Explanatory Note supporting the AFBV-WGG Initiative updated March 2021

Suggestions to enable the development of genome editing in Europe

A. The challenges facing Agriculture:

Agriculture is facing many challenges, the most important of which are a growing world population (9-10 billion people in 2050), the scarcity of arable land and the risks related to climate change and biodiversity loss. Hence the need for sustainable production using less land and inputs and reduction of its environmental impacts.

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 technologies referred to as NBT (New Breeding Techniques)[1] (to facilitate reading, notes and references have been grouped together in Annex 1) brings together a set of technologies enabling the targeted modification of genetic information by addition, deletion, or exchange (replacement) of nucleotides at a specific site of the genome sequence of a recipient organism[2]. In the case of plants these technologies will become essential enabling tools for desirable traits, such as, for example, resistance to biotic stresses, pathogens and aggressors, increased tolerance to abiotic stress such as drought or temperature variations; as well as improving sanitary, technological and nutritional qualities of harvested products.

Genome editing technologies have already demonstrated their strong potential for genetic improvement of crops in research and development. In fact, the first plants bred with the use of such technologies are on the market in North America[3] and a limited launch of an edited tomato variety has begun in Japan[4]. Various analyses and evaluations of these technologies undertaken by the French High Council of Biotechnologies (HCB), EFSA and the Scientific Advice Mechanism in Europe have concluded that plants developed with the use of such technologies are no different in their effects on health or the environment from those obtained from traditional breeding methods[5].

In response to climate and environmental challenges, in December 2019 the European Commission adopted the European Green Deal with the ambition of transforming its economy and society to put them on a more sustainable path. It considers that European food is famous for being safe, nutritious and of high quality, but that it should now also become the global standard for sustainability[6]. In its "Farm to Fork" strategy published in May 2020, the European Commission announced that it was carrying out a study that would look at the potential of new genomic techniques to improve sustainability along the food supply chain[7].

Given the potential of these technologies to enable the European Union to reach its sustainability goals, it seems essential for the EU to revise the regulatory framework for plants derived from genome editing technologies[8].

For this purpose, we present below our proposal for a revision of that framework.

D. Basis for our approach:

AFBV and the WGG believe 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 an interim solution involving the targeted amendment of Directive 2001/18/EC and related GMO Regulations and Directives, by introducing new provisions that will enable developers to integrate genome editing techniques in their breeding programs.

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

Without affecting the spirit and coherence of the Directive 2001/18/EC, we propose amendments that will reflect the current scientific knowledge and technological progress since the original drafting of the legislation. While these amendments only concern provisions in Directive 2001/18/EC, it is understood that the other GMO-related Directives and Regulations in the EU will have to be amended to incorporate the same changes.

Our proposals have been written specifically with the intention of addressing the regulation of plant products. They may be adapted, if necessary and where appropriate, to animals and microorganisms.

Our proposal addresses the following two points:
(1) the regulatory status and 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 obtain a broad range of modifications in the genome, going from a change in one nucleotide up to the incorporation of whole genes, we are proposing to establish different regulatory categories based on the type of edit that has been obtained. At this stage, we are proposing four categories of plants derived from genome-editing technologies which should be excluded from the scope of 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 methods[9]. The four categories will be described in a new Annex I C to the Directive and would include the following:

    - **Category 1:** A plant having a native allele that has been edited[10] to reproduce a functionality associated with a known allele present in its natural gene pool[11].
      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 breeding methods.
    - **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.
      Since the donor plant and the recipient plant are sexually incompatible, this category is an extension of Category 1 based on phylogenetic filiation (common ancestor between these two alleles).

- **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 can be obtained by spontaneous or induced mutagenesis.
  Using traditional breeding methods, 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 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). Using traditional breeding methods, one can select for "copy number" as a criterion. The addition of allelic copies by genome editing directly reproduces 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 2 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 to the Directive (see also point 4 below).

3. **Create a new, 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 EU.
  - 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[12] in the common catalogues of varieties of agricultural plant and vegetable species which can be marketed in the EU.

