Explanatory Note supporting the AFBV-WGG Initiative

Suggestions to enable the development of genome editing in Europe

A. The challenges facing Agriculture:

Global agriculture is facing many challenges, the most important of which are a growing world population (9-10 billion people in 2050) and the scarcity of arable land which will remain at best stable. Hence the need to produce more on the same area while taking into account:

- Environmental constraints related to climatic variations and the need to reduce inputs (crop protection products, fertilizers, water, etc.);
- The demands and constraints coming from consumers and the food chain.

B. The need to continue to innovate for crop improvement:

To meet these challenges, all stakeholders must continue to develop innovative and efficient agriculture in France, Germany, Europe and the rest of the world. Among the innovations which are required at all steps from seed to fork, those related to plant genetics play an important role. It is essential that all technologies available for the creation of new plant varieties can be used without exclusion in principle.

C. Genome editing:

Genome editing, one of the techniques referred to as NPBT (New Plant Breeding Techniques), brings together a set of technologies allowing the modification of genetic information by addition, deletion or exchange (replacement) of nucleotides at a targeted site of the genome sequence of a recipient plant. These technologies will become essential tools for quickly obtaining, for example, resistance to biotic stresses, pathogens and aggressors, increased tolerance to abiotic stress such as tolerance to drought or temperature variations; as well as improving sanitary, technological and nutritional qualities of harvested products.

These technologies have shown a significant potential for genetic improvement in research and development. In fact, the first plants derived from these technologies are on the market in North America. Various analyses and evaluations of these technologies in France, Europe (in particular the February 2017 report of the Scientific Advice Mechanism on new biotechnologies in agriculture) and other countries conclude that these new seeds are not different in their effects on health or environment from those obtained from traditional breeding techniques. See SAM (2017) "New Techniques in Agricultural Biotechnology", https://doi.org/10.2777/17902, and SAM (2018) "A Scientific Perspective on the Regulatory Status of Products Derived from Gene Editing and the Implications for the GMO Directive", https://doi.org/10.2777/407732.

Given the potential of these technologies, it seems essential for Europe to revise the regulatory framework for plants derived from genome editing techniques. For this purpose, we present below our proposal for a revision of that framework.

D. Basis for our approach:

AFBV and the WGG are aware that a complete revision of Directive 2001/18 / EC regulating GMOs will take a long time, which is difficult to reconcile with the need to maintain the competitiveness of research teams and seed companies. Pending a complete overhaul of the European Directives and Regulations concerning GMOs, as well as a harmonization with international treaties, our organizations

propose to quickly introduce in Directive 2001/18/EC and in related GMO Regulations and Directives, new provisions that will allow the use of genome editing techniques.

E. Proposed Additions to Directive 2001/18/EC:

Without affecting the logic and coherence of the whole Directive, we propose additions that take into account up-to-date scientific knowledge and technological progress. While these additions summarized below only concern changes in Directive 2001/18/EC, it is understood that the other GMO-related Directives and Regulations in Europe will have to be amended to incorporate the same changes.

Our proposals have been written with the intention of covering plants. They may be adapted, if necessary and where appropriate, to animals and microorganisms.

We propose to address (1) the conditions of use of technologies grouped under the term "genome editing" and (2) the regulatory status of null segregants, as follows:

1. **Define genome-editing techniques**. Include a definition of genome-editing techniques in the Directive (addition of a new point (4) to Annex I A, Part 1).
2. **Remove from the scope of Directive certain categories of plants derived from genome editing**. As genome-editing technologies can be used to create a broad range of plants with new traits, going from a change in one nucleotide up to the incorporation of whole genes, we are proposing to establish different categories of plants based on the type of edit that has been obtained. At this stage, we are proposing four categories of plants derived from genome-editing techniques which should be excluded from the Directive. Following confirmation of compliance of a proposed plant with an excluded category, in accordance with a confirmation process described below, such plant would then be regulated in the same way as plants derived from traditional breeding techniques. The four categories will be described in a new Annex I C and would include the following:

