final product is very similar or indistinguishable from what can be obtained with technologies that do not use recombinant DNA, such as conventional crossing. NPBT have taken advantage of knowledge deriving from the rapid progress of genomics, the science that investigates the structure and functional interactions of whole genomes. The production of cisgenic plants (cisgenesis) has been included among NPBT. The EU is now discussing whether the regulations for the release of cisgenic plants should be separated from those of intragenic and transgenic plants (see section 6 and our final recommendations). This discussion has been stimulated by a Scientific Opinion document of the European Food Safety Authority (EFSA), which concludes that similar hazards can be associated with cisgenic and conventionally bred plants⁴.

5) Genetic improvement through genome editing

Those NPBTs that allow to perform precisely targeted DNA modifications without moving the target from its natural position in the genome are called **genome editing**. The most promising technology is presently based on Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and the nuclease protein Cas9, hence the acronym CRISPR/Cas9. For brevity, we will therefore describe only this genome editing technology. Cas9 is an enzyme produced by the bacterium *Streptococcus pyogenes* and belongs to the large protein family of nucleases, enzymes that are able to cut DNA. Cas9 is targeted to a specific position of the genome thanks to a small guiding molecule, a small RNA that can be easily modified in the laboratory and inserted into cells together with the gene encoding Cas9, or with the Cas9 protein itself. Once it reaches its target, Cas9 cuts the targeted DNA sequence: the cell then uses its natural machinery to repair the break, with consequences that depend on how the technology is used. Three general uses can be distinguished, named SDN-1, SDN-2 and SDN-3, where SDN is the acronym for Site Directed Nuclease.

- SDN-1: after the DNA has been cut by the nuclease, the cell repairs the damage. Frequently, the repair is imprecise and results in random mutations at the target site of the nuclease: small nucleotide deletions, additions or substitutions. In this case, *genome editing* can be considered a true system of targeted biological mutagenesis and the most frequent final outcome is the inactivation of the target gene. This is very similar to the outcome of random mutation breeding through radiation or chemicals (section 3), the important difference being that a single mutation at the chosen target site occurs using SDN-1 genome editing, whereas the entire genome undergoes many random mutations when treated with chemicals or radiation.
- SDN-2: a short DNA template is inserted together with the nuclease. This DNA acts as a guide for the repair mechanism of the host cell, without being integrated in the genome, and can be designed to be identical to the targeted sequence except for one or a few nucleotide changes. Therefore, the outcome will not be a random mutation but a predetermined change in the target sequence. SDN-2 genome editing is therefore a technique of precisely planned biological mutagenesis: for a given gene, one can generate a useful allele already naturally existing in another variety of that plant or in a wild relative not suited for cultivation, or can accurately create a novel allele.
- SDN-3: when the DNA template that guides the repair involves an entire DNA sequence, ideally a complete gene, the outcome will be the integration of the new sequence at the target site. This will

⁴ http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2561.pdf

result in the production of a cisgenic, intragenic or transgenic plant, depending on the origin and characteristics of the inserted sequence. A scientific opinion paper provided by EFSA to the European Commission⁵ concludes that the hazard of plants produced through this technique are similar to those of plants produced by cisgenesis, intragenesis or transgenesis, with the difference that through SDN-3 gene editing the insertion occurs at a predefined location in the genome: this avoids the risks of inserting the sequence at a random site, where it can have unpredicted effects on the activity of other genes.

In many laboratories, genome editing has been successfully applied to major crops. Following the recent development of the CRISPR/Cas9 technology, SDN-1 has been most frequently used, with important results for both scientific knowledge and plant genetic improvement.

Any useful trait identified in a wild relative or a related species can be generated in a cultivated variety through genome editing,, without introducing any new gene and without requiring time consuming introgression by backcrossing: only the desired mutation is introduced. As described in section 2, it is unavoidable that, through crossing and selection, unidentified portions of the non-recurrent donor are transferred along with the desirable trait. It may take several generations to segregate out the undesired alleles by backcrossing, and this may even be prohibitive in woody plants, where generation time amounts to several years. Finally, it is important to underline that crossing destroys the legal identity of elite cultivars of heterozygous species like grapevine, olive and citrus. Genome editing avoids this problem altogether, because it allows breeders to introduce a desired characteristic without altering any of the traits that make each cultivar unique. For example, the use of fungicides in grapevine can be reduced by generating fungal resistance alleles identical to those found in certain wild grapes, an example of how innovation will safeguard tradition.