- **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); 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 and its origin (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. **Introduce a regular review and updating process for the Directive to ensure it reflects the advances of 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[13]. 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
Updated with notes and references, March 2021

Annex 1

Notes and References

(1) NBT (New Breeding Techniques: NBT is an umbrella term that captures a range of different technologies deployed in plant research and breeding, such as: genome editing, epigenetic modification (RNA-directed DNA methylation), grafting on GM rootstock, reverse breeding, agro-infiltration, intragenesis and cisgenesis. Van Der Meer et al. (2020) p. 7; SAM (2017) – p. 56-70. For plant applications the acronym NPBT (New Plant Breeding Techniques) is sometimes used. The EU Commission suggested using the term NGT (New Genomic Techniques) in its consultation with stakeholders which occurred in the first half of 2020. The term includes "techniques, which are capable to alter the genetic material of an organism and which have emerged or have been developed since 2001". In addition to genome editing technologies[2] and oligonucleotide-directed mutagenesis (ODM[2]) the Commission includes epigenetic modification (RNA-directed DNA methylation). https://ec.europa.eu/food/plant/gmo/modern_biotech/new-genomic-techniques_en

(2) Our definition does not restrict the list of genome-editing technologies in a rapidly evolving field and is consistent with the definition used by SAM. SAM (2018), p. 7. Without limitation, these technologies include, for example, site-directed nuclease-1 (SDN-1), site-directed nuclease-2 (SDN-2), site-directed nuclease-3 (SDN-3), oligonucleotide-directed mutagenesis (ODM), base editing and prime editing. EFSA (2020), p. 7 The nucleases can be of different types such as Meganucleases; TALEN (Transcription Activator-Like Effector Nuclease) or, more often mentioned or cited, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). Other technologies may be added to this list as technologies further develop in the field of genome editing.

(3) Gene-edited high oleic soybeans have been commercialized in the United States since 2019. https://calyxt.com/first-commercial-sale-of-calyxt-high-oleic-soybean-oil-on-the-u-s-market/

(4) A gene-edited tomato with a higher content of γ-aminobutyric acid (GABA), developed by Sanatech Ltd in collaboration with the University of Tsukuba, will be distributed to Japanese gardeners for their private use. The company is preparing simultaneously to produce necessary quantities for large-scale commercialisation. https://sanatech-seed.com/en/20201211-1-2/; http://p-e-s.co.jp/tomato/high-gaba-tomatoes-monitor/.

(5) In its 2020 opinion (EFSA (2020) – p. 11) EFSA's GMO panel concluded that it "did not identify any additional hazard associated with the use of the SDN-1, SDN-2 or ODM approaches as compared with both SDN-3 and conventional breeding techniques which include conventional mutagenesis. The SDN-1 and SDN-2 approaches can induce off-target changes but, like for SDN3, these would be fewer than those occurring with classical mutagenesis techniques, decreasing the risk of alteration or interruption of genes." In addition, for many species, field crops and vegetables in particular, the breeder is used to backcrossing to return to the elite variety containing only the new genomic fragment which provides the desired trait, the edited allele in this case. In its 2020 opinion EFSA recalled that in its 2012 opinion on SDN-3 it had pointed out that "backcrossing steps which follow the transformation process would likely remove off-target mutations from the genome of the final product […] The GMO Panel considers this aspect still applicable to plants generated via SDN-1, SDN-2 and ODM approaches." EFSA (2020) p. 10. See also SAM (2017) at pp. 87-91, Haut Conseil des Biotechnologies (2017), at pp. 48-55 (French version), 46-51 (English translation).

(6) Communication from the European Commission: "The European Green Deal", 11 December 2019, p. 11. https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1576150542719&uri=COM%3A2019%3A640%3AFIN

(7) Communication from the European Commission : A Farm to Fork Strategy for a fair, healthy and environmentally-friendly food system, 20 May 2020, p. 8. https://eur-lex.europa.eu/legal-content/FR/TXT/?uri=CELEX:52020DC0381
"Climate change brings new threats to plant health. The sustainability challenge calls for measures to protect plants better from emerging pests and diseases, and for innovation. The Commission will adopt rules to reinforce vigilance on plant imports and surveillance on Union territory. New innovative techniques, including biotechnology and the development of bio-based products, may play a role in increasing sustainability, provided they are safe for consumers and the environment while bringing benefits for society as a whole. They can also accelerate the process of reducing dependency on pesticides. In response to the request of Member States, the Commission is carrying out a study which will look at the potential of new genomic techniques to improve sustainability along the food supply chain."