   - **Category 1:** A plant having a native allele that has been edited[1] to reproduce a functionality associated with a known allele present in its natural gene pool[2].
     Making such a change would be equivalent, for instance, to the transfer of a known allele from a wild counterpart to a cultivated variety of the same species accomplished through traditional breeding.
   - **Category 2:** A plant having a native allele that has been edited to reproduce a functionality associated with a known allele present in a plant species that is outside the plant's natural gene pool.
     As the model allele exists in a non-sexually compatible plant species, there is no equivalent in traditional breeding. This category would constitute an extension of Category 1 if the donor plant and the recipient plant were sexually compatible.

[1]The terms 'Editing' or 'edited' refer to the application of 'genome editing' techniques.
[2]The term 'natural gene pool' refers to the gene pool of a plant species defined as all of the genes and alleles (i.e., different versions of the same gene) obtained from plants which can exchange genes by sexual crossing as well as from distantly related plant species with which genes can be exchanged by sexual crosses using traditional breeding techniques.

- **Category 3:** A plant having a native allele that has been edited to reproduce a new functionality, of which the sequence modifications obtained by genome editing are of the same type as those which be obtained by spontaneous or induced mutagenesis.
  In traditional breeding, such changes would be equivalent to those obtained by selecting a plant having a new allele due to a spontaneous or an induced mutation, which plant is then crossed with a cultivated plant in order to select the mutation of interest.

- **Category 4:** A plant in which a gene known and present in its natural gene pool[1] has been inserted into a targeted site of its genome.
  Amongst genotypes of a species there exists a variation in the number (from zero to N) of copies of certain genes (this may be due, for example, by duplication at the locus, uneven cross-overs or translocation via transposons). In traditional breeding one can select for "copy number" as a criteria. The addition of allelic copies by genome editing reproduces directly this breeding process.

With respect to all of the above categories, it is possible, through genome editing, to have in the same plant several edited alleles (or inserted genes). In such cases each edited allele (or inserted gene) shall be analysed independently according to the above-defined criteria. If all of the edited alleles or inserted genes fall under the same category, the plant belongs to such category. If the edited alleles or inserted genes belong to different categories, the plant must comply with each relevant category in order to be excluded. If a new edit is undertaken upon a different allele of a plant which has previously been determined to be excluded, only confirmation of exclusion for the new allele shall be required of the notifier.

Annex I hereof sets forth examples of plants belonging to the excluded categories based upon scientific publications or regulatory files accessible in public databases.

As scientific knowledge and technical progress evolve, additional new categories can be added to Annex I C (see also point 4 below).

3. **Create a new, specific, efficient and predictable regulatory pathway for the above categories of genome-edited plants**.

   Confirmation of the exclusion of an edited plant must be obtained by the notifier. The confirmation process is adapted to the exclusion category.

   - **Procedure for submitting the confirmation request**
     - The notifier shall file its confirmation request with the competent authority of the Member State in charge of GMO regulations (in France, the Ministry of Agriculture, and in Germany the Federal Ministry of Food and Agriculture) who will rely on its existing internal departments capable of evaluating GMOs (in France, ANSES or the HCB, and in Germany the BVL [Bundesamt für Verbraucherschutz und Lebensmittelsicherheit - Federal Office of Consumer Protection and Food Safety]);
     - The request for confirmation is made by the notifier whenever it wishes to benefit from the exclusion and remove its plant from the scope of Directive 2001/18 / EC, REGULATIONS (EC) No 1829/2003, No 1830/2003 as well as any other GMO regulations of the European Union.
     - The exclusion decision for an edited plant shall be valid for all progeny of such plant containing the same edit and binding upon all Member States;
     - Once the confirmation of exclusion is obtained, any variety obtained using the edited plant shall be subject to seed and plant variety regulations applicable to relevant crop species in the same manner as any variety obtained through traditional breeding techniques, including registration in the common catalogues of varieties of agricultural plant and vegetable species which can be marketed in the European Union.

