ERIC POUDELET ON NEW PLANT BREEDING TECHNIQUES (NPBT)

MULTILATERAL MEETING ON "THE FUTURE OF PLANT BREEDING TECHNIQUES IN THE EUROPEAN UNION"

WEDNESDAY 25 JUNE 2014, BRUSSELS

STEERING NOTE

1. SCENE SETTER

The **New Techniques Working Group** (NT WG) considered the following techniques:

1. Oligonucleotide directed mutagenesis (ODM)
2. Zinc Finger Nuclease Technology (comprising ZFN-1, ZFN-2 and ZFN-3)
3. Cisgenesis (comprising cisgenesis and intragenesis)
4. Grafting
5. Agro-infiltration
6. RNA-dependent DNA methylation (RdDM)
7. Reverse Breeding
8. Synthetic Genomics (only partially addressed)

In a number of cases (ODM, ZFN-1, ZFN-2, agro-infiltration, RdDM) the WG did not reach a consensus in the interpretation.

Competent Authorities or scientific institutions in some Member States (NL, DE, BE, UK) have issued position papers or reports on NPBT and their legal status, sometimes addressing socio-economic implications deriving from the inclusion of these techniques under the scope of the GMO legislation. Many other MSs have not taken any position yet.

The **New Breeding Technology Platform** issued a Legal Briefing Paper in 2013 taking into consideration the same techniques as above, except for intragenesis and synthetic genomics: the majority of techniques were considered out of the scope of the GMO legislation, except for ZFN-3.

Third Countries:

- Argentina
  Since 2012 a group of Argentine experts analyzed the issue and reached preliminary conclusions for most of the techniques. The National Advisory Committee on Agricultural Biotechnology (CONABIA) introduced the issue of NBT in its agenda during the last meeting held in late March 2013. It is expected that after a consultation process with developers, academia and researchers, decisions will be taken concerning certain techniques' inclusion in or exclusion from the GMO legislation.
- Australia, New Zealand
  In 2012 and 2013 Food Standards Australia New Zealand (FSANZ) convened an expert scientific panel to provide advice on a number of new plant breeding techniques that have come to the attention of regulators. It was not the role of the panel to make a legal determination as to whether the techniques or their derived food products would come within the definition

of 'food produced using gene technology' in the *Australia New Zealand Food Standards Code*. However, the expert panel were asked to provide their scientific opinion on whether derived food products should be regarded as GM food.

- USA

  The USA decided that no specific legislation was required for regulating plants derived from biotechnology. The USDA regulates the environmental release of certain genetically engineered organisms, which are, or are believed to be, plant pests under the Plant Protection Act. GM plants are regarded as a plant pest when genes from plant pests are introduced.

  Some decisions made by the USDA concerning crops derived by NPBTs are publically available:

  - A letter from 2004 states that under the current regulations, USDA has no authority to regulate products created by mutagenesis techniques such as Oligonulceotide directed mutagenesis.
  - Concerning plants derived by site directed nuclease techniques, USDA concluded that plants containing targeted gene deletions will not, in most cases, be regulated articles under the Plant Protection Act, unless the engineered plant is already a plant pest.
  - For applications where template DNA molecules are used (this corresponds to ZFN-2 and ZFN-3), the Agency will consider them case-by case.
  - USDA was approached by a plant breeder concerning the regulatory status of a grapevine transformed by intragenesis. The plant which carries a grapevine-derived anthocyanin regulatory gene and grapevine-derived regulatory elements is not considered to be a regulated article under the Plant Pest Act.

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2. SHORT DESCRIPTION OF THE TECHNIQUES

• OLIGONUCLEOTIDE DIRECTED MUTAGENESIS

Oligonucleotide directed mutagenesis (ODM) employs oligonucleotides for targeted (site-specific) induction of point mutations.

