The regulatory status of plants resulting from New Breeding Technologies

Summary Document produced by the NBT Platform[1]
22 May 2013

New Breeding Techniques

Recent scientific advances have led to the introduction of **New Breeding Techniques (NBTs)** that enable plant breeders to develop plants with new traits with greater speed, efficiency and precision; therefore offering **an improvement on traditional breeding techniques**. The NBT Platform has developed a Legal Briefing Paper[2] (LBP) for the purpose of elucidating the regulatory status of plants developed using these NBTs, since the practical use of these techniques will depend on the legal status according to Directive 2001/18/EC. This document summarises the main points raised in the LBP.

The LBP first explains the scope and purpose of Directive 2001/18/EC (from here on 'the European Directive'), which provides a definition of 'GMO' (Genetically Modified Organism) and 'non-GMO'. The analysis of the European Directive reveals that, contrary to what is often believed, the European legislator has chosen for a combined process and product approach. Consequently, a GMO is defined by a combination of the **process** (techniques of genetic modification) used to generate the plants and the characteristics of the resulting **product** (the plant itself)**.**

Cumulative analysis

Based on the abovementioned definition, a cumulative analysis has been developed, for which **every question needs to be answered in the affirmative** in order to lead to a Genetically Modified Organism covered by the European Directive. If this is not the case, it leads to a plant not subject to the European Directive. The analysis is composed of seven questions (see textbox below and the 'Cumulative Multilevel Analysis' on page (iv)).

1. Is it an organism?;
2. Is it non-human?;
3. Has the genetic material been altered (by 20bp or more) vis-à-vis the starting (parental plant) genetic material?;
4. The genetic alteration does not (and cannot) occur naturally (by mating and/or natural recombination)?;
5. Does the genetic modification occur at least through the use of the techniques listed in Annex I A part 1 of the Directive?;
6. Is the genetic modification not among the techniques listed in Annex I A Part 2?;
7. Is the genetic modification not among the techniques/methods listed in Annex I B?

[1] The NBT Platform is a coalition of SMEs, large industry representatives and members of prominent academic and research institutes which strives to bring clarity to the European debate on NBTs. Its aim is to provide policy makers and stakeholders with clear and precise information on NBTs and to generate awareness about their widespread benefits for the European economy and society as a whole. NBT Platform Secretariat, Rue Belliard 199, bte 22, 1040 Brussels, T: +32-2-5022008, info@nbtplatform.org.

[2] For access to this document, please contact the NBT Platform Secretariat using the contact information provided in this document.

The definitions in the European Directive form the basis for the analysis of each of the seven NBTs currently under evaluation **to confirm their legal and regulatory status** and thus to determine if, and for what reasons, any of these NBTs falls subject to the regulatory obligations imposed by the European Directive. The techniques covered are **1**) Zinc Finger Nuclease (ZFN) technology[3], **2**) Oligonucleotide Directed Mutagenesis (ODM), **3**) Cisgenesis, **4**) RNA-dependent DNA methylation (RdDM), **5**) Grafting (non-GMO scion on GMO rootstock), **6**) Reverse breeding, **7**) Agro-infiltration (Agro-infiltration '*sensu stricto*', Agro-inoculation). The analysis is carried out by applying a **cumulative multilevel test** to each technique, and is visualised in a **flowchart** (page iii of this summary) with supporting **checklist** (please refer to the main document).

This analysis shows that **none of the aforementioned NBTs necessarily lead to plants covered by the European Directive,** unless foreign DNA (20 bp or more) has been inserted has been inserted in the resulting plant. See page (iv) for the specific conclusions per technique. For an in-depth analysis of the techniques, please refer to the Legal Briefing Paper.

