Detailed comments on the Commission's proposal for NGT plants

AFBV and WGG recognize that the regulatory proposals for NGT plants (New Genomic Techniques) recently published by the Commission, if adopted, will enable the development in Europe of NGT plants that meet the needs of farmers and the demands of consumers and industry. The Commission proposes to establish two categories of NGT plants: the first, called category 1, would include NGT plants that could also occur naturally or be produced by conventional breeding techniques, as well as their progeny obtained by conventional breeding techniques. Following a declaration/verification process, verified category 1 NGT plants would not be subject to the rules and requirements of EU GMO legislation nor to the provisions of other EU legislation that applies to GMOs. They would be subject to regulations applicable to conventionally bred varieties. Other NGT plants would be classified as category 2. These plants would remain subject to the rules and requirements of EU GMO legislation, with an adapted risk assessment to cater for their diverse risk profiles and to address detection challenges.

While the draft regulation will have an overall positive impact, we believe that the bulk of NGT plants to be developed will concern category 1, and that the development of category 2 plants will be more limited, at least in the first few years. Comments by AFBV and WGG presented in this document are intended to help clarify the proposal, in order to increase chances of success for NGT plants and help make European agriculture more sustainable and better adapted to climate change.

Our comments are grouped into four points. The comment numbers refer to the texts of the Regulations (**Comment Rx**) or of the Annexes (**Comment Ay**) attached to this document. Comments concerning the text of the Regulations should be addressed as a matter of priority. Comments requesting explanations for the criteria and texts of the Annexes may be taken into consideration by the implementing acts provided for in Article 27 (a) and (b).

1. Criteria for classifying NGT plants as category 1 (NGT-1) - (Annex I):

It is important that these criteria be simple, precise and clear. Developers and competent authorities involved in the declaration and verification process should be able to determine unambiguously whether the NGT plant in question corresponds to one or more of these criteria. It should be possible for the competent authority to give a yes/no answer within the timeframe allowed by Article 6 or 7, as the case may be.

- **First paragraph of Annex I:**
  - **Ceiling for the number of modifications contained in a category 1 plant (Comment A1):** We understand that an NGT-1 plant may contain a limited number of modifications (20), which may correspond, for example, to the insertion of 20 cisgenes, or to modifications resulting from multiplexing using 20 CRISPR RNA guides. We suggest a **few simple rules to ensure that identical modifications are not counted twice.**

Proposed rules for calculating the number of modifications:

- **Homologous genes present in a diploid genome**
  In any case involving several copies of the same gene (homologous genes) we consider the nature of the modification made to the gene:
  - If the modifications made are of the same nature for all copies (same function obtained) they are counted as 1;
  - If the modifications made are different in nature and lead to different functionalities, each modification counts as 1.

- **Homologous genes present in an autopolyploid species (e.g., potato)**
  The above rules apply to plants with an autopolyploid genome.

- **Homeologous genes present in the genome - case of polyploid species (e.g., wheat)**
  - If the modifications on homeologous genes are of the same nature for all copies (same function obtained), they are counted as 1;
  - If the modifications on homeologous genes are different in nature and lead to different functionalities, each modification counts as 1.

- **Nature of modifications - examples**
  - Identical in nature
    - ✓ If SDN1 – same RNA guide or different RNA guides but targeting the same area of the gene. The modification obtained leads to the same function.
    - ✓ If SDN2 or SDN3 – same matrix.
    - ✓ Prime or Base editing – same change of base(s).
  - Different in nature
    - ✓ If SDN1 – different RNA guides targeting a different area of the gene.
    - ✓ If SDN2 or SDN3 – different matrices
    - ✓ Prime or Base editing – change of one or more other bases.

- **Off-target analysis (Comment A2):** The last part of this paragraph "*in any DNA sequence sharing sequence similarity with the target site that can be predicted by bioinformatics tools*" needs to be clarified. This could be understood as a reference to off-targets. If so, this needs to be made explicit. The consequence is that off-targets will have to be analysed or eliminated by backcrossing before a declaration can be filed which may be impossible for some species. Under these conditions, should the off-targets analysed be included in the modification count? Such obligation could severely limit the number of modifications needed to express the desired trait(s) in the NGT plant.

  Such an analysis can only be carried out in species for which a complete genomic sequence is available, whereas targeted mutagenesis can be carried out with partial sequences. What solution would be proposed for these species? Will it be necessary to determine the complete genomic sequence of NGT-1 plants? Such requirement would limit the availability of targeted mutagenesis in such species as well as in polyploid plants in general, or those with a large genome.

  If we wish to limit or avoid the presence of off-targets, we believe that certain procedures should be put in place, such as:

  - Define a level of similarity with the guide RNA sequence that is compatible with scientific knowledge of DNA/RNA hybridisation. A value of 80% could be appropriate for the analyses.
  - Request that information be provided in the declaration request for the NGT-1 plant regarding measures taken during targeted mutagenesis to limit off-targets and the analyses carried out after mutagenesis to verify the presence or absence of off-targets.

