# UNCOVERING AND REGULATING HIDDEN GMOS, PIRATE PATENTS AND HERBICIDE TOLERANT PLANTS

This document present the position adopted by french organisations who initiated a case to the french Conseil d'etat against commercial authorisations given for seeds and cultivation of varieties made tolerant to herbicides. A case which led to the referral to the EUCJ regarding the GM or non-GM status of mutagenesis and so-called New breeding techniques".

- Confédération Paysanne

- Réseau Semences Paysannes

- Les Amis de la Terre France

- Collectif vigilance OGM et Pesticides 16

- Vigilance OG2M

- CSFV 49

- OGM Dangers

- Vigilance OGM 33

- Fédération Nature et Progrès

*A growing number of Varieties made Herbicide Tolerant (VmHT) and plants that civil society organisations call"hidden GMOs" are grown and marketed without risk assessment, labelling or post-market monitoring. Beyond the infringment of the precautionary principle, and health and environmental protection, these hidden GMOs are also imposed on organic farmers and consumers who do not want them. They are also instruments of the appropriation of living beings through patents and the encouragement of biopiracy. This paper analyzes how a correct implementation of European regulations, taking into account international conventions, can put an end to such practices.*

**Summary**

## I – A simplified communication ignoring advances in knowledge

The talking points for promoting new GMOs have changed recently. They still include the same promises to solve, thanks to technical innovation, all the food, health, environmental and climate-related threats faced by humanity. Focusing on the how and not the why, they carefully avoid the political causes of these threats. However, while 20 years ago, industry and industry researchers boasted that they were able to modify, already with precision, living beings at will, they nowadays pretend to do the same thing as Nature, just going a little faster to make objects, plants and animals a little "smarter".

This shift in industry communication aims to respond to European and third country publics who refuse to accept GM food and crops although they do accept genetically modified drugs and industrial products. When humans are concerned or risks to biodiversity involved, these publics accept the genetic manipulation of non-hereditary somatic cells but oppose the manipulation of germ cells that carry the genetic heritage. Governments themselves increasingly express reservations about modifying the genetic heritage of any living organisms (see, for example, the scepticism toward new synthetic biology techniques)[1].

This is why the seed industry no longer speaks about genetic modification but about simple improvements of "traditional" or "conventional" processes. It aims to no longer modify the genome but just edit it in order to to improve it, as we edit a photo to make it a little more "real". The reference to an undefined tradition supposed to reflect common sense and values carried currently sought by citizens, would justify, in its view, the repeal of GM regulations, while "improvements" would legitimize patents.

The first recipe of this operation to rehabilitate genetically modified crop and food consists in instilling confusion by grouping processes that are very different from one another under a single undefined generic term, "mutagenesis", postulating that "mutagenesis = natural mutation". The second recipe consists in denying, or at least never evoking, the part of the induced modifications which are not genetic but epigenetic. The third recipe is not to mention related techniques that come with the technique described as the main one.

The various techniques described as mutagenesis are however very different from one another. The most recent ones involve related techniques that are also used to produce current transgenic GMOs, such as cell preparation, multiplication, selection and *in vitro* regeneration of transformed cells which exclude self-regulation of the organism that has been modified. The genetic and epi-genetic recombinations generated by these techniques are no longer of the same nature since they are deprived, by *in vitro* techniques, of this self-regulation, or homeostasis[2] we still know very little about. Those recombinations are subject to different legal frameworks, be it about biosafety, organic farming or intellectual property differently defined for each set of techniques.

The legal framework on biotechnological inventions in Europe was built in the last decades of the last century and dominated by an “all-genetic” conception roughly summarized by the central dogma "one gene codes for one protein that defines one function".

**Since then, a better knowledge of the genome, of the numerous interactions in networks and of epigenetics has thrown into question this mechanistic caricature of molecular biology which is still used in the industry’s communication.**

Plants and animals are part of multicellular organisms that are organized into tissues. Their cells, called eukaryotes, have a nucleus separated from the rest of the cell by a double membrane and several genomes that interact with one another. Although the majority of the genetic material is in the nucleus, there is also genetic material outside the nucleus in the cytoplasm (mitochondria, even chloroplasts). A living tissue is composed of cells that communicate with each other, either directly between two cells through their walls

1 See for instance decisions UNEP/CBD/COP/13/L34 (www.cbd.int/doc/c/5698/c27e/095104a8ef6563392098a2aa/cop-13-l-34-en.pdf), and UNEP/CBD/COP/DEC/XIII/17 (www.cbd.int/doc/c/78d2/b754/df5380c70ffc3fce80756de1/cop-13-dec-17-en.pdf) of the 13th conference of Parties (COP13) of the CBD. It can be found at www.cbd.int/conferences/2016/np-mop-2/documents

2 Homeostatis is the property of a system in which a variable is actively regulated to remain very nearly constant

(plasmodesmata of plants) or by vessels (phloem...) by means of hormones, proteins, nucleic acids (RNA and DNA)... This holds for plants as well as highly evolved mammals, for instance, the mother-embryo communication via the placenta. An organism is a set of tissues that communicate and interact with one another. It thus maintains a certain homeostasis while being able to adapt to its environment, for instance by epigenetic modifications.

The importance of epigenetics is enshrined in the two volumes of a recent report by the French Parliamentary Office for the Evaluation of Scientific and Technological Choices[3]. Its title highlights the novelty of the question: "Epigenetics, a new logic of life? ". The most fervent advocates of genetic engineering, such as Dr. Laurent Alexandre, also recognize this paradigm shift that must be taken into account in the interpretation of the law "*The genome alone does neither explain nor justify everything. Genetics has recently revealed the extreme complexity of our biology, which consists of a mixture of genetic determination, environmental response and chance. Far from the simplifying visions of the 2000s that the international sequencing program had brought to light, we now know that most diseases are the result of multiple genetic mutations associated with the individual specificities of our lifestyles. The environment, which modifies the expression of our genes, explains that two twins will diverge even on characteristics for which they are genetically identical. These environmentally induced differences are termed epigenetics; they result in changes in proteins surrounding the DNA molecule and / or the addition of chemical radicals to certain portions of the DNA.*"[4].

**II – Mutation and epimutation: what is it ?**

**II – 1. A mutation** is a change affecting the sequence of the genome's components (nucleotides A, T, G and C): insertions, deletions, translocations... It occurs mostly during cell multiplications. It results either from natural phenomena (local radioactivity, cosmic radiations...), or from human action. Mutations are an equally essential factor in the evolution of life as Darwin "natural" selection. The genome is not a code of life that must be "repaired" when it is broken by accidents resulting from unfortunate hazards of an imperfect nature. The genome is, on the contrary, sensitive, organized in multiple interacting compartments, and dynamic just as everything that is alive. It constitutes, together with the epi-genome and the other components of the organism to which it belongs, a set of networks (nucleus-plastid, cell-cell, tissue-tissue), interacting with one another. It perceives the information coming from its environment and reacts to it. Its environment acts as much on it as it acts itself on its environment. It adapts to the evolution of this environment by modifying, reorganizing and self-regulating the sequencing of the genetic and epigenetic elements that constitute it. Most mutations have no immediately visible effect. They are called "neutral". We know that their distribution in the populations largely escapes the laws of natural selection, but not much about their possible long-term existence or effects.

**II – 2. The epimutation** is a chemical modification of the spacial layout of DNA components and / or proteins. Epigenetics can be defined as the set of heritable or non-heritable changes in gene expression and genome integrity that do not come with DNA sequence alteration or mutations. Mutations and epimutations interact with each other and with all the constituent parts of the organism. Recent scientific articles highlight the importance of these epimutations as a factor of evolution, as some of them are heritable (then called para-mutations) and a sounding board for interactions between an entire living organism and its immediate environment. The study of its use in varietal selection of the resulting epi-alleles is in its early stages. The phenomenon of hybrid vigour might derive from epigenetic phenomena.

More generally, the improvement of analytical tools is making epigenetics a hot and permanently evolving scientific field. In view of the biological importance and very fragmentary knowledge of epigenetics (it is estimated in some model organisms that epimutations are twice as numerous as mutations), European Food Safety Authority (EFSA)[5] organized a series of consultations on how to assess the associated risks. The

3 https://www.senat.fr/notice-rapport/2016/r16-033-1-notice.html and https://www.senat.fr/notice-rapport/2016/r16-033-2-notice.html

4 *Le Monde* June 8th 2016

5 European Food Safety Authority

conclusion of a June 2016 symposium[6] can be summarized as: "***Researchers are beginning to know what they will have to work on***".

The environment of genomes (nuclear and cytoplasmic) and epi-genomes is the cell: they are therefore subject to external pressures that alter and / or penetrate into the cell. In unicellular organisms, each cell is directly subjected to the external environment. Together with horizontal gene exchanges (which are rare and sedlomly conserved during evolution), mutations and epimutations are the main factors of adaptation and survival of these organisms when their environment change.