Oligonucleotides of approximately 20 to 100 nucleotides are delivered to the cells by methods suitable for the different cell types (including electroporation, polyethylene-glycol-mediated transfection, natural uptake). The technique exploits the sequence specific interaction of the oligonucleotide with the resident DNA of the cells, resulting in gene targeting. This directs the attempted genetic modification to a specific region in the DNA or even to a specific base pair. The genetic modification can be the induction of a point mutation or reversion of an existing mutation which may lead to changes in the expression of a gene.

• ZINC FINGER NUCLEASES

Zinc Finger Nucleases (ZFN) are protein chimeras comprised of a zinc finger based DNA binding domain linked to a DNA cleavage domain. Zinc Finger domain(s) can be custom-designed to bind to a specific site within a given locus, thereby providing a highly specific targeting tool. The genes for the ZFN proteins are delivered for

instance by electroporation with plasmids or by infection with viral vectors into the cells; *Agrobacterium*-mediated transfer can also be used in plants. ZFNs are typically expressed transiently from a non-replicating vector (plasmid, virus) however, they may also be delivered directly as proteins or as mRNA.

This technique may be used in three different ways, which are designated ZFN-1, ZFN-2 and ZFN-3. ZFN-1 generates site-specific random mutations conferring changes of a single or a few base pairs, short deletions and insertions. In ZFN-2, a short homologous repair template is used together with the ZFN-complex, to introduce specific nucleotide sequence changes by homologous recombination. In ZFN-3 a large stretch of DNA (up to several kilobases) with ends homologous to the DNA sequences flanking the cleavage site is introduced together with the ZFN-complex. This allows insertions of entire genes at specific locations and ZFN-3 might therefore be used for transgenesis as well as cisgenesis and intragenesis.

- **CISGENESIS**

Cisgenesis is the genetic modification of a recipient organism with a gene (cisgene) from a crossable - sexually compatible – organism (same species or closely related species). The gene includes its introns and its flanking native promoter and terminator in the normal sense orientation.

- **INTRAGENESIS**

Intragenesis is a genetic modification of a recipient organism that involves the insertion of a reorganised, full or partial coding region of a gene frequently combined with a promoter and/or terminator from another gene (intragene) of the same species or a crossable species.

- **GRAFTING**

Grafting is an ancient technique used to combine desired traits of two different plants. It is a method whereby a vegetative top part (*the graft or scion*) of one plant is attached to a rooted lower part (*the rootstock*) of another plant.
Two possibilities are considered:

- ✓ Grafting a non-GM scion onto a GM rootstock;
- ✓ Grafting a GM scion onto a non-GM rootstock.

- **AGROINFILTRATION**

To perform agro-infiltration, plant tissues are infiltrated (in vivo or ex vivo) with a liquid suspension of Agrobacterium sp. containing a genetic construct in order to promote localised expression of a given genetic material. The benefits of agro-infiltration over stable transformation are speed, convenience, and the high level of expression usually reached.
Depending on whether or not the plant tissues contain germline cells/tissues, two types of agro-infiltration can be distinguished:

1. **Agro-infiltration "*sensu stricto*":**

Non-germline tissues (typically, leaf tissues) are agro-infiltrated in order to obtain localised expression, for instance:

a. to obtain large amounts of a given protein expressed in plant tissues;
b. to test the phenotypic effect of a given gene product in plants

2. **"Floral dip":**
Flowers or inflorescences containing germline cells are agro-infiltrated in order to obtain stable transformation of some embryos that can then be selected at the germination step.

- **RNA-DEPENDENT DNA METHYLATION (RDDM)**

RdDM is a technique that uses the effect of small RNA sequences e.g. micro RNA (miRNA) or small/short interfering RNA (siRNA) to alter gene expression through methylation of specific DNA sequences without changing the nucleotide sequence itself (epigenetic change). The purpose could be to shut down expression of specific genes. This gene silencing obtained by the methylation can be inherited through some generations, but will eventually disappear.

