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KENMERK	CGM/231124-01
ONDERWERP	Advice to revise Annex I NGT proposal

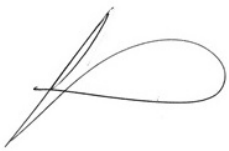
Geachte mevrouw Heijnen,

Mede naar aanleiding van de toelichting van de Europese Commissie aangaande de wetenschappelijke onderbouwing voor de criteria in Annex I van het NGT-voorstel, deelt de COGEM u het volgende mee.

Samenvatting:
Eerder dit jaar heeft de COGEM een advies uitgebracht over het voorstel van de Europese Commissie (EC) voor nieuwe wetgeving voor planten die zijn vervaardigd met behulp van bepaalde ‘nieuwe genoomtechnieken’ (NGT’s), dat wil zeggen gerichte mutagenese en cisgenese. In haar advies stelde de COGEM dat het EC voorstel in lijn was met de eerdere COGEM adviezen. Verder was ze van oordeel dat de veiligheid voor mens en milieu met het nieuwe wetsvoorstel gewaarborgd blijft.
Ten aanzien van de criteria in de Annex I van het voorstel die zijn opgesteld om een onderscheid te maken tussen planten die wèl en planten die niet vergelijkbaar zijn met conventioneel veredelde planten, merkte de COGEM op dat deze wetenschappelijke onderbouwing missen, en verduidelijking en aanpassing behoeven. Mede gezien de zeer korte deadline voor het advies heeft de COGEM destijds geen voorstel voor aanpassing van de criteria gedaan.
Inmiddels heeft de EC een Technical paper gepubliceerd waarin een rationale wordt gepresenteerd voor de criteria in Annex I. Naar aanleiding hiervan en ter ondersteuning van IenW met het oog op de raadsonderhandelingen over het EC voorstel, komt de COGEM in dit advies met een concreet tekstvoorstel voor aanpassing en verbetering van de Annex I.

De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies met een het tekstvoorstel voor aanpassing van de Annex I, treft u hierbij aan als bijlage.

Hoogachtend,



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c.c.

- Dr. T.N.V. Saaki, Ministerie van IenW, Directie Omgevingsveiligheid en milieurisico's, DG Milieu en Internationaal
- Drs. Y de Keulenaar, Hoofd Bureau ggo

Opinion to revise the criteria in Annex I of the EC proposal for new legislation for NGT plants

COGEM advice CGM/231124-01

1. Introduction

In response to a request from the Ministry of Infrastructure and Water Management, COGEM previously issued advice on the European Commission's (EC) proposal for new legislation for plants produced using certain "new genomic techniques" (NGTs), i.e. targeted mutagenesis and cisgenesis.[1]

The EC proposal distinguishes between NGT plants that could also be obtained by conventional breeding (so-called NGT1 plants) and plants that are not similar to conventionally bred plants (so-called NGT2 plants). To determine whether NGT plants belong to the first or second group, the proposal includes a number of criteria to determine whether the plants are equivalent to conventionally bred plants (Annex I of the proposal).

In its advice, COGEM stated that the EC proposal is in line with previous COGEM opinions. Also, with a view to possible future scientific developments, COGEM considered it prudent that the proposal provides for the regulation of any future NGT plants obtained by targeted mutagenesis, which cannot be said in advance to have a comparable safety profile to conventionally bred plants. COGEM commented that the criteria in Annex I designed to distinguish between plants that are (NGT1) and plants that are not (NGT2) comparable to conventionally bred plants lacked scientific foundation and needed clarification and adjustment.

Because of the very short deadline in which the advice had to be completed and the missing scientific substantiation for the criteria, COGEM did not make a proposal to adjust the criteria in the Annex. Recently, the EC has published a Technical paper presenting a rationale for the criteria.[2] Partly in response to this and in support of the Ministry, COGEM in this advice presents a concrete text proposal for adapting and improving Annex I.

In this advice and text proposal the rationale of the EC and various "position statements" of other EU member states and advisory bodies, such as the German ZKBS,[3] have been considered.

2. Considerations

The EC's proposal aims to exempt plants obtained using targeted mutagenesis and cisgenesis from the obligations of GMO regulations, because the risk profile of these NGT1 plants is similar to that of plants obtained through conventional breeding, which includes mutagenesis by radiation or chemical mutagens (classical mutagenesis). The genomic changes, such as mutations, deletions, or translocations, induced via targeted mutagenesis can also be induced via classical mutagenesis, or natural processes. The gene sequences introduced into the plant with cisgenesis can also be brought into the plant or crop via conventional breeding, because they are present in the breeder's gene pool.