Within so-called "superior" multicellular organisms (plants, animals including humans, etc.), which reproduce sexually and / or vegetatively, the environment of each cell is made up of other cells of the same organism. Only the cells of the periphery and the organs of exchange (digestion, respiration...) are in direct and permanent contact with the external environment or its constituents (food, water, air...). These are all somatic cells: when they mutate, the mutation is not inherited in sexual reproduction. Only mutations and epimutations (or transfers of horizontal genes, rare in multicellular organisms) of germ cells can be transmitted to subsequent generations. epimutations are transmitted, apparently more frequently in plants than in animals, by mechanisms still unknown despite "resetting" observed in sexual reproduction (meiosis). Germ cells are not in direct contact with the external environment except sometimes for very short periods during fertilization in plants or in certain aquatic animals.

Heritable mutations and epimutations therefore constitute responses to environmental stresses that are likely to penetrate germ cells themselves or to send signals capable of reaching them: radioactivity, UV radiation, electromagnetic radiation, chemical compounds, hormones, pheromones, proteins, nucleic acids (RNA and DNA), temperature changes, time of exposure to light...

The genomes and epi-genomes are modified and reorganized in response to these stresses while remaining subject to complex regulations within genomes, between genomes of the same cell and different cells, between cells of different tissues... These regulations are the result of the exchanges of the cell with its immediate environment which is made up of other cells and organs of the organism (the plant and / or its reproductive organs) as well as exchanges of the organism with the local ecosystem. The organism's self-regulation thus interfere with the changes of its environment. Between each heritable mutation, the modified organisms multiply sexually or vegetatively. They are therefore subject to natural and human selections during many generations which eliminate those that are ill-adapted to the local selection pressure. In agro-ecosystems that are constantly evolving, this process of local adaptation takes place permanently.

**II – 3. The selection of variants resulting from natural mutations and / or epimutations** consists in selecting and multiplying organisms that have only been modified "*in a way that occurs naturally by mating and/or natural recombination*"[7]. It does not produce GMOs according to the European Directive 2001/18 on GMOs. It also respects the basic principles of organic farming which rejects GMOs regardless of the genetic modification techniques used. It is an "*essentially biological* [process which] *consists entirely of natural phenomena such as cross-breeding or selection*"[8] according to the European Directive 1998/44 on the legal protection of biotechnological inventions and therefore cannot be patented[9].

In Europe, the marketing of seeds produced by such processes is regulated only by the different directives on the official catalogue of varieties of agricultural plant species and by the Regulation on plant health[10]. The catalogue provides for an evaluation of the phenotypic traits of the product alone, regardless of its production

6 https://www.efsa.europa.eu/en/events/event/160614

7 Article 2.2 of the 2001/18 Directive : « "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 ».

8 Article 2.2 of the 98/44 EC Directive on the legal protection of biotechnological inventions.

9 However, without patenting the process itself, the European Patent Office granted several patents on plants derived from "essentially biological processes" (selection of natural mutants followed by crosses), provoking numerous protests.

10 Regulation (EU) 2016/2031 on protective measures against pests of plants

process. The product has to belong to a variety with distinct, homogeneous and stable phenotypic characteristics and, in France, also have sufficient environmental value. For agricultural plants, Directive 2002/53[11] adds a requirement to assess the agronomic and technological characteristics. The "catalogue" directives also authorise EU member states to regulate or suspend the marketing and / or cultivation of any GMO or non-GMO variety which "*could be harmful from the point of view of plant health to the cultivation of other varieties or species*" or that "*present a risk for the environment or for human health*" (see Article 18 of directive 2002/53 as an example). However, they do not require an assessment of these risks *a priori*, nor do they require post-market monitoring.

The "catalogue" legislation also makes it possible to assess the long-term positive or negative externalities of any variety, whether GMO or not, as much on the environment as on the cultivation of other varieties, However, it does not make this compulsory. Such an assessment would be largely justified, for example, for Varieties made Herbicide Tolerant (VmHT). However, no such assessment has ever been done since it needs not more than one single Member State of the European Union to include one of these VmHT in its national catalogue, assuming no environmental impact, for this variety to have *de facto* access to the entire European market, including in countries which may have refused registration in the light of potential risks to the environment and / or the cultivation of other varieties.

**III – Random mutagenesis induced by chemical or physical agents**

**III – 1. *In vivo* induced mutagenesis** on whole plants or on their reproductive organs (seeds, flowers, pollen, cuttings, etc.) has been the subject of various scientific studies in the first half of the last century but has been commercially applied to produce crops grown at a large scale only after the 1950's.

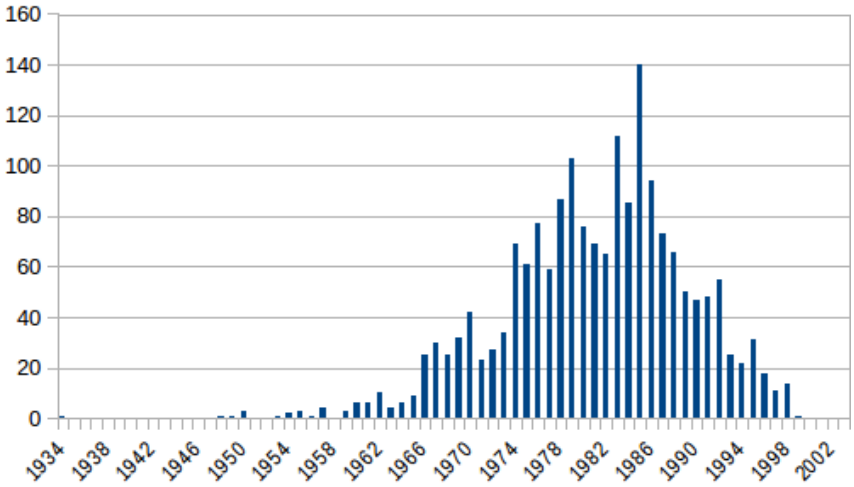


Figure 1 : Number of new varieties obtained through mutagenesis as provided by International Atomic Energy Agency (IAEA)

This process can be applied in the field or in a field station, using chemical or physical mutagens (radiation of various types, heavy metals, etc.). The mutagens used are either synthetic chemicals or products that are isolated from an organism and used at concentrations that do not occur in nature. As for radiation, itsuse as a mutagen makes it possible to subject the plants in one day to the equivalent of 1,000 years of natural radiation[12].

These *in vivo* mutagenic stresses do not modify the genome of the plants in a way that also occurs naturally, in particular given the doses received, their nature and the duration of application. They therefore produce GMOs according to the definition of the European Directive. The resulting genetic recombinations[13] are,

11 Directive 2002/53/EC on the common catalogue of varieties of agricultural plant species

12 http://www.fao.org/docrep/012/i0956e/i0956e.pdf

13 According to the Journal Officiel of the French Republic of September 22nd 2000, genetic recombination is "the phenomenon leading to the appearance, in a cell or in an individual, of genes or hereditary characteristics in a

however, "regulated" by the interactions with the immediate natural environment of the modified cells, which is made up of other cells and organs of the plant which itself is situated in a farming environment (cultivation of whole plant, storage for seeds...). Certain authors have therefore claimed that this procedure employs a "natural process", without however, specifying what they mean by "natural".

Among the plants that survive the mutations thus induced, the breeder selects those that express a new trait that is of interest to him. However, mutagenesis also causes many other mutations and epimutations, some of which can be damaging to the plant's cultivation, its nutritional and organoleptic properties... Therefore, the breeder crosses the mutated plant with other crop plants which are not known to be harmful in an attempt to introduce only the desired trait.

However, these backcrosses never succeed in transmitting only the desired trait. For a variety of reasons (non-Mendelian inheritance, proximity of trait / mutation, number and conditions of backcrossing cycles), an important "remainder" of mutations and epimutations is still present even after the fourteen backcrosses theoretically necessary to statistically obtain the highest possible similarity with the genome of the elite variety used. Seed companies never conduct these fourteen backcrosses. In addition, each backcross generally taking a year, breeders build sets of crops designed to reduce the duration of breeding cycles and to perform three cycles in 18 months for example[14]. At the end of the day, the homogeneity rate of visible (phenotypic) traits hardly exceeds 95%, depending on the species, without mentioning the part of the nuclear genome having a non-Mendelian segregation[15] and the genomes of plastids[16].

Among the other mutations and epimutations that are retained, some are not harmful for the first generations of plant multiplication under normal conditions, the breeder will not eliminate them. However they can be harmful :

- for the next generations,
- for some agro-ecosystems in which it will be cultivated,
- for the health of other organisms that are close to or will consume the crops.

These mutations and epimutations are not identifiable during selection but only when the new backcrossed plant is cultivated and / or consumed. In the absence of any specific assessment, these long-term effects may occur only after many years of cultivation or may remain unnoticed as long as they are not investigated and the crop is not grown under the conditions that make them visible. The existence of unforeseen long-term and / or remote effects, possibly on non-target organisms, is the basis of the “general surveillance” requirements of Directive 2001/18 (Annex VII C.3).

