



Council of the
European Union

Brussels, 13 November 2023
(OR. en)

**Interinstitutional File:
2023/0226(COD)**

15275/23
ADD 4

LIMITE

**AGRI 699
AGRILEG 285
ENV 1278
CODEC 2101**

CONTRIBUTION

From: General Secretariat of the Council
To: Delegations

Subject: Regulation on new genomic techniques (NGT) – common position of Sweden, Estonia, Denmark, Ireland, Portugal and the Netherlands

Delegations will find in annex a submission from delegations on the above subject, concerning drafting suggestions and comments on the Presidency compromise text for a Regulation on new genomic techniques (NGT) put forward after the meeting of the Working Party on Genetic Resources and Innovation in Agriculture (Innovation in Agriculture) on 30-31 October 2023.

**COMMON POSITION OF SWEDEN, ESTONIA, DENMARK, IRELAND, PORTUGAL
AND THE NETHERLANDS**

ANNEX I

Criteria of equivalence of NGT plants to conventional plants

A NGT plant is considered equivalent to conventional plants when it differs from the recipient/parental plant by no more than 20 genetic modifications per monoploid genome of the types referred to in points 1 to 5 4, in any DNA sequence sharing sequence similarity with the targeted site that can be predicted by bioinformatic tools.

Criteria specific for the use of targeted mutagenesis:

- (1) substitution or insertion of no more than 20 nucleotides;
- (2) deletion of any number of nucleotides;

Criteria specific for the use of cisgenesis:

- (3) on the condition that the genetic modification does not interrupt an endogenous gene or that the resulting combination of DNA sequences in the recipient plant already occurs in a species from the breeders' gene pool:
 - (a) ~~targeted~~ insertion of a continuous DNA sequence existing in the breeders's gene pool;
 - (b) ~~targeted~~ substitution of an endogenous DNA sequence with a continuous DNA sequence existing in the breeders's gene pool;
- (4) targeted inversion of a sequence of any number of nucleotides;
- (5) ~~on the condition that the resulting DNA sequences in the recipient plant already occur in a species from the breeders' gene pool:~~
 - (a) ~~targeted insertion of a continuous DNA sequence existing in the breeders' gene pool;~~

(b) ~~targeted substitution of an endogenous DNA sequence with a continuous DNA sequence existing in the breeders' gene pool.~~

Additionally we would like to add the following text to the new recital text of recital 14 :

Translocation occurs in nature and in conventional breeding and can therefore be classified as an event that can fall under NGT category 1, as long as this event follows the criteria as set out in Annex I, specifically under criterium 3 and 4.

Justification of the changes in annex I:

1. The specification of which criteria should be used for targeted mutagenesis (TM) or cisgenesis (CG).

It is crucial that Annex I is clear and leaves no room for interpretation, therefore we think it is important to explicitly state what criteria should be used for either TM or CG. With this addition annex I will leave no room for interpretation on this part. This separation of criteria is in line with the Commission's discussion paper that they put up for discussion in the Joint Working Group early in 2023, where this separation was also made.

2. "per monoploid genome"

Modification of recessive genes in relation to ploidy of the crop

Polyploid plant species are very common in nature. As opposed to diploid species polyploid plants carry more than two sets of chromosomes. Diploids are denoted as $2n=2x$, where x is the monoploid set of chromosomes and n is the haploid number of chromosomes present in egg or pollen cells. Many crops are polyploid. For instance, seedless banana and watermelon are triploid ($2n=3x$). Durum wheat, oat, potato, canola, leek, cotton and rose are tetraploid ($2n=4x$). Bread wheat, triticale (a hybrid of durum wheat and rye), kiwi and chrysanthemum are hexaploid ($2n=6x$). Strawberry and dahlia are octaploid ($2n=8x$). Sugarcane is decaploid ($2n=10x$). Higher numbers also exist. In addition, polyploids also arise occasionally in diploid species because of various mistakes in meiosis. For instance, several apple varieties are triploid although their parents were diploid, including Belle van Boskoop, Bramley, and Jonagold.

Mutations are more common in polyploid than diploid plants for two main reasons. First, polyploid plants naturally accumulate more mutations per generation than diploid plants due to their larger genome size (Konečná et al. 2021). Second, polyploid plants tolerate higher densities of induced mutations since loss of function mutations are masked by the redundancy of other gene homologs (Krasileva et al. 2017). As an example of the latter case, mutation densities of EMS mutant populations of hexaploid wheat are up to 10-fold higher than those of diploid barley (Tsai et al. 2013). We argue that the higher number of mutations observed in polyploid plants should be reflected in Annex 1 to ensure equivalence of NGT plants to conventional plants and therefore suggest the change to annex 1.