Main Conclusions

The following main conclusions were drawn from the analysis:

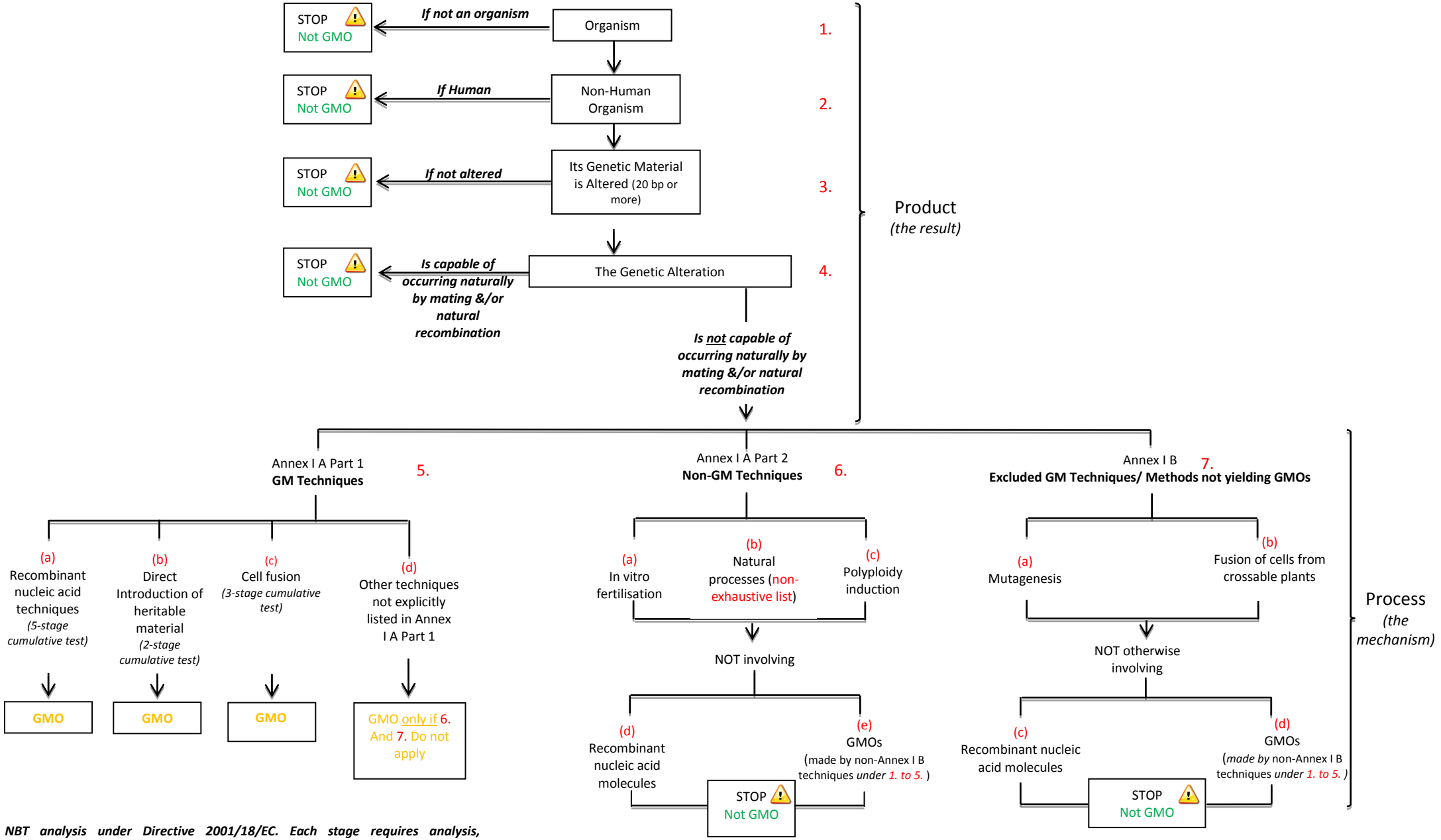
- ZFN-1 & ZFN-2 are not regarded as leading to a product covered by the European Directive for the following reasons:
    a) No foreign DNA is inserted into the genome (see LBP section 2.6).
    b) The alteration is capable of occurring naturally by mating and/or natural recombination.
    c) The oligonucleotides are not self-propagating entities and do not contain sequences necessary for replication.
    d) ZFN1 and ZFN2 are a form of mutagenesis. Mutagenesis is subject to Annex 1 B, and the resulting plants are therefore excluded from GMO regulation.
    e) Moreover, ZFN-1 falls within the 'natural processes' category in Annex I A Part 2 since in nature such deletions occur naturally (see point b). This technique replicates - in an accelerated and more efficient fashion - natural processes. Recombinant nucleic acid techniques (see LBP section 2.4) are absent because, among other considerations, ZFN-1 does not involve the *insertion* of *foreign DNA* produced *outside* the organism (see LBP section 2.6).
- ZFN-3 is regarded as leading to a product covered by the European Directive, except in the following situation:
    a) There is no formation of new combinations of genetic material (see LBP section 2.5 and point 4 in the flow chart below), provided the inserted genetic material is naturally present in the plant genome.
    b) The alteration is capable of occurring naturally by mating &/or natural recombination, provided the inserted genetic material is present in a naturally crossable plant. However, insertion of foreign DNA ($\geq$ 20 basepairs) does lead to a plant covered by the European Directive.