**Legal status of products derived from *in vivo* mutagenesis**

Mutagenesis has been classified since 1990 (Directive 90/220, then 2001/18) among the “techniques of genetic modification which have conventionally been used in a number of applications and have a long safety record”[17]. Developed since the 1950s, *in vivo* induced mutagenesis had already benefited in 1990’s of past growing experience which did not reveal any specific sanitary or environmental harm. In the absence of any requirements for traceability, monitoring and general surveillance , such possible harm had never been investigated. The European legislator concluded nonetheless that there is no risk and for this reason exempted GMOs resulting from such mutagenesis from the scope of the GMO regulation first in 1990 then again in 2001. Some experts conclude that these are not even GMOs. Such claims are contrary to the law.

**A necessary assessment …**

Some varieties have been made tolerant to various herbicides (VmHT) by *in vivo* induced mutagenesis. These VmHTs are GMO varieties excluded from the scope of Directive 2001/18. The GMO event they

different association from that observed in the parental cells or individuals".

14 http://www.lavoixdunord.fr/14840/article/2016-06-22/florimond-desprez-veut-faire-pousser-le-ble-plus-vite-que-dans-la-nature

15 The transmission of recessive traits - which are not expressed - is not subject to natural selection theorized by the laws of Mendel.

16 Organelle carrying part of non-nuclear genomes, female heredity which escapes the laws of Mendel.

17 Recital 7 of Directive 90/220 and Recital 17 of Directive 2001/18.

contain is therefore not assessed under EU GMO regulation. When the HT characteristic is claimed, its homogeneity and stability are assessed before registration under the catalogue. However, any possible impacts on health, the environment - including biodiversity, ecosystem services and disservices -, farming systems and socio-economic aspects are not assessed.

**... that is compulsory but not done.**
However, the European catalogue regulations require that an "environmental risk assessment equivalent to that laid down in Directive 90/220/EEC shall be carried out"[18] of all genetically modified varieties, including those that are excluded from the scope of the Directive 2001/18/EC[19]. However, these assessments are never carried out because information on the processes of genetic modification preceding the backcrossing of varieties is never provided since they are not GMOs falling within the scope of the Directive 2001/18.

As a result, only the active ingredient of the herbicide is assessed at EU level, in accordance with EU pesticide regulations. However, this assessment does not take into account its impact (or the impact of its metabolites) on the various plants that tolerate it (i.e. do not die of it), or on animal or human consumers. It does not include the impact of adjuvants nor the cocktail effects when it is used in combination with other pesticides in the various existing or potential agronomic paths, nor the short-, medium- and long-term impacts of changes in farming systems or the long-range and / or long-term changes in the environment...

**Non-existent traceability**
Several thousand varieties are derived from *in vivo* induced mutagenesis or from crosses performed to introduce the resulting traits into other varieties. Only some of these varieties are identified as such, notably by the International Atomic Energy Agency (IAEA). Even if this identification was to become mandatory, the initial lack of specific genetic markers and traceability makes it impossible to arrive at an exhaustive and reliable identification. The political choice not to seek to trace these varieties *ipso facto* has meant that these hidden GMOs are imposed on society.

**Consequences for organic farming**
This lack of identification of products resulting from induced mutagenesis is particularly disadvantageous for organic farming which rejects the use of synthetic chemicals and mutagenic radiation under artificial conditions. In the absence of an adequate supply of seeds obtained, selected and multiplied under organic conditions, the EU regulation of organic farming permits the use of non-organic seeds that are multiplied for one or two generations under organic conditions or simply not chemically treated after harvest. It rules out only those seeds that are labelled as GMO.

In the absence of appropriate information, organic seed production does not provide an absolute guarantee to the organic farmer that his cultivated varieties are not derived from induced mutagenesis. Organic farming can indeed exclude induced mutagenesis at the level of organic selection if it decides to label and control it, but it cannot control the processes of obtaining the non-organic varieties that it uses as long as it does not have sufficient seeds obtained from production processes in accordance with its own standards. The use in organic farming of seeds which may result from induced mutagenesis remains thus accepted by the European legislation on organic farming. Only certain private organic certifiers or brands have developed the means to research available information in order to exclude those which are identified as mutated. However, even those actors are powerless when no information is available.

This problem is, however, only relevant in the rich countries of Europe or America where almost all the seeds used are commercial varieties, a large part of which may be derived from induced mutagenesis. At global level three-quarters of the seeds used are local and peasant seeds that have never been genetically modified "*in a way that does not occur naturally*". These seeds constitute an immense gene reservoir that is available for new, safe breeding. This in situ and multi-local conservation of genetic diversity is known to promote adaptability to changes at the lowest cost. The bulk of the "genetic resources" enclosed in

18 Updated by the 2001/18/EC Directive. Art. 7. 4(a)
19 As indicated in Article 7.4 (a) of Directive 2002/55, for example. GMOs falling within the scope of Directive 2001/18 are subject to a comprehensive assessment (health, environmental, socio-economic), labeling and post-marketing follow-up.

genebanks come from this reservoir. But what will happen tomorrow if the dissemination of GMOs and the subsequent genetic contamination of local seeds is becoming more widespread? What will happen if new GMOs were to escape all regulation (see the following sections) and traceability leaving countries without any means to reject them or at least take measures against contamination?

**A non-patentable process**

Finally, the chemical or physical mutagenic stress caused by human intervention essentially remains an artifact and cannot be considered to be a "natural phenomenon". Random mutagenesis induced *in vivo* is not an "essentially biological" process which would therefore be excluded from patentability. While it may satisfy certain conditions of patentability (novelty, inventiveness and industrial exploitation), it is too unpredictable a process to be "reproducible by those skilled in the art"[20]. Random mutagenesis therefore remains a non-patentable process according to the basic requirements for patentability laid down in Directive 98/44.

**III – 2. *In vitro* induced mutagenesis** on cells or leaf tissues that are isolated and multiplied outside the plant requires to master several related techniques for the preparation of the protoplasts (partial or total suppression of the cell wall), *in vitro* cultivation, cell or tissues regeneration into whole plants, transformation of differentiated cells into totipotent cells[21], cellular fusions, euploidization[22], embryo rescue, anther[23] cultures... These related techniques have been extensively researched since the late 1960s. However, they were only deployed at scale since the 1990s in order to produce new transgenic varieties. For some species, regeneration remains impossible, while for other species, for which we know how to regenerate cells into plants, the techniques are still not fully under control[24].

A cell isolated from its original plant, or its reproductive organs if it is an embryonic cell, does not multiply and dies rapidly. The same applies to foliar tissues sometimes used for induced *in vitro* mutagenesis.

The first prerequisite for any *in vitro* technique is therefore to know how to keep these cells or tissues alive and how to multiply them. This is done in the laboratory in chemical baths which are themselves highly mutagenic.

The first of these procedures aims at eliminating the pecto-cellulosic wall, a kind of exoskeleton that protects the cells and keeps plants upright. A series of different baths aim at maintaining adequate osmotic pressure while providing nutrients and multiplication factors. Natural interactions and signals such as hormones, proteins and nucleic acids (DNA and RNA) can no longer be exchanged between tissues and between cells through their walls (plasmodesmata) or via vessels such as the phloem. The cellular processes that command and / or feed them are highly disrupted.

In order to survive, leaf or tissues cells adapt to the applied stress by modifying and reorganizing their genomes and / or epi-genomes in interaction with the artificial chemical environment rather than the other cells, tissues or organs of the mother plant. This can alter, in some cases, their metabolism and behavior. According to the French Association for Seeds and Seedlings (GNIS)[25], "somaclonal variation is the modification observed in some cells after a long cycle of *in vitro* cultures without regeneration. These cells are no longer identical to those of the mother plant. The variation may be due to a modification of the nuclear genome or the genome of the cytoplasmic organelles. By this method, variability could be obtained for traits such as herbicide tolerance, disease resistance, stress or salinity tolerance. »[26]

The second prerequisite for an *in vitro* technique is to be able, under hormone activity and other stresses linked to these conditions, to recreate whole plants from these foliar cells or tissues, which can be cultivated

20 A patent is granted only if the description of the invention enables a person skilled in the art to reproduce it.
21 A cell is called totipotent when it has the ability to differentiate into any specialized cell.
22 Process which consists in restoring to an organism its right number of chromosomes.
23 Terminal part of the stamen, which produces pollen.
24 http://www.nature.com/news/plant-genome-hackers-seek-better-ways-to-produce-customized-crops-1.20913
25 French Seed Interprofession
26 http://www.gnis-pedagogie.org/biotechnologie-amelioration-introduction-caractere.html for a French version.

and multiplied. This second operation is also extremely mutagenic. The genetic and epi-genetic changes provoked by this complex and stressful set of *in vitro* techniques do not therefore constitute any natural process. Many so-called recalcitrant plants do not lend themselves to regeneration for reasons that remain unknown. This limits the scope of induced mutagenesis techniques using an *in vitro* step to only non-recalcitrant species, which underlines the artificial nature of these techniques.

In order to obtain even more mutations and epimutations, it is possible to add mutagenic chemical agents to the culture broth or bombard cells with increasingly powerful ionizing radiations. This process is carried out until almost all cells or tissues die. The breeder must then regenerate whole plants from the survivors and cultivate these plants to identify any desirable trait(s), then multiply and backcross them in order to integrate these trait(s) into cultivated varieties, while trying to eliminate as many undesirable changes as possible (see above on the limits of backcrossing).