In nuclease-mediated genome editing, the nuclease and the guide RNA can be synthesised in the plant by expressing their coding genes. Such genes can then be easily selected out by backcrossing with the non-transformed plant or through selfing once the editing process has occurred. This crossing process is very simple because the genes to be selected out are just one or two, and they are easily identified. Therefore, **in this case, the intermediate product is transgenic but the final one is not.** Technologies to avoid even this intermediate step have been recently developed. It is therefore important to underline that **at the end of the procedure, SDN-1 and SDN-2 genome-edited plants are not GMOs and they are identical to the starting plant except for the desired mutation.**

Obviously, except for the SDN-3 procedure, *genome editing* cannot replace transgenesis when the desired trait requires gene(s) only present in species that are not sexually compatible. An example is the creation of rice capable of producing provitamin A in the seed (the notorious *golden rice*). Therefore, it is incorrect to state that genome editing is a non-GMO technology that can fully replace transgenesis. SDN-3 genome editing can be used to transfer genes from sexually incompatible species, but the result will be a GM plant.

6) Genetic improvement and the present European regulations for GMOs (Directive 2001/18/EC)

To be cultivated and commercialised, any new GM plant must obtain approval according to Directive 2001/18/EC of the European Parliament and of the Council. We report below the parts of the Directive that

⁵ http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2943.pdf

are relevant for the subject treated in this position document; for a complete analysis, readers can refer to the full text⁶.

Article 2 states:

Definitions

For the purposes of this Directive:

- (1) "organism" means any biological entity capable of replication or of transferring genetic material;*
- (2) "genetically modified organism (GMO)" means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination;*

Within the terms of this definition:

- (a) genetic modification occurs at least through the use of the techniques listed in Annex I A, part 1;*

.....

Article 3 states:

Exemptions

- 1. This Directive shall not apply to organisms obtained through the techniques of genetic modification listed in Annex I B.*

.....

Annex I A

TECHNIQUES REFERRED TO IN ARTICLE 2(2)

PART 1

Techniques of genetic modification referred to in Article 2(2) are inter alia:

- (1) recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation;*
- (2) techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation;*
- (3) cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.*

.....

⁶ DIRECTIVE 2001/18/EC: http://eur-lex.europa.eu/resource.html?uri=cellar:303dd4fa-07a8-4d20-86a8-0baaf0518d22.0004.02/DOC_1&format=PDF

Annex I B

TECHNIQUES REFERRED TO IN ARTICLE 3

Techniques/methods of genetic modification yielding organisms to be excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those produced by one or more of the techniques/methods listed below are:

(1) mutagenesis,

(2) cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods.

Clearly, **Directive 2001/18/EC regulates much more the technology used than the characteristics of the product.** On the other hand, it does not purely regulate a procedure; it is not sufficient to use recombinant DNA to fall under the Directive. Novelty is also required, that is “the formation of new combinations of genetic material” (Annex 1A of the Directive, paragraph 1). Therefore, the Directive also regulates the product, even if subordinately. If we also consider the rather imprecise “*inter alia*” in the first sentence of Annex 1, we can conclude that an unequivocal interpretation of the Directive could be rather difficult.

Based on Annex 1B, it is however clear that **an organism subjected to a genetic modification different from conventional crossing does not automatically become a GMO** from a legal point of view, even if it has undoubtedly been genetically modified. It follows that plants produced by random mutation breeding can be grown without falling under the Directive; as mentioned in section 3, they have actually been grown for many years also in many countries that do not allow GMO cultivations.

In conclusion, a specific trait that makes a plant resistant to a given herbicide can result from fortuitous natural mutation, random mutation breeding or through recombinant DNA. However, only the latter will fall under Directive 2001/18/EC, with all its extremely expensive and lengthy requirements, in spite of the fact that the three plants will be equivalent when referring to health and environmental safety.