[3] Since it is a well documented group of techniques, ZFN technology is used as an example of a much larger group of related new genome editing techniques, commonly referred to as Site Directed Nucleases (which includes ZFNs, but also TALENs and Meganucleases).

- ODM is not regarded as leading to a product covered by the European Directive for the following reasons:
    a) No genetic material is inserted into the genome (see LBP section 2.6).
    b) The oligonucleotides are not self-propagating entities and do not contain sequences necessary for replication.
    c) The alteration is capable of occurring naturally by mating and/or natural recombination.
    d) ODM is considered a form of mutagenesis (subject to Annex I B) not involving recombinant nucleic acid molecules (see LBP section 2.7 on 'mutagenesis'). The fact that it is more precise does not necessarily exclude it from Annex I B or subject it to GMO regulation.
    e) ODM falls within the 'natural processes' category in Annex I A Part 2 since in nature such mutations and deletions occur naturally (see point b). This technique replicates - in an accelerated and more efficient fashion - natural processes. Recombinant nucleic acid techniques (see LBP section 2.4) are absent because, among other considerations, ODM does not involve the *insertion* of *foreign DNA* produced *outside* the organism.
- Cisgenesis is not regarded as leading to a product covered by the European Directive for the following reasons:
    a) there is no formation of new combinations of genetic material, since the inserted genetic material can also be exchanged through traditional breeding methods;
    b) the alteration is capable of occurring naturally by mating &/or natural recombination; and
    c) The nucleic acid molecules are capable of occurring naturally in the plant.
- RdDM is not regarded as leading to a product covered by the European Directive for the following reasons:
    a) the offspring plants are substantially unaltered *vis-à-vis* the parental plant since the alteration is epigenetic and transient;
- A harvested product from a non-GMO scion grafted on a GM rootstock[4] is not covered by the European Directive for the following reasons:
    a) the scion itself is not genetically altered, nor are the fruits, seeds or other materials from the scion; and
    b) the process of grafting is not a recombinant DNA technique, nor a direct introduction of heritable material, nor a process of cell fusion.
- Reverse breeding is not regarded as leading to a plant covered by the European Directive for the following reason:
    a) the genetic material of the hybrid plants (the product[5]) is altered in a way that is capable of occurring naturally by mating and/or natural recombination.
- Agro-infiltration is not regarded as leading to a plant/product covered by the European Directive for the following reason:
    a) The progeny plant (the product) does not contain genetic material that is altered, since intermediate plants contain T-DNA and/or genetically modified *Agrobacterium* transiently and the foreign genetic material is not incorporated in the germline.

[4] The GM rootstock itself is covered by the European Directive.
[5] Intermediate plants that contain foreign DNA are covered by the European Directive.

Cumulative Multilevel Analysis



NBT analysis under Directive 2001/18/EC. Each stage requires analysis, ultimately allowing for classification of organisms developed with current and future breeding techniques.