Unlike a transgene, the intended genetic recombination can be described in a manner that does not differ from a natural genetic recombination. However, the numerous mutations and epimutations caused by the related techniques that are part of *in vitro* mutagenesis (cell multiplication, regeneration...) modify the structure of the genomes in a way that can be distinguished - by developing signatures - from genomes modified exclusively by natural recombination.

The regulations could require the creation of databases including all genetic modifications generated by these *in vitro* techniques. This would allow us to adopt a "matrix" approach to bring together a sufficient number of proofs to allow the identification of products produced by *in vitro* techniques[27]. Such a cluster of converging proofs, based on signatures, is currently used for the detection of unknown GMOs[28].

**III – 3. Marker assisted induced mutagenesis**

According to the French National Interprofessional Group for Seeds and Plants (GNIS), the techniques of somatic multiplication and induced random mutagenesis "are little used by breeders because the variability created can not be foreseen. Moreover, the obtained trait modifications are not very stable and are not always found in the regenerated plant or in its progeny. "[29]. Indeed, since the emergence, in the mid-1980s, of the first transgenic experiments that allowed the integration of a specific trait into a plant, breeders gradually abandoned induced random mutagenesis as shown in Figure 1. Seeds developed during 25 years of *in vivo* induced mutagenesis have remained on the market, whereas varieties produced through *in vitro* induced mutagenesis were only developed later.

Without any requirement to inform users and consumers, it is difficult to determine which process was used to obtain a variety except when it is described in a patent. The European Patent Office has granted patents on plants derived from mutagenesis only since the year 2000. A patent cannot be granted if the patented product is already on the market. Mutated varieties were, however, sold and grown before that date, and protected only by a Proprietary Variety Certificate (PVC) . It is unlikely, however, that any pre-2000 varieties were derived from *in vitro* mutagenesis. Scientific publications show that the techniques of cellular or foliar multiplication *in vitro* and regeneration were insufficiently mastered before the 2000s, and that their first improvements in the 1990s mostly served "classical" transgenesis which was developed following work on the Agrobacterium plasmids. Transgenesis was indeed the least "random" technique, presented as an innovation with regard to its precision higher than of random mutagenesis.

**High-throughput sequencing changes the context**

This situation changed only when genetic markers and high-throughput genome sequencing methods became widely used in the first decade of the 21st century. In little more than ten years, the cost of genetic sequencing went down by a factor 100,000, whilst the time required to complete it decreased from several years to a few days. While these techniques combine minimal cost with fast results, the techniques and

27 https://www.ncbi.nlm.nih.gov/pubmed/22333321

28 http://publications.jrc.ec.europa.eu/repository/handle/JRC67297 and http://eu.wiley.com/WileyCDA/WileyTitle/productCd-1444337785.html

29 http://www.gnis-pedagogie.org/biotechnologie-amelioration-introduction-caractere.html

platforms used, as well as the assembly software and comparison of sequences, still generate many errors [30].

With molecular markers, the induced *in vitro* mutagenesis then became a much less random technique and allowed patents to be granted. In addition to being more flexible, simpler, faster and cheaper than transgenesis, in vitro mutagenesis was finally considered exempt from the requirements of GMO regulation. It was then increasingly used to produce new commercial seeds for the European market.

Indeed, high-throughput sequencing has made it possible to rapidly build databases of genetic sequences to be targeted for a particular phenotypic desired trait and therefore, to quickly identify the plants possessing these sequences before submitting them to induced mutagenesis protocols. Molecular markers have made it possible to identify within a few hours those plants that possess one or more desired gene sequences, avoiding therefore to spend months multiplying tens of thousands of mutated cells, then thousands of regenerated plants, before distinguishing between those that express a new pertinent trait. The savings in terms of time and money are enormous...

With marker-assisted selection (MAS) of plants to be modified, induced mutagenesis has become a set of techniques sufficiently "reproducible" to obtain 70 to 80% of plants expressing the desired trait[31] and thus loses the randomness characteristic of mutagenesis used alone. With the rapid screening of cells, foliar tissues or mutated plants, it also loses its tedious and costly aspects and can be used at an industrial scale.

**Disappearance of phenotypic evaluation and selection**
However, the achievement of the desired mutation(s) does not, however, suppress the multiplication of other unexpected and unwanted mutations and epimutations, termed "unintentional", which are only partially cleared by backcrosses (see above) if they are not immediately lethal.

The use of molecular selection obviates the need for most of the regenerated plant multiplication steps that were previously indispensable to identify those plants expressing new desired traits. This removes many opportunities for the breeder to observe the most visible mutations and epimutations.

The molecular screening of regenerated plants is generally limited to targeting, by detection techniques such as PCR, the specific sequences of interest for the breeder. The whole genome is rarely sequenced.

However, even genome sequencing, which replaces observations in cell culture, in greenhouses or in the field, only allows breeders to identify the presence or absence of new genetic sequences. It does not allow them to identify their function (the traits expressed by the cultivated plant) if the link between sequence and function have not been established before. It also fails to detect any epimutations, which are more difficult to detect.

By drastically shortening the *in situ* observation time of plants, the use of molecular markers before and after induced mutagenesis therefore strengthens the need for a mandatory and complete assessment assessment of these GMOs' impacts.

## IV – Implications for the Application of International Conventions and European Regulations to Products Derived from Mutagenesis

### IV – 1. The Cartagena Protocol and Codex Alimentarius

The International Biosafety Conventions (Cartagena Protocol, Codex Alimentarius and OECD) consider that products derived from *in vivo*-induced mutagenesis are not modified living organisms (LMOs). According to these conventions, organisms resulting from "*modern biotechnology*", defined as "the application of in vitro nucleic acid techniques", are considered to be LMOs[32].

30 https://www.infogm.org/genetically-modifying-a-plant-is-far-from-harmless and https://www.infogm.org/genetically-modifying-a-plant-is-far-from-being-harmless-follow-up

31 As shown by various patents granted on plants obtained by these techniques.

32 Art. 3 of the Protocol (g): « “Living modified organism” means any living organism that possesses a novel combination

Under European regulations, a product resulting from mutagenesis is a GMO since it is modified in a way that does not occur naturally, especially as soon as the mutagenic agent is not natural. Likewise, a transgenic product is a GMO when the horizontal transfer of genetic information involves processes that do not occur naturally (synthesis of genetic events that do not occur naturally, the attachment of these events to viral and bacterial vectors, biolistics using "gene guns" with bullets covered in DNA, electroporation, etc.).

Under international regulations that are binding on the EU, which has ratified the Protocol and accepted the binding nature of Codex standards[33], a product is considered to be an LMO when genetic recombination is not regulated by a natural environment but by an *in vitro* environment, and when the process used overcomes "*natural physiological reproductive or recombination barriers"* and is not a technique "*used in traditional breeding and selection*" (see footnote 32).

The discrepancy between these regulations has no practical implications for the application of European GMO regulation to products derived from *in vivo*-induced mutagenesis which are also excluded from the application of the Cartagena Protocol, as well as products derived from cellular fusion of sexually compatible species.

However it is different for living organisms such as seeds, plants, animals and biological material intended for their reproduction, which result from induced *in vitro* mutagenesis. They could be seen, in a simplified interpretation, as excluded from the scope of European regulations. However they are clearly not excluded from international regulation. As previously mentioned (section III - 2), the "*natural physiological reproductive or recombination barriers*" specific to plant species mean that a cell or a leaf tissue does not reproduce when it is isolated from the plant to which it belongs, or its reproductive organs if it is an embryonic cell. Without human intervention, these cells and tissues die without leaving any offspring.

**The *in vitro* propagation of these cells or tissues requires by definition that natural physiological reproductive or recombination barriers need to be overcome. *In vitro* mutagenesis therefore produces LMOs within the meaning of international regulations.**

Of course, the Cartagena Protocol does not apply to LMOs traded within the boundaries of the signatory parties, and therefore it does not apply to LMOs traded between member countries of the single European market. However, as the Codex Alimentarius, the Protocol applies to trade between EU countries and third countries that are parties to the Protocol and / or signatories to the WTO or other Free Trade Agreements (FTA) referring to Codex as well as transboundary gene flows (wind, insects, freight transport, etc.).

- How will the EU comply with its legal obligations toward third countries on the export of seed resulting from induced *in vitro* mutagenesis if it has neither information on the process used to obtain these seeds, nor tools for their identification and traceability and their multipliable by-products?
- How can it prevent transboundary gene flows without public information on the identification of LMOs produced through *in vitro* induced mutagenesis?
- How can the EU defend itself at the WTO or apply FTAs signed with Codex Alimentarius countries if it does not meet its standards?
- Since third Parties signatories to the Cartagena Protocol would provide information on the LMO status of their products they exported to the EU, how could the EU allow this information to be erased on entry into the EU in application of a less restrictive internal rule, at the risk of having these products multiplied on its territory and then re-exported without any information to other parties to the Protocol?