- Grant a derogation from off-target analysis requirement for NGT-1 plants that have been backcrossed according to existing standards for the applicable plant species used in conventional breeding. Breeders are constantly confronted with "off-target" (undesirable or unwanted) modifications, for example when carrying out induced random mutagenesis or crossing an elite plant with a wild species. In such cases, backcrossing to revert to the elite plant containing the desired trait(s) is standard practice.
- When counting modifications made to achieve the desired trait, only consider *targeted* modifications in the calculation.

- **Criteria (1): substitution or insertion of up to 20 modified nucleotides (Comment A3)**: To remove possible ambiguities, the following points should be clarified:
  - Only the modified nucleotides in the area targeted by the guide RNA should be considered.
  - These 20 modified nucleotides correspond to nucleotides that can be modified by substitution or insertion during one editing experiment (at a targeted site) resulting in a modification.
  - These 20 modified nucleotides can be contiguous or dispersed in the area targeted by the guide RNA.
  - Furthermore, these modified nucleotides correspond to what is found on one strand of DNA, and the modification of one base leading to the modification of the corresponding base on the other strand of DNA should not be taken into account.

- **Criteria (3): Insertion or substitution**. We understand that these criteria concern cisgenesis (definition given in Article 3, paragraph 5), which includes intragenesis.

  - Definitions/remarks concerning cisgenesis and intragenesis can be found at various points in the text:
    - *Explanatory memorandum:* Page 1 (full text) - 2nd paragraph states that cisgenesis includes intragenesis.
    - *Explanatory memorandum: Page 1 (full text) - Notes N° 4 and 5,* cisgenesis is defined as: "[i]nsertion of genetic material (e.g. a gene) into a recipient organism from a donor that is sexually compatible (crossable). Exogenous genetic material can be introduced without (cisgenesis) or with modifications/arrangements (intragenesis)". "Crossable means that there are no natural barriers to the interbreeding of two plants from the same or different species. "
    - *Whereas N° 2: Page 1* - Intragenesis is defined as "a subset of cisgenesis resulting in the insertion in the genome of a rearranged copy of genetic material composed of two or more DNA sequences already present in the breeders' gene pool".
    - *Article 3 - section 5: Page 12* - Cisgenesis is defined as " techniques of genetic modification resulting in the insertion, in the genome of an organism, of genetic material already present in the breeders' gene pool".

    These different definitions help us to understand criteria 3 (a) and 3 (b) of Annex I, the wording of which suggests that they concern cisgenesis and possibly, at least in part, intragenesis.

  - Criteria 3 (a) and (b) are worded as follows:

    "Provided that the genetic modification does not interrupt an endogenous gene:
    (a) the targeted insertion of a contiguous DNA sequence existing in the breeder's gene pool;
    (b) the targeted substitution of an endogenous DNA sequence by a contiguous DNA sequence existing in the breeder's gene pool."

- **Criterion 3 (a):** Two points should be considered:
  - ***Comment A4:*** The term "**targeted insertion"** suggests that random insertion of contiguous DNA, even if carried out without interruption of an endogenous gene, is not permitted under this category.

    We do not understand the exclusion of this possibility. When a breeder chooses to cross two plants, one of which, the donor plant, contains a desirable gene not present in the other (recipient) plant (but originating from another crossable plant), he can select the progeny containing the desirable gene in the genome of the recipient plant without predicting the site where this gene will be found.

    We request modification of category 3 (a) to permit random insertions without interrupting an endogenous gene and suggest inserting the words "or random" in the proposed text: "***the targeted or random insertion of a contiguous DNA sequence existing in the breeder's gene pool***". It should be noted that this type of random insertion has already been used successfully to produce plants of interest to farmers and consumers. Two examples are cited by the JRC in its recently published report entitled: "Economic and environmental impacts of disease-resistant crops developed with cisgenesis" (doi:10.2760/715646). The examples cited (potato resistant to *Phytophthora* and apple resistant to scab) were obtained by random cisgenesis. This report confirms the value of this type of cisgenesis for NGT-1 plants.

  - **Comment A5:** Taking into account the formulation used in several places in the text (see above), we understand that the introduced **genetic material** corresponds to "**a contiguous DNA sequence**". To avoid any ambiguity, we propose that a definition of the term "contiguous DNA sequence" be provided. Does this sequence cover a gene (coding sequence and regulatory sequences - promoter and terminator) or does it correspond to *any* contiguous DNA sequence? At the limit two nucleotides constitute a contiguous DNA sequence. As an alternative, we suggest the following wording: "***targeted or random insertion of a gene existing in the breeder's gene pool***".

- **Criterion 3 (b): Comment A6:** This criterion covers "*The targeted substitution of an endogenous sequence with a contiguous DNA sequence existing in the breeder's gene pool*": should there be a relationship between the two sequences that are exchanged (e.g., coming from a homologous gene)? If this is not the case, could we take, for example, a coding sequence from a gene and replace it with another coding sequence from a different gene, or replace a gene's promoter with a promoter from another gene provided the coding sequence or the promoter come from the targeted plant or exist in the breeder's gene pool? What is, and what is not, permitted under this criterion needs to be clarified.