- **REVERSE BREEDING**

Reverse breeding allows to produce specific F1 hybrids in a much shorter timeframe and ambient numbers in comparison with conventional plant breeding techniques. In reverse breeding, an individual heterozygous plant is chosen for its elite quality, and, subsequently, homozygous parental lines are derived from this plant, which upon crossing, can reconstitute the original genetic composition of the selected heterozygous plant from which the lines were derived.
During reverse breeding, a genetic modification step is employed to suppress recombination during meiosis. However, the final heterozygous plants (and their homozygous parental lines) are non-transgenic (devoid of any new DNA).

- **SYNTHETIC GENOMICS**

Synthetic genomics is a field within synthetic biology that may include techniques of genetic modification. It involves the synthesis of stretches of DNA molecules and their combination into functional larger synthetic DNA molecules which are then transferred into a recipient structure. The synthesis of building blocks enables the easy introduction of changes into the genetic material, including mutations (exchanges, deletions and insertions of specific nucleotides), gene fragments or complete genes including those without any natural template.
Synthetic genomics also paves the way for the introduction of redesigned or newly designed combinations of biological parts that do not necessarily exist in nature and that, for instance, enable the reconstruction of new biological pathways.

3. QUESTIONS FOR DEBATE

3.2. What are the main difficulties in the legal analysis of NPBTs?

- The definition of GMO in the EU legislation is complex, because it is referring both to the characteristics of the organism obtained and to the techniques used
- The legislation was drafted when a number of these techniques were at an initial stage of development or application and therefore were not specifically addressed.

3.3. What is the procedure the Commission is going to undertake to deliver the result of the legal analysis on NPBT? When will the result be available?

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- A Commission position document is being prepared, which will be presented to MSs and stakeholders.
- It is unlikely that the document will be issued under this Commission's term.

3.4. In the case the Commission concludes that a product falls within the scope of the GMO legislation, what happens if a company has a different position and places on the market a product without aksing for the authorisation in accordance with the GMO legislation?

- The Commission expects that its conclusions will be shared by MS and stakeholders
- In the case above described, the Commission will have the same position than for any unauthorised product: it will remind to the Member State concerned that this product – which does not comply with the conditions of the GMO legislation- shall be removed from the market

3.5. In the case where the Commission would conclude that a product does not fall under the GMO legislation - could another legislation apply?

- The work that the Commission has undergone is limited to define whether a product of the new techniques falls within the scope of the GMO legislation

- If the Commission concludes that an organism created with the new techniques is out of the scope of the GMO legislation, it should be verified whether another legislation could apply (e.g. the Novel Food legislation)

3.6. Is the Commission considering the difficulties, for some NPBTs, to differentiate their products from naturally occurring alterations of the genetic material or from products obtained by means of traditional techniques?

- The Commission is actually focusing on the legal/scientific analysis, taking into consideration the definition of GMO set out in the EU legislation and the requirements for possible exemption.

- This issue was already addressed, although at a preliminary stage, by the New Technique Working Group

- Challenges for detection were also considered by the JRC in the report "New plant breeding techniques: state-of-the-art and prospects for commercial development".

- New technologies (e.g. next generation sequencing technologies) are rapidly evolving and they could provide additional tools to facilitate detection.

3.7. Why was EFSA consulted only for three new techniques (i.e. cisgenesis, intragenesis and ZFN-3)?

- The Commission decided to focus first on the techniques which entail the introduction of exogenous genetic material into the host, which is the case for these techniques as it is for transgenesis.

3.8. Is Synthetic Biology being considered under the ongoing legal analysis by the Commission?

- No, the Commission decided to focus first on the techniques which are at a more advanced stage of development and application.

- However DG SANCO, DG RTD, DG Enterprise and DG Environment requested an opinion to three independent non-food Scientific Committees (SCCS, SCHER and SCENHIR) on an operational definition for Synthetic Biology, on risk assessment methodologies and safety aspects and on research priorities in this field. The first opinion on an operational definition is currently under public consultation; the other evaluations are ongoing. The work should be completed by March 2015.