During the breeding process, the ploidy level is especially relevant for recessive traits. The difference between dominant and recessive genes is very clear in the case of plant disease resistance breeding. A resistance (R) gene that recognizes a pathogen and triggers the innate defense response, only needs to be present in a single copy to confer the desired trait. During crossing and selection it will therefore behave as a dominant trait. A different route is when resistance is created by

disrupting or removing plant genes that are hijacked and used by the pathogen during the infection process. This form of resistance is often very durable. However, to obtain disease resistance with such susceptibility (S) genes, often all copies of the S gene must be disrupted or deleted. For that reason, this behaves as a recessive trait.

A well-known example of a susceptibility (S) gene and one that has been used for generating disease resistance in several crop species, is Downy Mildew Locus O (*MLO*). *MLO* has first been identified in barley, where a natural *mlo* knockout mutation led to resistance to Downy Mildew (Jorgensen, 1992). This mutation was discovered more than 70 years ago (Pavan et al., 2010). It has been used in barley breeding since 1979 (Lyngkjaer et al. 2000). The function of *MLO* is universally conserved, therefore knockouts in other species also lead to disease resistance. *mlo* mutants have been found in many other species or induced and selected for using random mutagenesis techniques (Kusch & Panstruga, 2017). Targeted mutagenesis, such as CRISPR/Cas, has also been used to target *MLO* genes. Wang et al. (2014) used CRISPR/Cas9 to target the three homeologs of *MLO* located on the three genomes (A, B, and D) of bread wheat. Tek et al. (2022) used CRISPR/Cas9 gene editing to investigate three *MLO* candidate genes in cucumber.

The proposal to look at targeted gene edits on the basis of the monoploid chromosome set is directly relevant for targeted mutagenesis of *MLO* and other S genes. To generate a functional mutant variety of rye ($2n=2x$) that is resistant to downy mildew one would need to targeted mutate or edit one *MLO* gene in its genome, and self the progeny to make the mutated gene homozygous, as is the case in barley ($2n=2x$). To get the same result in durum wheat ($2n=4x$) one will need to mutate the two homologous genes, namely one on each of its two genomes. In bread wheat one will have to targeted mutate or edit the three homologous genes. In strawberry one will need to mutate the four homologous genes, to obtain the same result. As the targeted mutations or edits are functionally identical, it can be considered inappropriate to count each of them individually, and if you would nevertheless do that, the effective number of recessive targeted mutations that can be induced in NGT1 category plants will differ dramatically because of ploidy alone (20 mutations in diploid rice, 10 in tetraploid durum wheat, 6 in hexaploid bread wheat, 4 in decaploid sugarcane).

When one counts the number of changes related to the monoploid genome, all the cases would all count as one change (out of 20).

In addition, the change of “per monoploid” also clarifies that it is irrelevant if the same mutation is present in heterozygous or homozygous form in diploid and polyploid crops.

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- Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., & Qiu, J. L. (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology*, 32(9), 947–951. <https://doi.org/10.1038/nbt.2969>

3. Removal of “targeted” in criterium 3 and 5

It is sufficient to check the place of insertion after cisgenesis to ensure that no endogenous genes is interrupted. Targeted insertion of a cisgene is not necessary for his check. This was also concluded in the report of EFSA (Mullins et al., 2022).

Sources:

Mullins, E., Bresson, J. L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., Firbank, L. G., Guerche, P., Hejatko, J., Moreno, F. J., Naegeli, H., Nogué, F., Rostoks, N., Sánchez Serrano, J. J., Savoini, G., Veromann, E., Veronesi, F., Fernandez, A., Gennaro, A., Papadopoulou, N., ... Schoonjans, R. (2022). Criteria for risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis. *EFSA Journal*, 20(10). <https://doi.org/10.2903/j.efsa.2022.7618>

4. Addition of translocation to recital 14

We miss the presence of translocation in the annex, while it is explicitly mentioned in the Technical paper from the commission that these translocations do occur in nature and conventional breeding. E.g. transposons, which may form major parts of plant genomes, are a common source of genome translocations in plants. They can be activated by traditional breeding methods or natural environmental conditions, and they can be utilised as molecular markers in plant breeding. Therefore it would be logical to include translocation as an event that can fall under NGT category 1, under the requirement that it does not lead to intragenesis.

We understood from the Commission that translocation is covered by criterium 3, under substitution and insertion, however it would be good to clarify this in the recitals.

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