[8] SAM declared in 2018 “new scientific knowledge and recent technical developments have made the GMO Directive no longer fit for purpose.” SAM (2018) at p. 2. In addition, Julien Denormandie, French Minister of Agriculture, interviewed in *L’Opinion* in September 2020, answered a question on NBTs:
"What is your position on new genome editing technologies, which make it possible to speed up variety selection? It is a complex, legal subject. There is a red line in Europe that must not be crossed: that of GMOs. That said, plant innovation techniques are evolving. The European framework which regulates them dates from the beginning of the 21st century, it is undoubtedly unsuitable for these new technologies which make it possible to sift through what nature would undoubtedly offer, of itself, at a given moment, and present an agronomic interest. It should be made to evolve without crossing the red line."
https://www.lopinion.fr/edition/politique/julien-denormandie-il-faut-remettre-souverainete-alimentaire-coeur-224872. This topic was followed up by Reuters (Paris): France backs non-GMO regulation for crop gene-editing in EU. 18 January 2021. https://www.reuters.com/article/france-agriculture-gmo/france-backs-non-gmo-regulation-for-crop-gene-editing-in-eu-idINL8N2JT4A3

[9] For each of our four exclusion categories we have provided an example of equivalent genetic modification that can be obtained using traditional breeding methods. A few traditional breeding methods that have been compared to genome editing technologies are mentioned in SAM (2017) - pp. 29-36 and 94-100, EFSA (2012a) - pp. 13-18, EFSA (2012b) - pp. 7-8, and EFSA (2020) - note 7, p. 8.

[10] The terms ‘Editing’ or ‘edited’ refer to the application of ‘genome editing’ techniques.

[11] 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 methods of conventional breeding.

[12] In the EU, for plant species concerned by the "catalogue" regulation, any new variety offered for marketing must first be registered in the official catalogue of species and varieties of cultivated plants in at least one Member State. All the national catalogues constitute the Community catalogue. In France, registration in the Official Catalog is issued by order of the Ministry of Agriculture on the proposal of the Permanent Technical Committee for the Selection of Cultivated Plants (CTPS). As explained by France’s Scientific Committee of the HCB: “In France, marketing of varietal seed requires authorisation. This is provided through registration with the Official French Catalogue, the purpose of which is to guarantee users seed that is of sound and fair merchantable quality. Once a new variety has been produced, it must undergo a series of tests to check that it meets the three requirements of distinctness, uniformity and stability (DUS), as well as the requirements of value for cultivation, use and the environment (VCUE). Thus, for some species, assessment of cultivation covers yield and growth characteristics, while assessment of use may cover protein and antinutrient content, and environmental assessment may cover resistance to certain pests to reduce pesticide use and resistance to abiotic stresses to reduce use of resources (water, nitrogen, phosphorus, etc.). The VCUE tests are specific to each species.” Haut Conseil des Biotechnologies (2017), p. 57 (French version), p. 52 (English translation).

[13] In 2016 the Scientific Committee of the Haut Conseil des Biotechnologies concluded “In plant breeding, using negative segregation to remove a genetic modification event, of whatever origin (conventional crossbreeding, transgenesis, SDN-3, cisgenesis or intragenesis, agro-infiltration, etc.), is standard procedure. After molecular confirmation that the modification has been removed, the resulting plant should be exempt from risk assessment and could be considered to be a plant obtained by conventional breeding.” Haut Conseil des biotechnologies (2016) at pp. 13-14 (French), p. 97 (English).

References mentioned in the notes above:

EFSA (2012a) "Scientific opinion addressing the safety assessment of plants developed using Zing Finger Nuclease 3 and other Site-Directed Nucleases with similar function". EFSA Journal 2012;10(10):2943. https://doi.org/10.2903/j.efsa.2012.2943.

EFSA (2012b) "Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis. EFSA Journal 2012;10(10):2561. https://doi.org/10.2903/j.efsa.2012.2561.

