Opinion on the Regulation of Targeted Mutagenesis in Plant Breeding

Opinion adopted by the Academy of Agriculture of France and the Academy of Technology on July 7, 2016

Since the early 1990s techniques of induced mutagenesis targeted by biotechnological processes have been developed.

Targeted mutations obtained are of great interest for plant breeding as they can accelerate the creation of varieties of interest and therefore reduce costs associated with it.

In a regulatory landscape still confused at the European level, and in the absence of hindsight on concrete data from the field, the two French Academies in their opinion voted on July 7, 2016 call public authorities permit the development of the experiments in progress, including field experiments, and to use their results in order to prepare a regulatory framework which incorporates both biomonitoring and technical advances that these new technologies may provide.

I) Explanatory memorandum

Historically the Academy of Agriculture has been very early interested in advances in plant breeding and contributes to debates of society in science and technology. Since its creation the Academy of Technology has been interested in the evolution of techniques of the genome modification applied to the case of crop improvement in breeding programs. Its Committee of Biotechnology, in particular, follows the progress of molecular biologists in this field. It also watched attentively the successive regulatory and legal changes to varieties authorization procedures resulting from this work, both at the European Commission and national level. It will be recalled that in 2013 the Academy of Technology has partnered with the Academy of Sciences and the Academy of Agriculture to exchange their member expertise on the subject. Following this joint work the three Academies organized on November 19, 2013 a symposium open to discuss with a wider audience of advanced Genetically Modified Plants (GMP) in the world, the benefits and limits that could be allocated to them and the state of the French and European regulations. Anxious to bring their expertise to the greatest number, the Academies, following this conference, wrote a widely circulated opinion that concluded with these words: "To move forward in this debate, the academies require that scientific issues and agricultural affecting the GMP are thorough on an objective basis. This involves restoring the freedom to conduct research and testing, including field testing and long-term, under existing regulations."

The Biotechnology Commission supported the publication in 2014 of a book "*Ten Questions to Bernard Le Buanec on the Subject of GMOs*" that answers the many questions asked by the

public. The Academy of Agriculture working group on genetically modified plants also published in 2015 a book "*Plants Genetically Modified, Threat or Hope*" edited by Jean-Claude Pernollet.

At the end of 2015 a new working group was set up within the Commission of Biotechnology of the Academy of technologies to work on "New technologies and agriculture" which was associated the Academy of Agriculture. At the Academy of Agriculture working group "New Biotechnology for Food and Agriculture" examines the use of new biotechnologies, including those regarding changes to the genome. The joint working group of the two Academies brings together experts from the biotechnology of the Academy of Technology Commission as well as many of those of the Academy of Agriculture. It is therefore natural that the question posed by the availability of new techniques for genome modification without "foreign" DNA input as a selection tool in plant breeding process should be addressed. Several academic reports were considered, including in particular the report published by EASAC, the European Association of Academies of Sciences, that also published the same year by the German academies, which Acatech, our sister Academy and finally very recently, one of three American academies.

It seems important that the issue of access to new technologies for genome modification, targeted mutagenesis, is studied and discussed taking good account of the evolution of techniques and the benefits they bring in many applications derived progress facts in the life sciences (See Technical Appendix). Their low cost and ease of implementation would allow their use by many breeding companies regardless of their size, for public research laboratories, contributing to maintaining the diversity of plant breeding stakeholders. However their use in Europe, both in the field of research and in agricultural production, will depend on regulations that will be applied to them. It is important that the European Commission and its Member States quickly specify the status of these technologies in the plant area to avoid any uncertainty that might penalize the research, innovation and European agriculture in a socio-economic context increasingly globalized.

It is in this context that the two academies now offer the advice below. This is in line with the response of the French government in the issue of December 12, 2015 the member Brigitte Allain asked the Ministry of Ecology, Sustainable Development and Energy on the new plant breeding techniques: "It should ensure that the decisions taken at European level are proportionate to the risks and challenges of these techniques and take into account the purpose of the applications that can be developed with these techniques. Analysis of HCB (French biosafety committee) shows that the regulation of GMOs should not apply to some new techniques. The French government will also be alert to the legal certainty of decisions taken at European level."

This joint approach, to the field of plant breeding, does not exclude a broader joint reflection on the uses of these new techniques in the modification of animal genomes.

II) Opinion of the Academies of Agriculture of France and Technology on the regulation of targeted mutagenesis by editing the genome of plants

1) Since the early 1990s the techniques of induced mutagenesis targeted by biotechnological processes have been developed: they involve meganucleases, ODM (Oligonucleotide Directed Mutagenesis), ZFN (zinc finger nucleases), TALE nucleases (Transcription Activator-Like Effector nucleases) and the CRISPR system (Clustered Regularly Interspaced Short Palindromic Repeat) associated with a nuclease.

2) Targeted mutations obtained are of great interest for plant breeding as they allow to accelerating the creation of varieties of interest and therefore reducing costs associated with it. They also contribute to increase the genetic diversity of varieties created.

3) In the regulatory framework still confused at the EU level, and in the absence of hindsight on concrete data from the field, the two French Academies call on public authorities to permit the development of current experiments including field experiments and use the results in order to prepare a regulatory framework that incorporates both biomonitoring and advanced techniques and technical advances that these new technologies may provide.

From the perspective of both these French Academies, techniques of targeted mutagenesis can, in principle, be excluded from the techniques regulated by the European Directive 2001/18/EC on the deliberate release of genetically modified organisms into the environment, according to its Annex 1B.