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of genetic material obtained through the use of modern biotechnology; » and Art. 3 (i) : « “Modern biotechnology” means the application of:
a. In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or
b. Fusion of cells beyond the taxonomic family,
that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection ».

33 Codex texts on food safety serve as a reference when a trade dispute is brought before the WTO.

**IV – 2. Interpret EU Directive 2001/18 in the light of the Cartagena Protocol**

The exclusion of products derived from induced mutagenesis from the scope of European regulations dates back to 1990, and was confirmed in 2001. The similarity of the text of Annex 1B of the two Directives, which excludes mutagenesis and cell fusion from their scope reveals the legislator's difficulties with technical terms that have never been precisely defined, and processes which continue to evolve and for which there is no consensual definition.

Between 1990 and 2001, the only change concerns the fact that exclusion does not apply when the process uses recombinant nucleic acid molecules. Until 2001, the only use of recombinant nucleic acid molecules was in transgenesis. The so-called "site-directed mutagenesis" techniques using such molecules *in vitro* without being associated with transgenesis were only developed in the first decade of the 21st century and could therefore not be taken into account by the legislator in 1990 or 2001 as techniques which have "*conventionally been used in a number of applications*".

It can be inferred that the European legislator wished to establish the 1990s as a "starting point" of "*certain techniques of genetic modification which have conventionally been used in a number of applications and have a long safety record*", as it did in other texts (eg Regulation No 258/97 on novel foods / novel ingredients).

As previously seen (Section III - 3), cultivated varieties derived from mutagenesis were in 1990 and 2001 derived from induced *in vivo* mutagenesis. It is impossible to know with certainty whether any varieties derived from *in vitro* mutagenesis were developed and released into the environment before that date. If this were the case, they were obtained using techniques whose development was not completed, and grown without the public and the legislator knowing about them. They were therefore not submitted to any transparent evaluation. The methods of induced *in vitro* mutagenesis had not "*been used in a number of applications*", and had in no way "*a long safety record*".

It is legitimate to conclude that the intention of the legislator was to exclude from the scope of GMO regulation only induced *in vivo* mutagenesis and not induced *in vitro* mutagenesis.

The Cartagena Protocol was signed by the European Union on 24 May 2000, ie before the adoption of Directive 2001/18 on 12 March 2001. It entered into force on 11 September 2003.

Since then, the obligations arising from the Protocol have been applicable in the European Union, as have the Codex Alimentarius standards. Their implementation - by interpreting the European definition of regulated GMOs in line with the international definition – has become a matter of emergency since the introduction of new varieties resulting from induced *in vitro* mutagenesis techniques has been increasing in recent years in Europe. Moreover, various third Parties exporting to the European Union produce, without identifying them, varieties derived from new techniques applied *in vitro* to nucleic acids.

Most recent VmHTs have been obtained by *in vitro* induced mutagenesis techniques and a (non-exhaustive[34]) review of the patents issued by the European Patent Office (EPO) shows that this is true for many other new genetic and epi-genetics traits. It is still possible to identify most of these varieties, despite the fact that information on the process of production and of tools allowing strict traceability is not mandatory, which will make their identification extremely difficult and costly within a few years.

An interpretation of European regulations that ignores the Cartagena Protocol is also highly detrimental to organic farming. The standards of organic agriculture are defined at international level by IFOAM[35] and are approved by the Codex Alimentarius which refers to the definition of the LMOs of the Protocol. How is it possible therefore to reassure consumers in Europe and third countries to which European organic produce is

34 Comprehensive monitoring of patents is not within the reach of farmers and civil society organizations which are signatories to this text. In addition, a large number of patents describe several possible methods of obtaining without indicating which one was used for the patented invention.

35 International Federation of Organic Agriculture Movements

exported that these products have been produced without the use of LMOs, given that these LMOs are placed on the European market without information, labelling or traceability, and likely to be used by organic producers?

**IV – 3. Redesigning Nature *in vitro* to patent it?**

According to Directive 98/44, the patent protection can be based on a technical process (including "*microbiological*" processes) or *a* "*microbiological material*" but not an "*essentially biological*" process. According to a recent opinion of the European Commission[36], biological materials that have been obtained exclusively through "essentially biological" processes would also not be patentable.

A cell that is isolated from its natural environment (the plant) in order to be multiplied *in vitro*, constitute a "microbiological" material as defined by the patent law[37]. It can therefore be patented as an invention, even when it also exists in a natural state in a non-isolated form[38]. Methods of in vitro induced mutagenesis which involve an intervention on such "microbiological material" are basically "microbiological"[39] methods and not "non-patentable"[40] essentially biological processes. The use of such methods does not therefore preclude the grant of a patent on the products resulting therefrom, contrary to products exclusively derived from "essentially biological" processes.

However, the “microbiological” method used does not make induced *in vitro* mutagenesis a patentable process since it remains a technique that is as random as the induced *in vivo* mutagenesis. In order for the European Patent Office not to oppose patentability, *in vitro* mutagenesis has to be preceded by a marker assisted screening of the biological materials likely to mutate in order to express the desired trait and described in a manner which enables the person skilled in the art to reproduce it with a sufficient success rate sufficient[41]. This product then becomes patentable within the meaning of current European law[42]. According to the established case-law of the European Patent Office, a product is indeed patentable, even if its process of production is not patentable, if it is new, an invention and can be reproduced at industrial scale by a person skilled in the art. Only products derived from "essentially biological" processes would escape this rule on the territory of the European Union, according to the opinion of the European Commission[43].

However, some industry continue to characterize this set of techniques as random in the hope that this will give it a "natural" character that allows it to escape the application of GMO regulation.

According to these authors, induced *in vitro* mutagenesis assisted by molecular screening is a random natural phenomenon when the aim is to exclude products obtained from it from regulated modern biotechnologies. But it would become a reproducible biotechnological invention in order to obtain a patent. The same ambivalent rhetoric is used in relation to the new techniques of modification used to modify the genomes and

36 Notice on certain articles of Directive 98/44/EC of the European Parliament and of the Council on the legal protection of biotechnological inventions (08/11/2016, C 411/03) http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:52016XC1108(01)&from=FR

37 Decision T356 / 93 of the European Patent Office: 3. “*Plant cells as such cannot be considered to fall under the definition of a plant or of a plant variety. Rather they are considered to be "microbiological products" in the broad sense*” https://www.epo.org/law-practice/case-law-appeals/recent/t930356ex1.html

38 Art. 3.2 of the 98/44/EC Directive : “Biological material which is isolated from its natural environment or produced by means of a technical process may be the subject of an invention even if it previously occurred in nature.”

39 Art. 2.1.b) of the 98/44/EC Directive : “ ‘microbiological process’ means any process involving or performed upon or resulting in microbiological material.”

40 Art. 2.2 of the 98/44/EC Directive : “ A process for the production of plants or animals is essentially biological if it consists entirely of natural phenomena such as crossing or selection.”

41 A patent application is not admissible if it does not contain a sufficiently clear and complete statement of the invention for the person skilled in the art to perform it.

42 It should be noted that the notion of "reproducibility" used by the EPO has nothing to do with the definitions of the European CEN and International ISO standards which are required for the marketing of the same products.

43 See Notice (08/11/2016, C 411/03) of the EC. This does not mean that the process is also patentable because it consists of two steps that are both non-patentable: a selection stage that is "essentially biological" associated with a mutagenesis step that is random on its own.

epi-genomes resulting from the use of "NBT"[44] (discussed in section V of this document).

Most of these patents describe the novel trait obtained by induced *in vitro* mutagenesis in an imprecise manner that does not allow to distinguish it from a similar trait obtained through a processe which "*consists entirely of natural phenomena such as crossing or selection*". This deliberate confusion of products resulting from fundamentally different technical processes or natural phenomena aims to extend the protection of these patents to plants with similar native traits. The strict application of GMO labelling and traceability requirements to all living organisms "obtained through the use of modern biotechnology" would rule out this form of "biopiracy"[45].

**V – New GMO techniques**

**All the new GMO techniques also called NBT (New Breeding Techniques) produce LMOs as defined by the Cartagena Protocol**. These new techniques of genetic modification of genomes and epi-genomes include a step of "In vitro [...] *recombinant deoxyribonucleic acid*" and/or of "*direct injection of nucleic acid into cells or organelles*", and more generally steps of "*application of* in vitro *nucleic acid techniques*" which "*overcome natural physiological reproductive or recombination barriers*" and include, *inter alia*, the related techniques that are also used in transgenesis which has given rise to the majority of GMOs currently on the market (cf. Supra).

In 2001, as in 1990, the legislator carefully defined:
- in the first part of the directive Annex IA, an open list ("*inter alia*") of techniques of genetic modification to which it is possible to add any new techniques unknown at that time.
- in the second part of the same annex, a closed list of techniques which are not considered to give rise to a genetic modification. It is therefore not possible to add any new techniques to this list.

It is therefore possible to consider that some new techniques, to which the definition of GMO provided by article 2.2 of the directive applies, do give rise to GMOs and it is not possible to invoke Annex IA second part to claim such technique do not produce GMO. The Cartagena Protocol does not allow the exclusion of products derived from these new *in vitro* techniques from its requirement of transparency and prior consent.

