HIGH COUNCIL FOR BIOTECHNOLOGY

NEW PLANT BREEDING TECHNIQUES

General introduction: First stage of HCB deliberations

Paris, 20 January 2016

At the suggestion of its Board, the High Council for Biotechnology decided to address the issue of new plant

breeding techniques (NPBTs) through a self-referral. These NPBTs, some of which have already led to development and market release of new plant varieties in North America, raise a number of questions, currently being debated, concerning their risks and opportunities and the manner in which they should be regulated.

HCB has begun the first stage of its deliberations concerning NPBTs

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by clarifying the terms of the debate and assessing some of the issues involved. It has focused, in this first stage of its work, on the following points:

- Description of the main NPBTs currently being examined by the European Commission;

- Related issues: potential opportunities, particularly for development of varieties with new traits that cannot be obtained using the breeding techniques already available (conventional breeding, transgenesis); possible risks to health and the environment as well as in the socio-economic and ethical fields;

1 See statements of decisions of the Board meetings of 5 November, 1 December and 15 December 2015. It should be noted that this work had to be done in relatively short order, given the announcements by the European Commission on the discussion timetable for NPBT regulation, and members of HCB had to study a large volume of technical literature on a complex subject in a very short space of time.

- The question of whether or how, in the light of these aspects, development of NPBTs and marketing of their products should be regulated.

To deal with these issues, HCB enlisted the expertise of its Scientific Committee (SC) and Economic, Ethical

and Social Committee (EESC) on the following basis, decided by the Board.

SC: The Board asked the Scientific Committee to address two questions: a description of NPBTs, concentrating initially on the eight techniques being discussed by the European Commission

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, and the possible risks of NPBTs to health and the environment, together with information likely to be of

use when deciding whether or not to classify NPBT products as GMOs. A working group [3] described these techniques and identified the questions they raised. A memorandum drafted on the basis of this material was then discussed and approved at a full meeting of the committee [4].EESC: Qualified individuals and representatives of organisations belonging to the EESC were invited to provide summary papers clarifying their positions; these papers, together with a summary of the many legal analyses of NPBTs, contributed to the debate within the EESC and were discussed at a full meeting of the committee [5]. Further to this discussion, other EESC members made known or explained their positions. A briefing paper summarising all these contributions and the discussions to which they had given rise in the EESC was reviewed and approved by committee members.

This first stage thus produced the following material.

1. Work by the Scientific Committee

- A memorandum introducing the issue of NPBTs and the scientific questions that they raise (Scientific *Committee, Memorandum on New Plant Breeding Techniques (Document 1));*
- A set of fact sheets (one for each technique) as an appendix to this memorandum.

2. Work by the Economic, Ethical and Social Committee

- Papers from EESC members:

2 See list of NPBTs in following Scientific Committee documents. During the second stage of its deliberations, the Scientific Committee will be invited to consider other potentially relevant techniques, such as those involving epigenetic mechanisms, that might be used to produce plants in future. 3 Group set up at the end of HCB's first term. Details of its composition are given in the Scientific Committee memorandum. 4 Scientific Committee meeting of 16 December 2015. 5 EESC meetings of 10 November and 16 December 2015.

. Joint paper by the Coop de France farming cooperatives' association (CdF), the FNSEA farmers' union, the National Seed Association (GNIS), the Young Farmers (JA) and the French Union of Seed companies (UFS): Appendix 1.

. Joint paper by Friends of the Earth France (AdT), the Confédération Paysanne farmers' union (CP), the Organic Farming Federation (FNAB), France Nature Environnement (FNE), Greenpeace (GP), the Farm Seed Network (RSP) and the French Beekeepers' Association (UNAF): Appendix 2. This paper, which was subsequently supplemented with information from D. Evain (FNAB) and B. Bonzi (Friends of the Earth France), is divided into five parts (Appendices 2.1 to 2.5).

. Paper by the Trade and Retail Federation (FCD): Appendix 3.