- **Contents of the confirmation request application**

  The information requirements to be supplied by the notifier shall be adapted to the plant category:

  - *Standard requirements for all categories :*
    (i) Name of the notifier and contact information ;
    (ii) Taxonomic description of the plant which has been edited or in which a gene has been inserted;
    (iii) Technique used and main steps that have been followed, including, if applicable, whether or not an intermediate GMO was produced in the editing process, and the modalities of elimination of any inserted recombinant nucleic acid sequence, and confirmation of the elimination of any such inserted sequence (null segregant);

  - *Requirements that are Category specific :*
    - *For Categories 1 et 2 :*
      (i) Taxonomic description of the plant containing the model allele and a description of the model allele ;
      (ii) Description of the edit realized in the final plant (addition, deletion or replacement) and confirmation that the resulting edited sequence has been obtained and comparison of the functionality of the model and edited alleles ;
    - *For Category 3 :*
      (i) Description of the new allele and its functionality obtained after genome editing and available background information on the reasons that led to editing such allele (research work, for example) ;
      (ii) Description of the edit realized in the final plant (addition, deletion or replacement) and confirmation that the resulting edited sequence and its functionality have been obtained;
    - *For Category 4 :*
      (i) Taxonomic description of the donor plant containing the inserted gene and a description of such gene ;
      (ii) Confirmation of the sequence of the inserted gene in comparison to the original gene before insertion ;
      (iii) Confirmation that the inserted gene is located at the site targeted by genome editing.

Any information supplied by the notifier for which it wishes to claim confidentiality must be marked "Confidential".

The processing time by the competent authority of a Member State to determine whether or not an edited plant falls under one of the four Categories for exclusion should be no more than sixty days. .

4. **Permit periodic updating of the Directive if justified by advances in scientific knowledge and technical progress**. As indicated above, these proposals are based on the current state of scientific knowledge and technical progress achieved based upon that knowledge. As scientific knowledge and technical progress evolve rapidly in this field, we propose that every five years, after consulting the relevant stakeholders and in collaboration with the competent authorities of the Member States, the Commission reports to the European Parliament on developments in scientific knowledge and technical technological progress and, if necessary, proposes a revision of the annexes.

5. **Address the status of null segregants (progeny of a GMO plant from which the GMO feature has been removed).** As part of this revision of the Directive, we propose that null segregants be confirmed as being excluded from the scope of the Directive. A null segregant that is obtained after genome editing and that is also an edited plant is subject to the confirmation process to confirm exclusion under one of the four Categories above.

These different proposals are included in a draft amendment which you will find attached hereto.

Frankfurt and Paris, January 2020

Annex 1

Examples of plants falling under excluded categories, based upon scientific publications or regulatory files accessible in public databases

These examples are taken from the literature or from regulatory files. We tried to find, from available public information, the origin of the model alleles. Thus, for each example, and when available, the first reference discloses the edited plant and the other references describe the probable origin of the model alleles. Except for the plants already marketed in North America, these examples do not prejudge the fate of these edited plants and their commercial opportunities.

Methodology and criteria used:

- The example must describe an edited plant that has been achieved;
- For the examples of Categories 1 and 2, a model allele is identified in a plant that is sexually compatible (Category 1) or non-sexually compatible (Category 2);
- For the examples of Category 3, information is provided on the approaches used to obtain the edited gene, including results in transgenic plants (RNAi experiments for example);
- For category 4, information is provided on the inserted gene;
- For the edited plants we tried to use the original publication; for the model alleles we sought to find them in the publications cited by the inventors of the edited plant.

