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DIRECCION GENERAL DE CALIDAD Y EVALUACION AMBIENTAL Y MEDIO NATURAL

Subdirección General de Calidad del Aire y Medio Ambiente Industrial

Secretaría de la Comisión Nacional de Bioseguridad

COMMENTS OF THE SPANISH NATIONAL COMMISIÓN OF BIOSAFETY ON NEW PLANT BREEDING TECHNIQUES

General Comments

According to the legal definition of GMOs, *a priori* it could be unclear if the products of some of the NPBTs (New Plant Breeding Techniques) would be considered a GMO given that they do not generate new combinations of genetic material that may not occur naturally or that they are included in the exemptions provided for the current legislation. In these cases, a 'case by case' assessment would be always necessary. Considering the rapid advance in molecular biology techniques the GMO regulation should focus on the final organism (product) and not on the process.

Regarding some specific NPBTs we would add the following:

Oligonucleotide-directed mutagenesis (ODM)

During the process of obtaining, oligonucleotides are introduced into the cells inducing site-specific changes in the genetic material. In addition, the oligonucleotides are not self-propagating entities and do not contain sequences necessary for replication, i.e., they are not transmitted to offspring. The ODM technique cause changes in the genome that are indistinguishable from those obtained by traditional mutagenesis techniques.

ODM should not be considered a technique that results in GMOs.

Zinc Finger Nuclease technology (ZFN o SDN)

This technology is based on using proteins that bind to a given sequence and produce a break in it. The natural DNA repair machinery repairs the break by homologous recombination. The double strand break and subsequent repair can introduce specific changes in the original sequence. This technique was initially designated as ZFN for the use of Zinc Finger Nucleases. Similar techniques have been developed that use other types of proteins so that currently, the name of SDN (Site Directed nucleases) is used.

There are three types of SDN. All produce changes in specific locations in the genome, but they differ is in the type of change that occurs:

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- SDN1: change in DNA is randomized resulting in small deletions, additions or substitutions of nucleotides. SDN1 effects are similar to those that could be obtained by mutagenesis methods so that the resulting organism should not be considered GMOs.
- SDN2: the change is site-specific and involves the deletion, addition or substitution of one or a few nucleotides.

If the effect is the replacement of one nucleotide by another the result of SDN2 is similar to those that could be obtained by mutagenesis methods so that the resulting organism should not be considered as a GMO. Therefore, SDN-2 is already captured by Annex IB and thus excluded from the Directive 2001/18/CE.

If the effect is the removal or addition of one or more nucleotides then the consideration as GMO should be determined "case by case". In any case, the SDN2 aim is not to introduce new genes but modify the existing ones, which should be considered in the assessments, regardless of whether it is considered as leading a GMO.

- SDN3: the change produced is the introduction of a DNA fragment in a specific location in the genome. The size of the inserted fragment may be from a few nucleotides to a few thousand. In this sense it is similar to what can be obtained by more "traditional" methods of genetic transformation, but differs in that the insertion site is unique and specific.

Therefore, the organisms obtained by SDN3 should be evaluated as GMOs but considering that the insertion site is known.

Another point to be considered about the SDN is that they do not require the insertion into the genome of the genes that encode for nucleases. These genes can be introduced into cells temporarily or, in some cases, the nuclease protein can be introduced directly.
Related to this issue would be the technology CRISPR/CAS whose use should be considered at some point.

Many experts are of the opinion that it should be clarified in Directive 2001/18/CE that "mutagenesis" includes SDN1/SDN2.

Cisgenesis and Intragenesis

Cisgenesis is the genetic modification of a recipient plant with a natural gene from cross-compatible species. It allows the transfer of specific genes without the need for long periods of recurrent selection. This allows to enter a character, for example, from a variety to another, at least 4 times faster than by traditional methods and is especially useful in difficult crossing species with very long periods of generation.

Cisgenesis products should be classified as GMOs. However, the effects of introduced gene are indistinct from those produced in the variety of origin and the only differences could result from the cisgen insertion in the genome. The safety analyzes should be focused in this point.

This technique may in some cases meet the criteria of self-cloning as described in Annex II, Part A of Directive 2009/41/EC and when that is the case it may be considered as falling outside the scope of Directive 2009/41/EC. We guess that cisgenesis and cisgenesis with T-DNA borders sequences identical or highly similar (≥85% identity) already present in the same or sexually compatible species, may be considered as yielding organisms equivalent to those resulting from self-cloning and therefore that could be considered as out of the scope of Directive 2009/41/EC.

Grafting

The technique of grafting is a method of propagation of plants whereby a vegetative component of a plant (the graft) is attached to a rooted part of another plant (the rootstock) to produce a chimeric organism with improved cultivation characteristics.

The graft is used primarily to allow the growth of value trade varieties in unfavorable conditions, taking the more resistance of the rooted part used, or to ensure that the productive characteristics of a specimen remain unchanged compared to the dispersion introduced by genetic sexual reproduction. Grafting is possible only between more or less closely related species. Once the graft is established, the branches of the rooted part are usually completely removed so that if it is a plant cultivated for its fruits, none come from the rootstock

If the graft is performed on a genetically modified rootstock, the beneficial characteristics acquired by the transgenic rootstocks increase fruit quality by improving plant vigor but the genetic alteration of the rootstock does not affect to the pollen or the seeds.
In these cases the rootstocks should be considered a GMO but not the graft and the derived products.

Agro-infiltration

This technique involves the use of a genetically engineered *Agrobacterium* as a vector to produce certain effects in certain tissues of the plant but not in the reproductive organs. Typically, it is applied to leaves. This technique is primarily used to identify, from a plant population, individuals that have resistance mechanisms. The genetic material introduced in the vegetative tissue of the plant is not incorporated to the germline and thus is not incorporated to the seeds or pollen.

Therefore, in those cases where Agro-infiltration is just used for transient expression in plant tissue/cell, and therefore the recombinant DNA is not replicated and/or integrated in plant genome and progeny of

the Agro-infiltrated plant is not generated, we consider that the Agro-infiltrated plant used for the generation of the product should not be considered as GMO. However, the *Agrobacterium* used are a transgenic organism that should be regulated as it does have the ability to reproduce.

RNA-dependent DNA methylation (RdDM)

RdDM is a technique which makes use of a small double strand RNA molecule that induces methylation at a target DNA sequence by the natural defense mechanism of the organism, thereby inhibiting the transcription of the target gene. As a result, the target gene is silenced without changing the DNA sequence.

As there is no alteration of the DNA sequence, the resulting organism should not be regarded as GMO.

Reverse breeding

Reverse breeding is a new technique that produces homozygous parental line to be use for the reconstruction of any hybrid plant with (un)known parents by the suppression of genetic recombination.

This is achieved by a step of genetic modification in the process to suppress the natural meiotic recombination in the plant genome. After obtaining the desired intermediate plants, the parental plants without the transgenic events are selected.
The intermediate plants containing transgenes should be classified as GMO but not the end products.

Synthetic Genomics

It involves the synthesis of DNA molecules that are then transferred into a recipient. The product of this technique should be considered as GMOs.

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