2. The possibility of crossing NGT-1 plants – Article 3, section 7

The section provides: "An NGT category 1 plant" is an NGT plant which:

(a) fulfils the criteria of equivalence to conventional plants, set out in Annex I, or

(b) is progeny of the NGT plant(s) referred to in point (a), including progeny derived by crossing of such plants, on the condition that there are no further modifications that would make it subject to Directive 2001/18/EC or Regulation 1829/2003;

(a) We suggest modifying paragraph (a) as follows: "fulfils **one or more criteria of equivalence to conventional plants, set out in Annex I"**. **Comment R1 – pages 12 and 14**: As written the paragraph could be interpreted to mean that the NGT-1 plant must fulfil all of the criteria listed in Annex I. This comment also applies to Article 6, Section 3 d (ii).

(b) **Comment R2 – pages 12 and 14:** We understand this paragraph to mean that when two NGT-1 plants are crossed, their progeny is an NGT-1 plant (subject to the condition stated in the paragraph) which can be used in conventional breeding programs. However, certain points must be clarified:

- **The case of hybrids:** Crosses of two NGT-1 parental plants can produce hybrids (in corn for example). Both parents having been declared and verified, could the resulting hybrid be placed on the market as an NGT-1 plant, after registration in the seed catalogue? Could we produce and commercialise hybrids originating from two parents each having more than 10 modifications, containing as a result more than 20 modifications?

  When such hybrids are made available to a third party, does their labelling need to mention two identification numbers (one for each parent)?

- A breeder can also cross two NGT-1 plants and further breed with the progeny. Will he be allowed to keep only the plants having all the modifications of the parents or will he be able to choose plants having some but not all modifications from each plant, i.e., those most relevant for the selected plant and useful for the environment chosen for its cultivation? Will such selected combination of modifications have to be declared and validated before the resulting varieties are entered in the seed catalogue? Regarding their labelling, will it be necessary to indicate the two identification numbers of the original parents, including in those instances when all parental modifications are not present in the final variety?

To sum up:

- Can an NGT-1 plant enter the breeder's gene pool and be used like any other conventional plant?
- Is it necessary to keep track of the modifications in the progeny?
- Can these modifications be present in combinations that are different from those of the original verified NGT-1 plant(s) from which they were derived?
- Does the limit of 20 modifications per plant still apply to progeny, or can this ceiling be exceeded?
- What information must be provided when registering a variety containing modifications resulting from breeding programmes using one or more verified NGT-1 plants?

Comments on labelling – Articles 9 and 10 – page 17:

The current proposal is that the label of the plant made available to third parties bears the words: "*«cat 1 NGT»*, *followed by the identification number of the NGT plant(s) it has been derived from*". Furthermore, a public register will be set up containing verfied NGT-1 plants with a description of the trait(s) and characteristics which have been introduced or modified, a summarised description of the technique(s) used to obtain the genetic modification, and an identification number.

If the modifications introduced concern several traits, there will be several descriptions. During the crosses carried out not all traits will necessarily be conserved in the final plant and others, coming from another NGT-1 plant, may be present.

In order for the information found in the public register and on the label to be informative, shouldn't an identifier be assigned per trait?

We suggest that the identification consist of three parts: two alphanumeric digits corresponding to the applicant, followed by two corresponding to the species of the declared plant and ending with five corresponding to the trait introduced or modified (for a total of nine alphanumeric digits).

3. **Proposed ban for organic farming - Article 5 (2) and Whereas 23:**

Comment R3 – pages 6 and 13: We believe that organic farmers and markets for organic products should have the freedom to choose to use NGT-1 plants and their products in the same way as modified plants and products currently excluded from Directive 2001/18/EC.

Indeed, category 1 NGT plants will provide traits that can help organic farmers address cultivation challenges during attacks by pests when they cannot use synthetic pesticides. Why not let organic farmers decide themselves which NGT-1 plants they wish to grow, particularly those that fulfil criterion 5 of Annex I? Currently in France this situation exists for hybrid cabbage the parents of which were obtained from protoplast fusion (a technique excluded from the scope of GMO legislation – Annex IB of Directive 2001/18/EC). Such cabbage are used by some organic farmers and not by others. As Europe wishes to increase the share of organic farming in agriculture, why deprive organic growers of technologies that can only help them to achieve this objective?

4. **European legislation on GMOs obtained by transgenesis – Comment R4 – Whereas N°9, page 4:**

We suggest deletion of the sentence: "*Moreover, there is no indication that current requirements in the Union GMO legislation for GMOs obtained by transgenesis need adaptation at the present time*." How transgenesis is regulated is not the subject of the current mandate but this topic remains heavily debated. Indeed, for several years no application for GMO cultivation has been submitted in Europe while new GMOs, such as GM corn or GM wheat tolerant to drought, or GM potatoes resistant to *Phythopthora* are cultivated throughout the world. Is this absence linked to European regulations or other reasons? For many years, many stakeholders and EFSA have been asking for an adjustment of the studies to be carried out depending on the nature of the GM plant. Flexibility must be introduced and the systematic need for animal feeding experiments revisited. The treatment of transgenesis could be the subject of a future specific revision.

Attachments: Text of the Regulation and its Annexes with additional comments.