Category 1:

- An edited, salt-tolerant rice plant, following inactivation of the *OsRR22* gene (known allele). Zhang *et al.*, 2019; Takagi *et al.*, 2015.
- A potato plant edited by inactivating the *StGBSSI* gene (known allele), leading to the accumulation of amylopectin (waxy starch) in the tuber. Based on the availability of potato mutants rich in amylopectin and on knowledge of the synthesis of amylopectin in cassava, corn and wheat. Veillet *et al*., 2019; Hovenkamp-Hermelink *et al*., 1987.
- A rice plant in which the promoter of three genes coding for sucrose transporters, *SWEET11, SWEET13 and SWEET14* has been edited (modification of nucleotides) to no longer be sensitive to the transcription factor produced by *Xanthomonas oryzae pv. Oryzae*. There are rice mutants for these genes; several have been associated in this edited plant. Oliva *et al.*, 2019; Zaka et al., 2018.
- A pink-fruited tomato plant following inactivation of the *SlMYB12* gene (known allele). Deng *et al.*, 2018; Fernandez-Moreno *et al.*, 2016.
- A maize plant tolerant to *Setosphaeria turcica* (*Helminthosporium turcicum*) following the replacement, by edition, of the sensitive allele of the *NLB 18* gene coding for a membrane kinase and responsible for the interaction with the fungus by the resistant allele identified in a corn tolerant to this fungus (known allele). Schmidt 2018; Hurni et al., 2015; Li & Wilson 2006.
- A maize plant accumulating only amylopectin in the seed following inactivation of the waxy (*Wx1*) gene coding for the Granule Bound Starch Synthase (*GBSS*) (known allele). Based upon the waxy maize mutant which has been marketed for many years. Schmidt 2016.
- A soybean plant with a high oleic acid content following inactivation of two fatty acid desaturase genes (*FAD2-1A* and *FA D2-1B*) (known alleles). Haun *et al.*, 2014; Pham *et al*., 2010.
- A rapeseed plant edited to be tolerant to imidazolinone and sulfonylurea herbicide families by modifying a single nucleotide of the *BnAHAS1* gene in Genome C and a single nucleotide of the *BnAHAS3* gene in Genome A of *Brassica Napus*. https://www.inspection.gc.ca/plant-health/plants-with-novel-traits/approved-under-review/decision-documents/dd-2013-100/eng/1427383332253/1427383674669; https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-

products/novel-food-information-cibus-canola-event-5715-imidazolinone-sulfonylurea-herbicide-tolerant.html. Many mutants of this enzyme are known to exist in rapeseed, conferring tolerance to sulfonylureas. Magha *et al*., 1993.

Category 2:

- A tomato plant whose gene *SlJAZ2*, orthologue of the *AtJAZ2* gene of *Arabidopsis*, has been edited (modification of the nucleotide sequence) to reproduce the dominant mutant version of *Arabidopsis* (absence of the C-terminal - jas motif) to obtain the resistance to bacterial spot disease (*Pseudomonas syringae pv*. tomato (Pto) DC3000). This modified receptor, SlJAZ2Δjas, no longer fixes the coronatine synthesized by the bacteria and as a resultthe stomata do not open. Ortigosa *et al.*, 2019; Gimenez-Ibanez *et al*., 2017.
- An edited grape cultivar in which (i) the *Mlo* gene has been suppressed to obtain powdery mildew resistance and (ii) the *VvDMR6* gene has been suppressed based upon knowledge of the suppression of the analogous gene in *Arabidopsis thaliana* resulting in downy mildew resistance. Giacomelli *et al*., 2019; van Damme *et al*., 2008.
- A cassava plant resistant to potyvirus [Cassava brown streak disease (CBSD)] obtained by editing (modification of the nucleotide sequence) of the gene coding for the translation initiation factor elF4E. Many isoforms of this factor giving potyvirus resistance are known in many plants: chilli, tomato, pea, *Arabidopsis* mutants. Gomez *et al.*, 2019; Bastet *et al*. 2019.
- An edited wheat plant in which the three genes corresponding to the Mildew resistance Locus (*Mlo*) called *TaMlo-A1, TaMlo-B1* and *TaMlo-D1*, located on chromosomes 5AL, 4BL and 4DL, are simultaneously inactivated to reproduce a phenotype resistant to powdery mildew, based upon the knowledge of *Mlo* alleles naturally present in barley. Wang *et al.*, 2014; Büschges *et al*., 1997.