EFSA (2020) "Applicability of the EFSA Opinion on site-directed nucleases type 3 for the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide directed mutagenesis". EFSA Journal 2020;18(11)/6299. https://doi.org:10.2903/j.efsa.2020.6299

Haut Conseil des Biotechnologies (2016). Comité Scientifique, Note sur les « Nouvelles Techniques », Paris, le 19 janvier 2016, http://www.hautconseildesbiotechnologies.fr/fr/system/files/file_fields/2016/03/30/cs_1.pdf.

Haut Conseil des Biotechnologies (2017). Comité Scientifique, Avis sur les Nouvelles Techniques d'Obtention de Plantes (New Plant Breeding Techniques -NPBT), Paris le 2 novembre 2017 (adopté par le CS le 26 avril 2016). http://www.hautconseildesbiotechnologies.fr/fr/avis/avis-sur-nouvelles-techniques-dobtention-plantes-new-plant-breeding-techniques-npbt

http://www.hautconseildesbiotechnologies.fr/sites/www.hautconseildesbiotechnologies.fr/files/file_fields/2018/01/11/publicationtraductionanglaise-171201aviscsnpbtfinale.pdf

SAM (2017) "New Techniques in Agriculture Biotechnology". https://doi.org/10.2777/17902

SAM (2018) "A Scientific Perspective on the Regulatory Status of Product Derived from Gene Editing and the Implications for the GMO Directive". https://doi.org/10.2777/407732.

Van Der Meer *et al*., The Status under EU Law of Organisms Developed through Novel Genomic Techniques, European Journal of Risk Regulation (2020), doi:10.1017/err.2020.105

Annex 2

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.

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 result the 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:

- A tomato plant where the *SIGAD3* gene has been inactivated to obtain three to five times higher content of γ-aminobutyric acid (GABA), useful in the prevention of life-style diseases (hypertension, diabetes). Although the *SIGAD3* gene has been identified in tomato since 2008, its role in bioaccumulation of GABA in tomato fruits was discovered in transgenic experiments (Nonaka *et al*., 2017; Lee, 2018).
- 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

References cited in the above examples:

Bastet *et al*. 2019. Mimicking natural polymorphism in eIF4E by CRISPR-Cas9 base editing is associated with resistance to potyviruses. Plant Biotechnology Journal **17:** 1736–1750**-** doi: 10.1111/pbi.13096

Borejsza-Wysocka *et al*., 2004. Silencing of apple proteins that interact with DspE, a pathogenicity effector from *Erwinia amylovora*, as a strategy to increase resistance to fire blight. Acta Horticulturae **663**: 469–474 - doi:10.17660/ActaHortic.2004.663.81

Bull *et al.*, 2018. Accelerated *ex situ* breeding of GBSS- and PTST1-edited cassava for modified starch. Science Advances **4**:eaat6086 - doi.org/10.1126/sciad v.aat60 86

Büschges, R. *et al*., 1997. The barley Mlo gene: A novel control element of plant pathogen resistance. Cell **88:** 695–705.

Degrave *et al*., 2013. The bacterial effector DspA/E is toxic in *Arabidopsis thaliana* and is required for multiplication and survival of fire blight pathogen. Molecular Plant Pathology **14**: 506–517 - DOI: 10.1111/mpp.12022.

Deng *et al.,* 2018. Efficient generation of pink-fruited tomatoes using CRISPR/Cas9 system. Journal of Genetics and Genomics **45**: 51-54 - doi.org/10.1016/j.jgg.2017.10.002.

Fernandez-Moreno *et al.* 2016. Characterization of a new pink-fruited tomato mutant results in the identification of a null allele of the SlMYB12. Plant Physiology **171**: 1821-1826.

Giacomelli *et al.,* 2019. Generation of mildew-resistant grapevine clones via genome editing, ISHS Acta Horticulturae 1248: XII International Conference on Grapevine Breeding and Genetics - DOI: 10.17660/ActaHortic.2019.1248.28.

Gimenez-Ibanez *et al*., 2017. JAZ2 controls stomata dynamics during bacterial invasion. New Phytologist **213**: 1378–1392 - doi: 10.1111/nph.14354.

Gomez *et al.,* 2019. Simultaneous CRISPR/Cas9-mediated editing of cassava *eIF4E* isoforms *nCBP-1* and *nCBP-2* reduces cassava brown streak disease symptom severity and incidence. Plant Biotechnology Journal **17**: 421–434 - doi: 10.1111/pbi.1298.