7) Which regulations for genome editing?

As explained in section 5, *genome editing* allows to obtain plants indistinguishable from those obtained by natural or random mutation, when the SDN-1 technique is used or when a natural mutation identified in another sexually compatible plant is introduced using the SDN-2 technique. Directive 2001/18/EC is now 15 years old, a rather long period of time considering the speed of scientific research and technological innovation in the field of molecular genetics. Plants obtained through genome editing are therefore presently in a regulatory limbo, waiting for the decisions of the European Commission.

The main purpose of regulating plant cultivations should be the safeguard of public health and of the environment. The large majority of scientists therefore agree that a new cultivated plant should be evaluated in consideration of what it is, instead of considering the underlying technology. Directive 2001/18/EC, on the contrary, mainly evaluates and regulates the technology. The severe EU regulations on GM plants impose heavy economic burdens on plant registration (in the order of tens of millions) as well as on management and continuous monitoring of GM cultivations. In practice, only large, multi-national companies can afford these costs. The present regulations therefore favor them, to the detriment of small and medium enterprises and public research institutions. An extension of Directive 2001/18/EC to the products of genome editing will

even affect field trials in countries that presently do not allow GMO testing in experimental open field conditions, like Italy. The losers will mainly be public research and specific crops of national interest, giving way to other, less technology-hostile countries.

The inclusion of plants produced by SDN-1 or SDN-2 genome editing among GMOs can finally have another important negative consequence: since it is often difficult, and sometimes impossible, to distinguish them from natural or random mutated plants, obvious problems will arise about compliance with Directive 2001/18/EC. In this context, European agriculture risks an invasion of genome-edited plants generated outside the EU, without being able to identify them or to compete globally in their production. In a sad remake of European GMO history, our public research and companies will be excluded also from this innovation, in spite of the fact that every year we import millions of tons of transgenic soybean that we are not allowed to grow and the soy meal derived from it. By repeating this mistake with genome editing, the outcome will be a nonsense of logic, a scientific absurdity, a legal mess and an economic damage altogether.

Within this framework, it can be useful to observe that two distinct national authorities, the Swedish Board of Agriculture (the Government's expert authority in matters of agri-food policy, responsible for the agricultural and horticultural sectors) and the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL, the German Federal office of consumer protection and food safety), recently asked for their opinion on how to regulate plants obtained through SDN-1 genome editing, concluded that genome-edited products that do not contain exogenous DNA should not be considered GMOs and should therefore be excluded from Directive 2001/18/EC⁷.

Concluding statement

SIGA and SIBV represent the vast majority of Italian researchers involved in plant molecular biology and genetic improvement. Based on the considerations reported in this document, these two societies bring the following observations to the attention of all citizens, and particularly of the legislators responsible for defining regulations and norms, hoping that this will help to keep the discussion on solid scientific grounds rather than ideology and dogmas:

- Genetic modifications caused by mutations that occur randomly in nature have been fundamental in the evolution of all organisms, including cultivated plants, and are therefore at the basis of our life. Plant breeders select the plants perceived as having the most useful gene combinations. All plants presently cultivated result from a process that started long ago from naturally occurring wild plants characterized by a series of genetic modifications that made them suitable for cultivation and thus were selected and gradually improved by farmers and, recently, by breeders.
- These plants now provide us with an unprecedented amount of nutritious, safe and reasonably inexpensive food.
- To improve cultivated plants, many mutations have been produced using technologies that provide highly random and equally unpredictable results. These new plants have not undergone specific regulations. In recent years, new technologies have emerged, such as the production of GM plants

⁷ Swedish Board of Agriculture:

http://www.upsc.se/documents/Information_on_interpretation_on_CRISPR_Cas9_mutated_plants_Final.pdf

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit:

http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/Opinion_on_the_legal_classification_of_New_Plant_Breeding_Techniques.pdf?__blob=publicationFile&v=2

and, even more recently, genome editing: these technologies are more precise and therefore their effects can be more easily predicted. However, these plants are either the object of strict regulations (GMOs) or are in an uncertain regulatory limbo (genome editing).