Appendix to the Opinion of the Academies of Agriculture and Technology on the Regulation of Targeted Mutagenesis by Editing the Genome in Plants

A mutation is a biological phenomenon occurring in two steps: first, in general, the breakage of a DNA molecule of a chromosome by a transient effect of various physical or chemical or biological factors, then repair of this break by cellular enzymatic reactions making possible the emergence of new morphological and/or physiological characters (traits) or the removal previously existing traits.

Spontaneous mutations

Spontaneous mutations naturally occur in the environment. It is generally accepted that they are the source of genetic diversity. Their existence and the new features they control have been exploited by humans to identify species and varieties suited to their needs. These spontaneous mutations have had and still have major consequences on agriculture. One example is the control of the adhesion of rice grains to the panicle that allowed the domestication of this cereal. Another example, among many, is illustrated by point mutations of a gene of two-row barley turning it into six-row barley called winter barley.

Random mutation induction

In the late 1920s breeders sought to increase the genetic diversity through various means including the "induced" mutations. These mutations are caused by either electromagnetic radiations or by chemical products applied to seeds in the laboratory. These seeds are then sown and plants derived from them are sorted for new desired traits.

Regarding radiation the first attempts were using X-ray. Subsequently, at the dawn of the atomic age, gamma-rays and neutrons have taken over as this type of radiation could be routinely obtained in newly installed nuclear research power plants. Mutations have also been induced by ultraviolet radiation on cells in culture. The changes produced by these physical processes can result in loss of chromosomal fragments or rearrangements in the genome. Regarding chemicals the EMS (acronym for ethyl methanesulfonate) is most often used.

These induced mutations are random. Therefore very large cohorts of mutant plants are required to identify those having agronomic value, with correspondingly high costs. However, many mutations have been well selected and introduced into a number of varieties which are

still cultivated such as 200 varieties of rice in the world, sunflower, yams, many fruits and ornamental plants.

Induced mutations led by biotechnological processes

Two advanced techniques allowed the development of induced mutations which are directed or targeted (for the so-called genome editing): (i) genome sequencing which from the 1980s precisely identifies the nucleotide sequences of genes on the DNA molecule; the first sequencing of an entire plant genome was published in 2000 and (ii) the discovery of "scissors" (DNA nucleases) to cut biological chromosomal DNA at a specific location.

The discovery of these "scissors" is derived from an enzyme that has been called meganuclease identified by the team of Bernard Dujon in 1992 at the Pasteur Institute, France. This restriction enzyme has the particularity to recognize a sequence of 18 consecutive nucleotides in a genome. The probability of finding another suite of 18 nucleotides in the DNA molecule being close to zero, this meganuclease cuts DNA at a targeted site. This observation was the beginning of new targeted mutations in the genome. Once the DNA cut, it can be repaired independently following the same mechanism as in the case of spontaneous mutations described above.

Since the discovery of these new tools ODM (Oligonucleotide Directed Mutagenesis) were used in the late 1990s, nucleases zinc finger (ZFN, Zinc Finger Nuclease) in the early 2000s, nucleases effector TALEN (Transcription Activator -Like Nuclease Effector) in the late 2000s and, since 2012 the CRISPR system (Clustered Regularly Interspaced Short Palindromic Repeats). In the latter case it is not a protein that recognizes the DNA sequence but a RNA sequence of approx. 20 nucleotides which associate with a DNA nuclease such as nuclease Cas9. This RNA, which serves as a guide to "scissor", is easily synthesized in vitro with minimal cost by copying the sequence of the region of the target gene in which one seeks to create a new mutation.

These CRISPR techniques are getting better controlled than the other techniques and the risk of off-target mutations becomes extremely low or zero, as explained in the previous paragraph. At this level, we must distinguish their applications in plants of those in animals and man. In plants they are considered as a new tool to create targeted new mutations to be studied in the field by the breeder.

These techniques allow the creation, when the genome sequence of the plant is known, a new mutation at the same site of a gene that controls a previously identified trait: mutations can be specified in advance and sorting plants is then greatly simplified, thus they allow acceleration of the work of the breeder and to reduce costs.

We now have effective tools to induce targeted mutations of genomes. These mutations are promising for the development of new plant varieties to meet the current challenges of food production which is in strong growth and demand globally while preserving the environment

Our analysis of the situation of these new techniques in relation to the European Directive 2001/18 / EC on the deliberate release of genetically modified organisms into the environment is as follows:

A) - The Directive defines in Article 2 a genetically modified organism (GMO) as a "body, with the exception of human beings, whose genetic material has been altered in a way that does not occur naturally by multiplication and / or natural recombination."

- Annex 1B of the Directive excludes from its scope mutagenesis "provided it does not involve the use of recombinant nucleic acid molecules or GMO [regulated]."

- Annex 1A states that are regulated "recombinant techniques deoxyribonucleic acid involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced in any way out of a body, inside of 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 can grow continuously." The latter specification defines a recombinant nucleic acid in the spirit of the directive.

B) - The targeted mutagenesis techniques implemented by ODM, meganucleases, zinc finger nucleases or TALEN nuclease or the CRISPR system (Cas9 or Cpf1) can be used in different ways in which these molecular tools will be introduced into the cell either by transgenesis, by transient expression of the corresponding genes, or by direct injection of the nuclease (and its guide in the case of RNA CRISPR) or oligonucleotide (ODM) mutagenesis.

The choice depends on the technical possibilities of the method used and the type of cell of the considered plant species. In all cases it is possible to obtain a final product, directly or after genetic segregation, without incorporation of recombinant nucleic acid capable of multiplying continuously in the host organism, which is easily demonstrable by modern techniques of sequencing.