. Paper by the National Council of Secular Family Associations (CNAFAL): Appendix 4.

. Paper by Sarah Vanuxem, qualified individual in the field of law: Appendix 5.

. Paper by Estelle Brosset, qualified individual in the field of law: Appendix 6.

Other organisations and qualified individuals have provided the HCB secretariat with information and points of view in different forms (included in the briefing paper mentioned below): Serge Boarini (qualified individual in the field of sociology), Sophie Fonquernie (Association of French Regions), François Lucas (Coordination Rurale farmers' union) and René Mazars (Collectif Interassociatif Sur la Santé, a health umbrella group).

- Rounding off this set of documents is a 'Summary of the main legal analyses of applicability of Directive 2001/18/EC to NPBTs': Appendix 7.

- Lastly comes a briefing paper based on all the above information and the discussions to which it gave rise in the EESC (EESC, Overview of papers and discussions (Document 2)).

As decided by the HCB Board, and given the short time-frame for this first stage of work, HCB is now

embarking on the second stage to examine a number of points in greater detail – NPBT traceability, consumer information, legal protection of techniques and products, regulation, forward-looking review of techniques, maturity analysis of techniques actually used, etc. – in order to provide the public authorities with all the information they require to form an opinion

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. All these points will be developed further in the second stage of HCB work.

Christine Noiville, President of the High Council for Biotechnology

6 One member of the Scientific Committee made a specific request regarding some of these points – points that will be discussed at the next Scientific Committee meetings.

ANNEXE 3 : Traduction anglaise de la Note

SCIENTIFIC COMMITTEE MEMORANDUM ON NEW PLANT BREEDING TECHNIQUES

Paris, 19 January 2016

The HCB as issued a self-referral on the “new plant breeding techniques” questions. The HCB Scientific Committee has set up a working group (WG) specifically to study a range of techniques and their possible environmental, health and technological impacts.

Its work is presented in two parts:

- The first part introduces the issue and examines the questions underlying the debate on the regulation of these techniques.

- The second part consists of a set of fact sheets produced by the WG for each technique.

1. Introduction: Background and clarification of terms

The rapid expansion in new plant biotechnology, with design and use of new plant breeding techniques (NPBTs), raises a number of questions. The current NPBT debate in Europe hinges on the regulatory framework for products obtained through use of new breeding techniques, as it is unclear whether they come under the directives on GMO use1. It is important, even critical, to settle this question if these techniques are going to be adopted by the breeders, who need an answer. It is also important to clarify the terms of the question for everyone concerned, including supply chains and consumers.

It should be noted that, because of the history of this question2, the list of techniques discussed is quite heterogeneous3, and the generic term 'new plant breeding techniques (NPBTs)' may give rise to confusion. Thus:

(1) While they all apply to plant breeding, the techniques considered are not necessarily specific to the plant kingdom (site-directed nuclease (SDN) technology, for example, or genome editing is also frequently employed for animals);

(2) Plant breeding techniques that could be considered new and could assist in producing plants obtainable by an NPBT are not included in the list if they raise no problems regarding GMO regulation: thus the fast-developing technique of genome breeding is not considered;

(3) These techniques are not necessarily new (grafting, for example, is an old technique, but the question arises of whether or not a genetically modified (GM) status must be ascribed to products derived from grafting of a non-GM scion onto a GM rootstock);

(4) Some items on the list are not techniques as such but entail use of genetic modification: for example, innovative plant-breeding strategies. This consequently raises the question of their status under the regulations, as mentioned above (case of negative segregants, for example);

(5) One last level of complexity is added by the fact that it is possible to have multiple

combinations of NPBTs (see Table 2: cisgenesis targeted using SDN-3, for example).