The seed industry has nonetheless started an intensive international lobbying campaign aimed at excluding most of them from the scope of GMO regulation. With regard to the European Union, such a claim is contrary both to the letter of Directive 2001/18 and the legislator's intention, not to speak of the fact that it would make it impossible to implement the Cartagena Protocol and Codex Alimentarius..

**V – 1. « Natural » techniques ?**

The industry claims that the majority of those new techniques of genetic modification do "the same thing" as any "natural" spontaneous mutations. This ignores the fact that those techniques give rise to epimutations, some of which are unintentional ("off-target"), and that the techniques used to obtain null segregants from GMOs[46] rely the introduction, if not stable insertion, of various recombinant nucleic acid sequences.

It is undeniable for example, that cisgenesis and intragenesis belongs to the family of techniques of transgenesis. Excluding them from the scope of directive 2001/18 as the industry asks would amount to rewriting the directive.

The industry also claims that **grafting** of a GMO part and a non GMO part (or vice-versa) might give rise to non-GMO plants. This claim is based on a surprising ignorance of the GMO regulation which also apply to products derived from GMOs. It is without question that the plant consisting of a GMO rootstock and a non-

44 New Breeding Techniques or New Technologies of Genetic Modification

45 Defined by the countries that oppose to it and by numerous NGO as the "exploitative appropriation of indigenous forms of knowledge by commercial actors".

46 Process that uses a GMO to obtain another genetic or epi-genetic modification, then suppress it from the modified plants which then will be multiplied

GMO graft is a GMO, and that the fruit produced by this plant is derived from a GMO. It is equally clear that the plant from which a transgene have been removed to shorten the selection delays is also derived from a GMO. Legally speaking, they are GMOs. It has also been biologically shown that, for example, circulating DNAs and RNAs between the different tissues of a grafted plant (one component of which is a GMO) induce protein synthesis and various changes in the non-GMO part that are caused by the GMO part. They are therefore GMOs from a scientific point of view.

The techniques using nucleases (SDN[47], meganuclease, ZFN, TALEN and Crispr/Cas-like techniques) introduce into a cell, temporarily or permanently, coding or non-coding genetic material[48] that induce a genetic recombination but which is itself not always the intended result of the introduction in the cell of exogenous material. The last quoted technique, using Crispr sequences and nuclease (Cas9, Cpf1, C2C1, C2C2…), modified or not and anchoring itself to genetic sequences called PAM[49] which narrow their field of action, is particularly fashionable because rather cheap and easy to use. In a relatively short time, a technician can test many different forms until he finds the combination of guide-RNA allowing the desired modification in the targeted site without pre-empting effects in other places of the genomes and epi-genomes.

Depending on the way those nucleases are used (notably depending on the use or not of DNA matrix and depending on the cell repair system used to repair double stranded DNA break: NHEJ[50] or HDR[51]), it has become customary to classify the results depending on the expected actions:

- SDN1 : mutations directed to genome sequences with random results – insertion / deletion / translocation… - with no DNA matrix for repair and based on the most complex NHEJ repair systems, the most efficient but involving many mistakes;
- SDN2 : attempts to alter sequences by providing short DNA matrix to replace a few nucleotides by trying to stimulate the HDR repair system;
- SDN3 : insertion of long fragments of DNA by using a long DNA matrix and trying to stimulate the HDR repair system. Depending on the origin and the possible modification of the DNA matrix, it will be called cisgenesis, intragenesis or transgenesis.

47 Site Directed Nuclease. Actually, it would be better to talk about “sequence directed nuclease” by not focusing on the practical aspects: the targeted site (coding DNA or not) but on the more or less homologous sequences present many times in the targeted genome, allowing to better explain why numerous off-target effects are observed.

48 Oligonucleotides, zinc finger, TALEN, Crispr/Cas9...

49 Protospacer adjacent motif (PAM), nucleases Cas-like anchor sequence in the vicinity of which the DNA cut can be guided by two RNAs assembled in one in the 2012 technological variant.

50 NHEJ : Non Homologous End Joining, very complex double-stranded DNA cut repair system under canonical or alternative form (operating at high-frequency and source of numerous errors) of ligation of non-homologous ends, repair of double-stranded DNA cuts by joining.

51 HDR: Homology Dependent Repair, system for repairing double-stranded DNA breaks with homologous recombination (intervening at very low frequency compared to NHEJ, more "reliable" less subject to errors than NHEJ), ie repairing of double-stranded DNA cuts using a partially homologous DNA template with cut ends, matrix that is copied (to introduce a more or less long sequence, modified or not, of variable origin) during the repair.

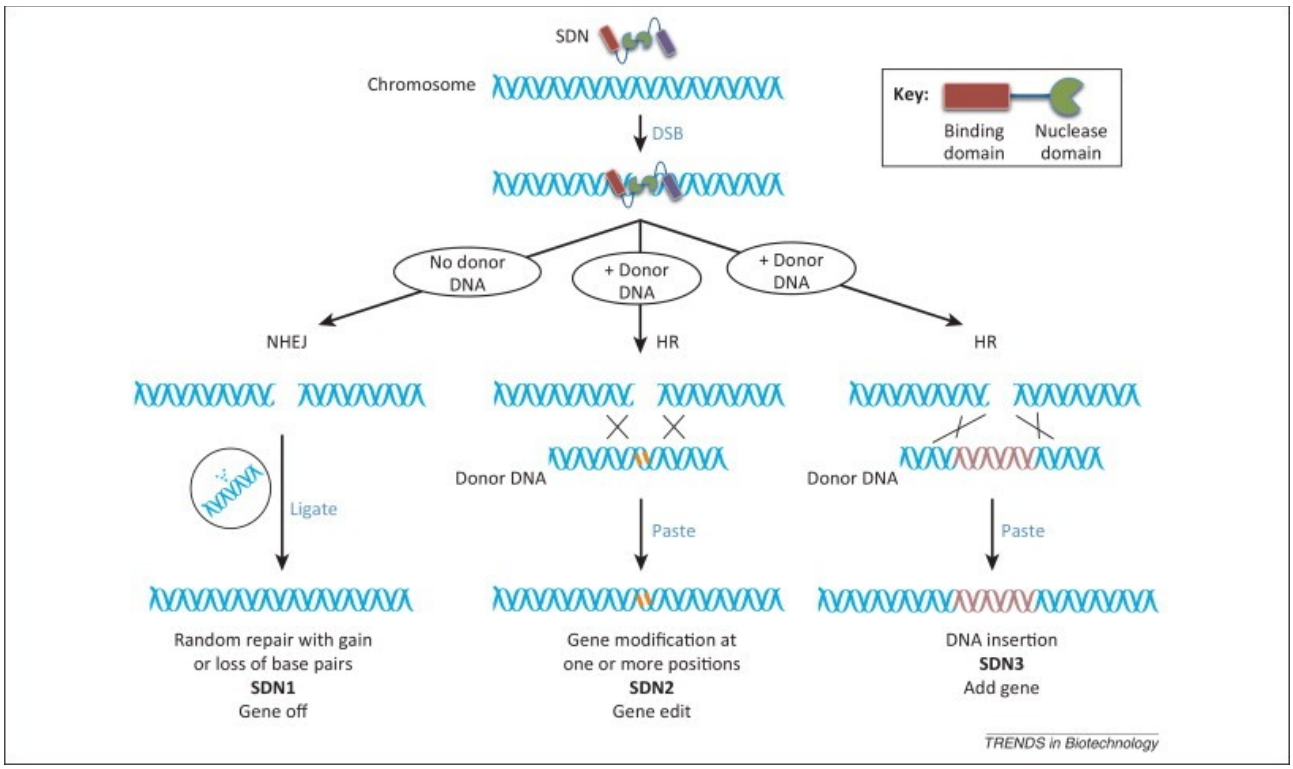


Figure 2 : Classification of SDN 1 to 3 with their obtained DNA modifications depending on the system used to repair double stranded DNA cut implemented through the reagents provided to the genome.

Various combinations of these tools are possible. Crispr/Cas systems can be used to induce epimutations while other versions like the Crispr-C2C2 or Crispr-Cas9 systems can be used to modify RNA.

All those techniques rely, as for any *in vitro* modification of genomes, on the use of related techniques such as protoplastisation, cell culture and selection of the transformed cells, vectorization of reagents (often done by using *Agrobacterium* – the most efficient system – which can leave fragments of its plasmid or of its genome in the one of modified plants), cell wall breaks and plant regeneration[52].

**V – 2. Twisting words to escape from regulation and mislead consumers**

These techniques are accompanied by a communication component that uses semantic distortions, metaphors and omissions regarding when it comes to molecular problems caused. Except for SDN3 with transgenic sequences, the industry claims:

- that the genetic material introduced in the cell would not be recombinant since it would only induce recombination of DNA or RNA and would be – in certain forms of these techniques – no longer present in the final product. It should therefore escape from the application of the european GMO regulation which cover products obtained through mutagenesis or cell fusion only when those techniques involve “the use of recombinant nucleic acid molecules”;

- that those techniques, often referred to as “directed mutagenesis” would only be a specific improvement of random mutagenesis and should therefore be considered as excluded from the scope of the european regulation;

- that these techniques would induce fewer off-target effects and would be more secure than random mutagenesis which is not regulated and that they should therefore also be deregulated.