Category 3:

- An apple cultivar where the *MdDIPM4* gene (a kinase receptor) is inactivated by editing to obtain resistance to scab (*Erwinia amylovora*). By analogy with *Arabidopsis* mutants and studies of receptor interaction with the bacterium effector (DspA / E) a sequence of MdDIPM4 was deleted in the apple gene. Pompili *et al*., 2019; Degrave *et al*., 2013; Borejsza-Wysocka *et al*., 2004.
- A petunia plant with prolonged flowering by inactivation of the *Ph ACO1* gene which codes for a 1-aminocyclopropane-1-carboxylate oxidase involved in the production of ethylene (reduced quantity in the edited plant). By analogy with the results obtained by expressing antisense in petunia. Xu *et al*., 2019; Huang *et al.*, 2007.
- A durum wheat plant that has been edited to inactivate up to 35 of the 45 α-gliadin genes (known alleles) on three chromosomes, causing a reduction in the production of α-gliadins and a drop in immunoreactivity by 85%. Sanchez Leon *et al*., 2018.
- A tomato plant of which the promoter of the SlCLV3 allele (new allele) has been edited in order to increase fruit size. Rodriguez-Leal *et al*., 2017.
- In several citrus species, the promoter of the *CsLOB1* gene (LATERAL ORGAN BOUNDARIES 1) has been edited by deletion of the sequence $EBEP_{thA4}$ (which fixes the effector produced by the bacteria) conferring resistance to citrus canker [Xanthomonas citri subsp. citri (Xcc)]. Based on knowledge of the interactions between the promoter and the effector of the bacteria and on similar works on rice. Jia *et al*, 2016a (grapefruit tree); Jia *et al*., 2016b (lemon tree); Peng *et al*., 2017 (orange tree). In order for these edited plants to benefit from the exclusion provided by this Category 3, the recombinant DNA used for the editing will need to be removed (null segregants).

Category 4:

We did not find any plants that met the criteria for this category. There are many examples of plants containing one or more cisgenes (see two examples below), but none are the result of insertion at a

targeted site and homologous recombination. The cisgenes introduced into the plants described below were obtained by transgenesis. With genome editing, a cisgene may be inserted in a chosen site by double homologous recombination, without any residual vector sequence.

- A potato plant in which several mildew resistant genes identified exclusively in wild potato species have been inserted using *Agrobacterium tumefaciens*, selected on the criteria that (i) all R genes are expressed and (ii) conformity to the varietal type is maintained. Haverkort *et al.,* 2016.
- An apple cultivar made resistant to scab by inserting the cisgene *FB_MR5* from the wild variety Malus × robusta 5 (*Mr5*) in chromosome 16. Kost *et al*., 2015.

Examples of edited plants having alleles in different categories:

As indicated earlier in this Explanatory Note, the same edited plant may contain alleles which correspond to different categories. Two examples are presented below.

- A tomato plant that has been edited by inactivating (1) the *SIER* gene (which regulates tomato stem length), (2) the *SP5G* gene (linked to rapid flowering) and (3) the *SP* gene (linked to precocious growth termination), all three genes having known mutant alleles, to make it compact and early yielding, suitable for urban agriculture. This plant contains edited genes corresponding to Category 1 for the alleles of the *SlER* and *SP* genes and to Category 3 for the allele of the *SP5G* gene. Kwon *et al*. 2019; Xu *et al*., 2015; Soyk *et al*., 2017, and Menda *et al*., 2004.
- An edited cassava plant accumulating amylopectin (waxy starch) instead of amylose following inactivation of the *PTST1* gene encoding the Protein Targeting to STarch and the *GBSS1* gene encoding the Granule Bound Starch Synthase. Based on the availability of cassava mutants rich in amylopectin and knowledge of the synthesis of amylopectin in potatoes, corn and wheat. This plant contains two edited genes, the allele of the *GBSS1* gene corresponds to Category 1 and the allele of the *PTST1* gene to Category 3. Bull *et al.,* 2018; Morante *et al*., 2016

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