Haun *et al.*, 2014. Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. Plant Biotechnology Journal **12**: 934–940 - doi: 10.1111/pbi.12201.

Haverkort *et al.*, 2016. Durable Late Blight Resistance in Potato Through Dynamic Varieties Obtained by Cisgenesis: Scientific and Societal Advances in the DuRPh Project. Potato Research - DOI 10.1007/s11540-015-9312-6.

Hovenkamp-Hermelink *et al.*, 1987. Isolation of an amylose-free starch mutant of the potato (*Solanum tuberosum L.*). Theoretical Applied Genetics **75**: 217–221 - https ://doi.org/10.1007/bf002 49167.

Huang *et al.*, 2007. Delayed flower senescence of *Petunia hybrida* plants transformed with antisense broccoli ACC synthase and ACC oxidase genes. Postharvest Biol. Technol. **46**: 47–53.

Hurni *et al*., 2015. The maize disease resistance gene Htn1 against northern corn leaf blight encodes a wall associated receptor-like kinase. Proceedings of the National Academy of Sciences **112**: 8780-8785 - doi/10.1073/pnas.1502522112.

Jia *et al.*, 2016a. Modification of the PthA4 effector binding elements in Type I CsLOB1 promoter using Cas9/sgRNA to produce transgenic Duncan grapefruit alleviating XccDpthA4:dCsLOB1.3 infection. Plant Biotechnol. J. 14, 1291–1301.

Jia *et al.*, 2016b. Genome editing of the disease susceptibility gene CsLOB1 in citrus confers resistance to citrus canker. Plant Biotechnol. J., doi.org/10.1111/pbi.12677.

Kwon *et al.,* 2019. Rapid customization of Solanaceae fruits crops for urban agriculture. Nature Biotechnology - doi.org/10.1038/s41587-019-0361-2.

Kost *et al*., 2015. Development of the first cisgenic apple with Increased Resistance to Fire Blight. PLoS ONE, 10, e0143980 - DOI:10.1371/journal.pone.0143980.

Lee, Jeongeun, Utilization of High GABA Tomato via CRISPR/Cas9 for Hybrid Breeding, A Dissertation Submitted to the Graduate School of Life and Environmental Sciences, the University of Tsukuba in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Agricultural Science (Doctoral Program in Biosphere Resource Science and Technology), February 2018, https://tsukuba.repo.nii.ac.jp/?action=repository_uri&item_id=48214&file_id=17&file_no=1

Li & Wilson, 2006. Composition and methods for enhancing resistance to northern leaf blight in maize. World Intellectual Property Organization, Application No. PCT/US2011/041822.

Menda *et al.*, 2004. In silico screening of a saturated mutation library of tomato. Plant Journal **38**: 861–872.

Morante *et al.*, 2016. Discovery of new spontaneous sources of amylose-free cassava starch and analysis of their structure and techno-functional properties. Food Colloids **56**: 303-395 - doi.org/10.1016/j.foodhyd.2015.12.025.

Nonaka et al., 2017. Efficient increase of ɣ-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. Scientific Reports 7 - DOI:10.1038/s41598-017-06400-y

Oliva *et al.*, 2019. Broad-spectrum resistance to bacterial blight in rice using genome editing. Nature Biotechnology **37**: 1344-1350.

Ortigosa *et al.,* 2019. Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of SlJAZ2. Plant Biotechnology Journal **17**: 665–673 - doi: 10.1111/pbi.13006.

Peng *et al.,* 2017. Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene CsLOB1 promoter in citrus, Plant Biotechnology Journal **15**: 1509–1519 - doi: 10.1111/pbi.12733.

Pham *et al.*, 2010. Mutant alleles of *FAD2-1A* and *FAD-1B* combine to produce soybeans with the high oleic acid seed oil trait. BMC Plant Biology **10:** 195-206 - biomedcentral.com/1471-2229/10/195.

Pompili *et al.,* 2019. Reduced fire blight susceptibility in apple cultivars using a high-efficiency CRISPR/Cas9-FLP/FRT-based gene editing system. Plant Biotechnology Journal - doi: 10.1111/pbi.13253.