Anticipating that the French competent authorities would ask HCB for an opinion in preparation for consultation of Member States by the European Commission, the Scientific Committee has set up a working group to:

(1) Provide a concise, intelligible and instructive description of NPBTs, with information about their potential applications, the state of the art and their various stages of development and adoption based on talking to stakeholders (Appendix 1);

(2) Identify the questions raised by NPBTs.

1 The EU directives on GMO use are (1) Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and (2) Directive 2009/41/EC on the contained use of genetically modified micro-organisms. 2 Initial report by COGEM in 2006, followed by establishment of a European Commission working group and publication in a journal with a high impact factor (Nature Biotechnology) of the findings of a parallel investigation by Commission's Joint Research Centre (Lusser et al., 2012). 3 List of NPBTs discussed by the European Commission working group: oligonucleotide-directed mutagenesis (ODM), zinc finger nuclease (ZFN) technology, cisgenesis (including intragenesis), grafting, agro-infiltration, RNA-dependent DNA methylation (RdDM), reverse breeding, and synthetic genomics.

Basing the list on the NPBTs under discussion in the European Commission, the following techniques are considered in this report4:

1. Genome-targeting NPBTs

i. Site-directed nucleases (SDNs5: ZFN6, MN7, TALEN8, CRISPR9/Cas)

ii. Oligonucleotide-directed mutagenesis (ODM10, RTDS11, etc.)

2. Epigenetic techniques

i. Gene expression control by RdDM12

3. Various methods related to use of genetic engineering techniques

i. Specific contexts in which genetic engineering techniques are used

- Agro-infiltration

- Grafting of a non-GM scion onto a GM rootstock or a GM scion onto a

non-GM rootstock

ii. New concepts relating to the nature of the modified sequence

- Cisgenesis / Intragenesis

iii. Offspring of modified individuals whose genetic modification has been removed

by segregation

- Negative segregants, produced as a result of innovative breeding strategies (e.g. reverse breeding, various accelerated breeding methods, Seed Production Technology, etc.)

2. General characterisation of NPBTs

In comparison with “conventional” transgenesis, molecular targeting of genetic modifications in the genome is the most significant advance offered by some of these new techniques.

Site-directed nucleases (SDNs: ZFN, MN, TALEN and CRISPR/Cas9 (see Appendix 1)) can be used to target selected DNA sequences. This targeting can have three different purposes: (1) Mutation (insertion or deletion) of a single base pair or a small number of nucleotides (even several dozen) that is random although targeted at a specific site on the genome, this is known as SDN-1; (2) Allele conversion, modifying part or all of a gene sequence, which is known as SDN-2; (3) Targeted integration of a DNA sequence, known as SDN-3.

The diagram below shows how some of these techniques fit in with the existing landscape (Figure 1):

4 Each technique covered by a specific fact sheet is shown in bold type. An additional fact sheet is devoted to ‘conventional’ transgenesis for comparison purposes. 5 SDNs: Site-directed nucleases. 6 ZFN: Zinc finger nuclease. 7 Meganucleases (MNs) have not been covered by a fact sheet, since the working group considered the technology already to have been overtaken by other targeting tools. 8 TALEN: Transcription activator-like effector nuclease. 9 CRISPR: Clustered regularly interspaced short palindromic repeat. 10 ODM: Oligonucleotide-directed mutagenesis. 11 RTDS: Rapid Trait Development System. 12 RdDM: RNA-dependent DNA methylation.

Figure 1. NPBTs: SDN-1 differs from conventional mutagenesis in that it targets a specific site, usually, but not automatically, leading to loss of function in a given gene (gene knockout). Nucleases are introduced into the cell to target a mutation site, but the nature of the mutation is not predefined. With SDN-2, a DNA template is introduced into the cell together with the site-directed nucleases, enabling the nature of the modification to be defined. The template itself is not incorporated into the genome. The same purpose can be achieved using oligonucleotide-directed mutagenesis (ODM, RTDS). SDN-3 allows targeted integration of a sequence. It is this targeting of the transgene insertion site that distinguishes this last technique from conventional transgenesis.