- During more than 20 years of cultivation, no health or environmental hazards have been specifically associated to GM plants as such. On the contrary, advantages for the environment and the economy have often been reported. It is right and proper to continue monitoring these plants, and similarly to accurately monitor the effects of any agricultural practice and cultivation, traditional or innovative. Nevertheless, the restrictive norms that regulate GM plants impose economic burdens that *de facto* favor large multinationals to the detriment of small and medium enterprises and of public research institutions. It is time to move to a system that modulates regulations based on both the technology and the product, and therefore **regulates a new plant taking mainly into account its final characteristics, instead of considering only the technology used to generate it**⁸.
- Within this perspective, it is correct to revise Directive 2001/18/EC, envisioning norms based on the actual possible hazard of the product, which depends on the combination of the genes and the species involved in a given modification.
- It is not scientifically or legally acceptable to define as “altered in a way that does not occur naturally by mating and/or natural recombination” an alteration that cannot be distinguished from a natural one. A decision to regulate as GMOs the products of genome editing, in particular those obtained through the SDN-1 technique or when a natural mutation identified in another sexually compatible plant is introduced through the SDN-2 technique, would be a serious mistake in terms of science, logic, norms and economic implications.
- In summary, until Directive 2001/18/EC will remain in force in its present form, **we recommend to exclude from it the products of genome editing when they do not contain gene combinations different from those in plants produced through crossing or random mutation.**

GLOSSARY

Alleles: alternative forms of the same gene. Two alleles are distinct when their sequence is different, even for a single nucleotide. Some differences do not have effects on gene function and therefore on the characteristics of the individual. Others can affect gene function to variable extents and can be of great practical interest in cultivated plants.

CRISPR-Cas9: *Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR-associated protein 9.* A bacterial mechanism that can recognise and cut exogenous DNA. It has been adapted to plants and animals to perform genome editing.

Domestication: when referred to plants, it indicates those genetic modifications that have made wild plants suited to be cultivated and utilised, and have therefore been selected by humans.

EFSA: *European Food Safety Authority.* The agency established by the European Union to be a source of scientific advice and communication on risks associated with the food chain. EFSA also has a duty to communicate its scientific findings to the public.

⁸ See also Kuzma (2016) Reboot the debate on genetic engineering, *Nature* 531:165-167, <http://www.nature.com/news/policy-reboot-the-debate-on-genetic-engineering-1.19506#/b8>

Gene expression: a series of biochemical reactions that start from a DNA sequence that constitutes a gene and finally lead to the production of a protein, or an RNA molecule, that will perform a specific function. DNA transcription produces RNA molecules. RNA translation produces proteins thanks to the genetic code. Many RNAs are translated and thus produce proteins, others directly participate in cell functions.

Gene: a portion of DNA containing the information to produce an RNA molecule. It is the structural unit of biological heredity.

Genome: the entire set of genetic material present in a cell and inherited when cells divide. It is made of DNA and consists of millions or billions of nucleotides organised in a variable number of chromosomes, depending on the species. Each chromosome is a long double-stranded DNA molecule. At least two copies of each chromosome, and therefore of each gene, are present in animal and plant cells. Mature germ cells (gametes) are peculiar, because they have only one copy for each chromosome: In sexual reproduction, two gametes fuse and re-establish the chromosome pairs.

Mutation: any heritable modification in the nucleotide sequence of a genome.

Nucleases: enzymes able to cut DNA or RNA.

Nucleotide: the structural unit of nucleic acids. It is composed of phosphoric acid, one five-carbon sugar (ribose or deoxyribose) and one nitrogenous base. Deoxyribonucleotides form DNA, whereas ribonucleotides form RNA. The four nitrogenous bases are adenine, guanine, thymine (uracil in RNA) and cytosine. They determine the nucleic acid sequence and are usually represented by their initials: A, G, T (U) and C.

SDN: *Site directed nuclease*. Enzyme that cuts DNA at a specific target site along the nucleotide sequence.