Rodriguez-Leal *et al.,* 2017. Engineering Quantitative Trait Variation for Crop Improvement by Genome Editing, Cell 171, 470–480, http://dx.doi.org/10.1016/j.cell.2017.08.030.

Sanchez Leon *et al.*, 2018. Low-gluten, non-transgenic wheat engineered with CRISPR-Cas9. Plant Biotechnology Journal **16**: 902–910 - doi: 10.1111/pbi.12837.

Schmidt 2016. Corn with high content of amylopectin developed by CRISPR/Cas technology. 15-352-01_air_inquiry_cbidel Pioneer. https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regulated/regulated_article_letters_of_inquiry/regulated_article_letters_of_inquiry.

Schmidt 2018. Corn with Improved Resistance to Northern Leaf Blight developed by CRISPR-Cas technology. 17-076-018_air_inquiry_a1_cbidel revised Pioneer, https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regulated/regulated_article_letters_of_inquiry/regulated_article_letters_of_inquiry.

Soyk et al., 2017. Variation in the flowering gene *SELF PRUNING 5G* promotes day-neutrality and early yield in tomato. Nature Genetics **49**: 162–168.

Takagi *et al.,* 2015. MutMap accelerates breeding of a salt-tolerant rice cultivar. Nature Biotechnology **33**: 445–449.

van Damme *et al*., 2008. Arabidopsis DMR6 encodes a putative 2OG-Fe(II) oxygenase that is defense-associated but required for susceptibility to downy mildew. The Plant Journal **54**:785-793 -. doi: 10.1111/j.1365-313X.2008.03427.x.

Veillet *et al.*, 2019. The *Solanum tuberosum* GBSSI gene: a target for assessing gene and base editing in tetraploid potato, Plant Cell Reports **38**:1065–1080, https://doi.org/10.1007/s00299-019-02426-w

Wang *et al.*, 2014. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nature Biotechnology **32**: 947-952 - doi:10.1038/nbt.2969.

Xu *et al.,* 2020. CRISPR/Cas9-mediated editing of 1-aminocyclopropane-1-carboxylate oxidase1 enhances Petunia flower longevity. Plant Biotechnology Journal **18**: 287-297 - doi: 10.1111/pbi.13197.

Xu *et al*., 2015. A cascade of arabinosyltransferases controls shoot meristem size in tomato. *Nature Genet.* **47**, 784–792.

Zaka *et al*., 2018. Natural variations in the promoter of OsSWEET13 and OsSWEET14 expand the range of resistance against *Xanthomonas oryzae pv*. PLoS ONE 13(9): e0203711 - doi.org/10.1371/journal.pone.0203711.

Zhang *et al.*, 2019. Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. Mol Breeding **39**: 47-56 - doi.org/10.1007/s11032-019-0954-y.

A few published reviews - For additional information on the production of plants by genome editing:

- Chen *et al.*, 2019. CRISPR/Cas genome editing and precision plant breeding in Agriculture. Annual Review of Plant Biology **70**: 28.1-28.31 – doi.org/10.1146/annurev-arplant-050718-100049.
- Jaganathan *et al.*, 2018. CRISPR for crop improvement. An update review. Frontiers in Plant Science - doi:10.3389/fpls.2018.00985
- Metje *et al.*, 2020. Genome edited plants in the field. Current Opinion in Biotechnology **61**: 1-6 - doi.org/10.1016/j.copbio.2019.08.007.
- Modrzejewski *et al*., 2019. Environmental Evidence - What is available evidence for the range of application of genome-editing as a new tool? Environmental Evidence 8- https://doi.org/10.1186/s13750-019-0171-5.
- Sharma *et al.*, 2019. Recent advances in developing disease resistance in plants, F1000Research, 8(F1000 Faculty Rev):1934 Last updated: 19 NOV 2019, doi.org/10.12688/f1000research.20179.1.
- Soda *et al.,* 2018. CRISPR-Cas9 based plant genome editing: significance, opportunities and recent advances. Plant Physiology and Biochemistry **131**: 2-11 – dx.doi.org/10.1016/j.plaphy.2017.10.024.
- Zhang *et al*., 2018. Applications and potential of genome editing in crop improvement. Genome Biology 19: 210-XXX – doi.org/10.1186/s13059-018-1586-y.