RNA-dependent DNA methylation (RdDM) uses epigenetic mechanisms13 to control expression of a given gene without altering its base sequence. In plants, the technique is employed to alter (increase or reduce) expression of an endogenous gene. This can be used to control metabolic activity, for example. It is also possible to control gene expression in an organism interacting with the plant, enabling pathogens (for example) to be targeted. The important point to consider is the targeting of the locus of the proposed epigenetic modification. The latter can be achieved through expression of a molecule rather than a transgene or through transient expression of a transgene or targeted proteins (e.g. agro-infiltration, CRISPR with a fusion protein having methyltransferase activity (Cas9- MT), or modification induced by a transient viral infection (VIGS)). In transient transfer14, epigenetic alteration of gene expression control could be transmitted over several generations.

The other NPTBs listed on page 89 concern methods related to use of the above techniques, not excluding ‘conventional’ transgenesis. They depend on the context in which they are used (agro-infiltration, grafting), the nature of the gene modified (cisgenesis/intragenesis) or their use in innovative breeding strategies resulting in removal of genetic material by segregation through conventional cross-breeding (negative segregants).

3. Questions raised by NPBTs

With regard to the current EU regulatory framework on GMOs, questions about the status of the above NPBTs chiefly concern their similarity to techniques covered by existing directives (mainly Directive 2001/18/EC). In particular a decision is expected as to whether or not these techniques are considered to generate GMOs under the relevant legal definition and whether they must be regulated as such or should be exempt from assessment.

Under current provisions, a product has GMO status when obtained by techniques including in particular insertion of a new molecule of recombinant DNA. The provisions cover products derived

13 Epigenetics describes the molecular mechanisms involved in controlling expression of a genetically encoded trait. The present memorandum discusses modifications produced by DNA methylation. Such DNA modifications are reversible, and, although they can be transmitted from one generation to the next, whether they are retained will depend on the environment. Other modifications of DNA- associated proteins are possible. 14 Longer-lasting expression is possible with genome integration by transgenesis or SDN-3 targeted integration. Non-permanent modification is again observed if transgenes are removed by segregation.

from techniques involving a modification that is deemed to be a mutation. It should be noted that products of mutagenesis are exempt from assessment owing to a history of safe use.

Moreover, the targeting capability of some of these genome modification techniques has been highlighted as a possible argument for easing assessment regulations.

The question of a modification's transmission and heritability is also important when considering the regulatory framework for products derived from these techniques. It turns on two issues: (1) Transient (or non-transmissible) presence versus heritability of the genetic modification itself (modification of somatic cells versus germ cells, since modification of somatic cells can also be transmitted by plant propagation); (2) Transient presence versus heritability of the epigenetic consequences of a genetic modification (epigenetic changes can be induced without stable insertion of genetic material in the genome; in the event of stable insertion, they may continue to exist after this material has been removed by segregation).

- One possible approach would be to consider whether or not the products (fruit, seed, fodder, etc.) contained transgenes. Thus the products would be studied separately from other parts of the plant that did not enter the marketing chain. This does not mean that these other parts would not be assessed, particularly if their genetic characteristics were covered by Directive 2001/18/EC, but the product, if not distinguishable from a similar product obtained by a technique not falling within the definition of GMOs, should not be subject to specific assessment.

- Another set of questions concerns the possibility of detecting products of a particular technique by identifying the technique used. If molecular detection technology is unable to tell the difference between the techniques used to obtain products, non-DNA traceability methods (technical or documentary traceability) could provide information if necessary.

As noted above, EU legislation is being debated against a background of continuously evolving genetic engineering techniques. One proposal is that regulation should be based on the characteristics of the products obtained rather than the techniques used to generate them. There is also the problem of assessment if one organism cannot be differentiated from another obtained by practices not subject to assessment.

The tables below, together with the subsequent comments and overview, provide information for the French competent authorities in preparation for discussions at the EU level.

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