52 http://www.infogm.org/genetically-modifying-a-plant-is-far-from-harmless and http://www.infogm.org/genetically-modifying-a-plant-is-far-from-being-harmless-follow-up or http://www.infogm.org/5975-ogm-modifier-plante-pas-anodin and http://www.infogm.org/5982-ogm-modifier-plante-pas-anodin-suite

These claims are contrary to the industry's own communication which promotes its patents on their own techniques such as TALEN for example, certifying that it does have fewer off-target effects than others like Crispr/Cas-like techniques for example. We therefore witness the publication of many articles announcing undetectable "off-targets" for which a close examination leads to the conclusion that those "claims" of undetectability are more linked to deficiency of the detection techniques than to a real absence of detectability (which is also never claimed). It is true that the softwares used to predict and detect "off-targets" and the techniques used to sequence and assemble (without forgetting the software used to analyse the sequences) suffer from multiple deficiencies which obviate the reliability of many sequence databases and publications that account for them (cf. Supra).

None of the industry's arguments permit the exclusion of those techniques from the obligations established by the Cartagena Protocol and the Codex Alimentarius. Regarding the application of the european regulation and beyond the fact that it must be interpreted in a way that allows the fulfilment of requests from the Cartagena Protocol and the Codex:

- Regarding the first claim :
  - When using the SDN-1 type techniques of genetic modification of the genome and epi-genome, induced mutations are the result of cuts targeting one or several specific sequences but also similar sequences. High frequency DNA repair through NHEJ systems is an important and well-known source of errors. They are therefore GM techniques induced *in vitro*, that are more precise than previously implemented techniques at a particular location of the genome, while remaining as random with regard to other mutations and epimutations ("off-targets").
  - With the SDN2 and SDN3 version which aim at stimulating the HDR DNA repair system – leaving aside NHEJ DNA repair systems – the risk of off-target effects modifying the sequences that are similar to the targeted sequence is the same. However these techniques introduce in the cell – in addition to the reagents – genetic sequences aiming at being the matrix for repair. It is not necessarily transgenesis since these sequences are not necessarily coming from different species but it looks like – whether cis, intra- or trans-genesis depending on the origin and the modification or not of the introduced sequences -.
  - In each case, the cause of the modification is the introduction of genetic material and reagents into in the cell, which recombine with the cell's own DNA, whether there is insertion or not (stable or not) in the DNA itself. European regulations do not define the recombinant genetic material used as being itself, *per se*, the result of the obtained recombination nor as having to be inserted in a stable and definitive manner. It does apply as soon as it is used. In other words, the messenger can have disappeared while having left a message of recombination.

- Regarding the second claim :
  - When an important shift in techniques and scientific paradigm occurs, as many users and promoters of the patented techniques like to highlight, it cannot be stated at once that it is a "revolution" and a mere refinement of older techniques.
  - All the techniques so-called "NBT" are indeed patented. It is not the case for random *in vivo* induced mutagenesis which is, for Directives 90/220 and 2001/18, the ground level of techniques admissible for exclusion of the european GMO regulation application. A revolution, as proclaimed by the proponents of NBT is a disruptive change especially considering the recent advances in our knowledge on genome and epi-genome. It is an abuse of language to refer to "mutagenesis" in general and *in vivo* induced random mutagenesis in particular to qualify NBT as traditional techniques whilst also claiming patentability which relate only to innovation.
  - A process that involves the introduction into a cell of genetic material prepared outside of the cell (regardless if it remains in a stable manner or not and regardless of its size and origin) is closer to transgenesis than to mutagenesis, especially considering the related techniques and the unintentional effects modifying the genomes and epi-genomes. Calling it mutagenesis is a misleading use of words.

- Regarding the third claim,

- The term “Off-target effects” assume there is a target. But targets and therefore off-targets only exist with directed techniques called « directed mutagenesis », not with techniques like the random mutagenesis which is, by its very nature, not targeted.
- However, as soon as those techniques are implemented *in vitro*, the related techniques will cause as many unintended mutations and epimutations[53], together with off-target effects due to the introduction and sometimes insertion (stable or not) of genetic material prepared outside of the cell for so-called « directed mutagenesis » techniques.
- Under normal conditions of cell culture and screening, most of these unintended and off-target mutations and epimutations will pass unnoticed. Their effects are all the more unpredictable.
- Genome sequencing, particularly high throughput sequencing, is a still very imperfect technique with many errors, varying according to the sequencing platforms. Moreover, assembly and comparison software are still unreliable and, in most cases, there is no reliable reference genome for comparisons. This explains that new elements continue to be discovered even in the best known genomes (bacteria, man[54]), while the notion of pan-genome is increasingly used to describe its complexity and the large variations within the same species[55].
- In addition, sequencing takes little or no account of epimutations due to intrinsic technical difficulties. Finally, as seen above, backcrosses aimed at "eliminating off-target effects" remain unable to eliminate all of them. A single non-eliminated off-target sequence is sufficient to generate deleterious effects on health, environment or farming systems. In the case of maize, with nearly 98% of non-coding DNA, 1% of the genome (localized in open chromatin = the active nuclear genome) may be responsible for 40% of the phenotypic variations of the agronomic traits[56].

### V – 3. Traceability of products derived from NBT techniques

It has often been said that NBT techniques are untraceable, except SDN3 with intra- or transgenesis, because the modifications obtained are too similar to those that can occur naturally.

Apart from the clever confusion between

- Traceability (documentarion subject to the ISO standard and applicable as soon as retailers are willing to);
- Detection of modifications (easily feasible by an appropriate technique such as PCR, LCR, LAMP... associated or not in various forms such as SNPLex (see Annex)) and
- Identification of a specific modification (easy when it is about insertion, deletion, translocation...) with the technique used or the owner of the modified variety,

one may notice that many authors have not really looked for the scientific elements that would make it possible to achieve the last (i.e. to identify the specific modification and technique used).

The cut and paste approach did work perfectly between many authors superficially addressing these different issues. However, various elements should make it possible to trace “NBT” products coming from the "NBT", and even quantify them through quantitative techniques, or to determine this quantity against a pre-established threshold by qualitative techniques with sub-samplings, as used in seed certification[57].

The annex to this document briefly explores some ideas which may provide key answers to the question of the identification of products obtained through “NBT”. A coordination of ENGL[58] laboratories is starting to coordinate their work on this.

53 See last footnote 52.
54 Hehir-Kwa, *et al.* 2016. *Nature Com.* 12989
55 Golicz *et al. Nature com*. 2016. 13390. Hirsch et al. *Plant Cell* . 2014 http://dx.doi.org/10.1105/tpc.113.119982
56 Rodgers-Melnick *et al.* 2016. *PNAS* E3177-E3184
57 Remund 2001, *Seed Science Research*. 101-119. Kobilinsky and Bertheau 2005. *Chemometrics and Intelligent Laboratory Systems* 189-200.
58 European Network of GMO Laboratories : Network of research laboratories and of control of competent authorities. They develop guidelines for detection (eg of unreferenced GMO) by validating between the labs methods of detection and quantification of OGM in support of the EC reference lab : EURL-GMFF located at the JRC of Ispra.

As described above, the related techniques used both in classical transgenesis, *in vitro* induced random mutagenesis and for in the majority of the "NBT" techniques induce numerous mutations and epimutations. A certain number of them remain even after numerous backcrosses with usually an Elite variety. This pattern of mutations and epimutations could constitute a signature, a molecular profile, characteristic of the *in vitro* mutated plants.

Another set of markers, some of which make use of the inherent properties of adaptive immunity that Crispr systems are, are also under scrutiny to differentiate the various NBT techniques and to monitor the obtained modifications.

**V – 4. Weapons of mass destruction ?**

The relative simplicity and low cost compared to their powerful capacity to alter living organisms (even in a dirty way) means that risk "NBT" techniques pose an additional risk which has partly motivated the request to US agencies to update GMO risk assessment regulations. Their ability to induce at the microscomic level numerous modifications targeting certain places of one organism, without ever coming back to natural selection, makes it almost impossible to control the impact of their dissemination at the macrocosmic level of ecosystems of which we know so little.

For example, we know only a few percent of the microbes or insects present in ecosystems, whose extreme diversity and variability cannot be captured by the computer models desperately trying to predict the impact of LMO releases. Gene-drive technique is a striking example of this kind of risk. It enables a skilled biology student to release in the environment organisms that can eradicate a whole specie.

Human societies are even less controled, which is why US national intelligence experts and members of the presidential council on science and technology have considered those new GMO techniques to be potential “weapons of mass destruction”[59]. This is why a failure to regulate these techniques would be would be more detrimental than the lack of regulation of *in vitro* induced random mutagenesis. Note that the FBI and the US Homeland Department have recently started to finance demonstrations on NBT and synthetic biology, with some external “observers” being present.

**VI – What are the proposals for regulation ?**

**VI – 1 Industry proposals**

*The assertions and claims of the industry* (in italic below), pronounced in France by the *Union Française des Semenciers* (UFS) and at international level by the International Seed Federation (ISF), are clear:

*- products obtained through new techniques of genome and epi-genome modification that could be categorised as mutagenesis should be excluded from GMO regulation and therefore accepted by organic farming.*
*- the decision on whether to apply GMO regulation should be based on the product’s traits, without considering the process.*

This demand aims at avoiding *a priori* any assessment of unintended impacts resulting from the process, such as any possible genetic and epigenetic modifications which can give rise to unintended risks. For example, a non-GMO graft on a GMO rootstock would not be considered. This approach would also mean a stricter assessment of all varieties, which would not be justified and discriminatory for conventional, low-volume, local varieties or for specific production methods that will not have the capacity to bear the cost.

*- the breeding process used should remain confidential in order not to trigger refusal by consumers* [60].

59 www.whitehouse.gov/sites/default/files/microsites/ostp/PCAST/pcast_biodefense_letter_report_final.pdf - www.dni.gov/files/documents/SASC_Unclassified_2016_ATA_SFR_FINAL.pdf

60 The argument of industrial secrecy to protect the monopoly of operating innovative processes does not hold in the mouths of those who make public the description of the same processes in order to benefit from the protection of

- *any assessment should be based exclusively on the catalogue requirements and should concern only the newly created traits, the rest of the product being a priori considered as "substantially equivalent". According to the industry, a variety that continuously produces an insecticide (such as Bt GMOs) would be considered as of good environmental value since it would reduce insecticide spraying* (which has been observed occasionally in one field but shown to be false in the few larger studies carried out on Bt cotton in China!)*, as well as a Variety made Herbicide Tolerant would allow for a better carbon storage by eliminating the need to till* (until it is invaded by weeds that have become herbicide tolerant[61] and not to mention the constant increase of herbicide levels in surface waters, the return of more toxic herbicides or the eutrophication of North American lakes where non-tillage has increased leaching of nitrogen inputs!).

- *the identification of varieties protected by plant variety protection should be based, as with numerous patents, on recognizable molecular markers in the harvested products and in the new varieties resulting from crossing the protected variety (SNP[62], micro-satellites[63]…), contrary to the phenotypic traits actually used which limit the scope of the PVP to the sole capacity to observe the plant when cultivated.*
- *The breeder's exception should be discontinued for at least the first five years following the grant of a PVP.*

Those last two demands *ipso facto* involve the conversion of the PVP into a patent exempted from the mandatory description of the invention and therefore, from the information of the breeding process, even though the temporary monopoly confered by patent is supposedly given in exchange of disseminating the knowledge. The new GMOs could therefore stay hidden while being "patented" with such new "plant variety protection / patents". The industry pretends to be ethical by proposing that the classical patent protection should not be given to products obtained through "essentially biological" process. So implicitly, the industry thereby acknowledges already being granted patents on "essentially biological process".

**VI – 2 Demands underlying the legal challenge of French farmers organisations and civil society** who initiated the case to the french Conseil d'Etat that has led to the referral to the EUCJ :

1) Application of GMO regulation to all genetically modified organisms as defined by the Cartagena Protocol, including those derived from *in vitro* mutagenesis. For products that are already marketed, an immediate obligation to declare, label and trace the GMO trait with the cost being borne by the breeder, obligation to monitor with the cost being born by the GMO chain; provisional extension of the marketing authorisation until a scientific evaluation can be carried out (as for the European regulation "Reach").

2) Strict implementation of the catalogue regulations which require an assessment of environmental risks equivalent to that required under the GMO regulation for all genetically modified varieties, including those obtained through techniques excluded from the scope of the GMO directive.

3) Regarding non-GMO varieties displaying a trait that can possibly have a negative environmental or health impacts (such as herbicide tolerance), mandatory assessment, before any marketing authorisation, of potential long-term impacts on health, environment and existing farming systems. With the obligation of general surveillance in case of commercialisation, in order to take into account any unexpected effects as for phyto or pharmacovigilance.

4) Exemption of low-uptake peasant or artisanal varieties for which additional assessments are not justified.

5) Moratorium on all VmHT (regardless of the breeding process) until the assessment of their long-term impacts on health, environment, cultuvation of other varieties and existing farming systems has been completed.

6) Obligation to provide the information on the origin of the genetic resources used and on the breeding and

patents.

61 49 % of the cultivated areas of cotton in Arkansas, Mississippi and Tenesee are so invaded by amaranths that have become tolerant to the Roundup that they must be hand-weeded, Riar *et al. Weed Technology* 2013 27:778–787

62 Most used molecular markers

63 Small sequences

multiplication processes of any newly marketed varieties.

7) Ban on any process leading to LMOs, as well as the use of synthetic chemicals and unnatural radiation in organic breeding.

8) Ban on the use of GMOs as defined by the Cartagena Protocol in organic agriculture, as soon as the information is available.

9) Ban on patents on any plant or animal, on their parts and on the genetic informations they contain, that have been obtained, or may be obtained, through an “essentially biological” process.

10) The only acceptable industrial protection for farmers is a return to the 1978 UPOV Convention without any limitation on the use of farm seed by other regulations.

The ECJ is not looking into all these issues. Its decisions will only concern the questions submitted by the French Conseil d'Etat. However, the ECJ's responses need to take into account the entire legal framework, including the international framework and the obligations arising from it.

## Annex

## Detection strategies / identification vs. traceability

The legal requirement to label and therefore detect and trace GMOs all along the chain was adopted by the EU in the late 90s. To allow the controls inherent to this obligation, research programs and a laboratory network (ENGL) in support of the european reference laboratory EURL-GMFF was set up by the European Commission do define the techniques, strategies and procedures needed to routinely implement the controls and self-controls.

The question about the regulatory status to be applied to products obtained through the new techniques of genetic modification brings some of the stakeholders to declare those products are neither identifiable nor traceable as such. And therefore, it is impossible to submit those products to the requirements of the european legislation on GMOs.
But, on the scientific aspects alone and as it was extensively developed in this document, the implementation of a technique of genetic modification induces genetic and epigenetic off-target effects and, the upstream and downstream steps of such an implementation *in vitro* induces genetic and epigenetic unintentional effects. For plants, the ones promoting those new techniques argue that such effects disappear because of the backcross steps aiming at introducing in an elite variety the modifications obtained *in vitro* in a "guinea-pig" variety. But, the number of backcrosses effectively done with the elite variety for commercialised modified products (six) are lower than the minimum number of fourteen backcrosses needed to obtain 95% of "purity". Which, depending on the size of the genome, could leave millions of "uncleaned" base pairs, without mentioning the phenomenon of genetic segregation which could raise the number of unintentional and off-target effects still present (Hollick, 2007). In addition to this is the lack of knowledge regarding the genome (Ingvarsson and Street, 2011). Last: it should not be forgotten that the movement of nucleic acids (DNA, RNA…) between different part of the plant makes it possible to detect the presence of a genetic modification in another part of the plant which was not modified initially, even for loose parts as fruits in the case of grafting.

Conclusion? There is a high probability to find in the commercialised plants most of the unintentional modifications (mutation and epimutation) occurring in a variety modified *in vitro* by a technique of genetic modification and/or because of the related techniques[64]. Therefore, used as a signature of an unnatural origin of the modification found in the plant, it would allow to detect, identify and trace those modifications.

Targets, techniques and strategies are already available to detect and identify all the products obtained through new techniques with the possibility to identify the implemented techniques of genetic modification itself and above all, a high probability to distinguish between mutations coming from *in vivo* mutagenesis and from *in vitro* mutagenesis. Methods of detection exist like multiplex PCR, SNPLex… as well as for identification (pattern / profile and decision support tools…). Providing they are validated by the EURL-GMFF with the support of the ENGL, those methods could be used in a "matrix approach" which would aim at gathering together bodies of evidence or presumptions as national control organisms do. An approach which would need databases and decision support tools allowing a routine work as it is already the case of unknown transgenic GMOs[65]. It would also be mandatory for companies to provide methods of detection / identification and reference material (as for transgenic GMOs) which would speed up the implementation of validated methods.
Documentary traceability would ease the detection for case-by-case (self)control of genetic modifications, identification of the used technique while lowering the cost. It would also allow the minimum time required by research programs to finalise the procedure of technical controls.
Those procedures and databases would possibly be filled with a watch of patents obtained or requested by companies. Databases which would also be modified on long term to take into account a higher number of elements acting directly or indirectly as "signature".

Would it be expensive? Such a traceability would not be of a prohibitive cost as it would only require most of the techniques used to aim at insuring the only presence of modifications, not necessarily the identification of the techniques nor their proprietary. Without mentioning the announced the price decline of materials used for high-throughput sequencing.

64 See www.infogm.org/spip.php?article6026 and www.infogm.org/spip.php?article6027
65 http://www.sciencedirect.com/science/article/pii/S0734975017300058