COGEM notes that even conventional breeding does not involve zero risk. Through conventional breeding, it is possible to introduce increased toxicity, allergenicity or feral potential, for example. The history of safe-use of plant breeding is based on the fact that breeders want to avoid these unfavorable traits. Genotypes or plants with adverse traits are not selected or the traits are removed by selfing or outcrossing. If necessary, adverse traits are controlled for. In potato breeding, for example, alkaloid content in ware potatoes is controlled.[4]

The criteria in Annex I are therefore not intended to exclude all possible risks, but to ensure that even with the future state of science and technology, the products (NGT1 plants) of targeted mutagenesis in particular remain within the range of the risk profile of conventionally bred plants. Plants that do not meet these criteria (NGT2 plants) must undergo a risk assessment.

The Annex I from the EC proposal consists of the following text:

ANNEX I
Criteria of equivalence of NGT plants to conventional plants

An NGT plant is considered equivalent to conventional plants when it differs from the recipient/parental plant by no more than [20] genetic modifications of the types referred to in points 1 to 5, in predictable DNA sequences. A predictable DNA sequence is any DNA sequence that shares sequence similarity with the targeted site.

(1) Substitution or insertion of no more than [20] nucleotides
(2) Deletion of any number of nucleotides;
(3) On the condition that the genetic modification does not result in an intragenic plant:
    (a) Targeted insertion of a contiguous DNA sequence existing in the breeder's gene pool;
    (b) Targeted substitution of an endogenous DNA sequence with a contiguous DNA sequence existing in the breeder's gene pool;
(4) Targeted inversion of a sequence of any number of nucleotides;
(5) Any other targeted modification of any size, on the condition that the resulting DNA sequences already occur (possibly with modifications as accepted under points 1 and/or 2) in a species from the breeders' gene pool

COGEM has the following considerations and comments on the criteria in Annex 1:

2.1 Targeted mutagenesis and cisgenesis are both NGTs but differ widely

The current Annex I can be interpreted in several ways, partly because attempts have been made to capture both targeted mutagenesis (gene editing) and cisgenesis in five seemingly linked criteria. However, cisgenesis, the insertion of sequences that can also be introduced into the crop via conventional breeding or crossing, is a fundamentally different technique from (targeted or classical) mutagenesis. Mutagenesis introduces genetic variation that is (possibly) not yet present in the breeder's gene pool. Creating criteria to cover both the products of cisgenesis and targeted mutagenesis creates ambiguity about the scope of the criteria. This will be discussed further in some of the sections below.

Separation between these two different techniques and the consequences of genetic modification in the criteria of Annex I increases the readability and clarity of the criteria to be used.

2.2 Limiting the size of insertions

The justification provided by the EC[2] for the criteria in Annex I, that a maximum of 20 contiguous nucleotides may be inserted because a sequence above 20 nucleotides constitutes a unique sequence that is not present in the genome of the plant species or the breeder's gene pool, is debatable. COGEM acknowledges that approx. 20 nucleotides might be a lower limit of a 'unique' sequence, depending on the size of the plant genome in question. However, whether an inserted sequence is 'unique' is less relevant in determining whether the resulting plant falls into the same risk profile as conventionally bred plants. After all, with (classical or targeted) mutagenesis the plant breeder aims to obtain a unique sequence. By introducing a small number of mutations into the existing sequence, a unique sequence is obtained.

On the other hand, the COGEM recognises that a limit must be drawn on the number, and size, of insertions and targeted modifications in order to prevent (currently theoretically) the creation and insertion of a synthetic gene or transgene. From pragmatic considerations, COGEM can therefore agree to a limitation for the insertion of at most 20 contiguous nucleotides.

2.3 Number of allowed modifications in the plant genome

Annex I states that a maximum of 20 modifications (of the types 1 to 5) may be introduced. COGEM notes that in classical mutagenesis, hundreds of mutations, deletions, translocations, and substitutions are introduced into the genome of a plant during the breeding process. These kinds of changes may also occur naturally due to mistakes during DNA replication and chromosome segregation needed for cell division and the formation of gametes (meiosis).[5,6,7,8] Therefore, the imposed restriction of 20 modifications is inconsistent with the number of modifications generated by classical mutagenesis or occurring naturally.

Furthermore, the limitation to 20 modifications per NGT1 plant does not consider polyploidy (multiple sets of chromosomes) and gene copies, resulting in 'target' sequences appearing multiple times in the plant genome. Introducing mutations in multiple gene copies can be highly desirable for breeders, especially for so-called recessive traits. In a diploid plant (2n) with a recessive, usually inactive, allele underlying the desired trait, currently breeders can use self-crossing of a heterozygous genotype for that trait to obtain a homozygous plant in which the dominant allele is no longer present. With polyploid plants (> 2n), this is more difficult or impossible with increasing polyploidy. Many crops are polyploid, such as potato (4x), rape seed (4x), groundnut (4x), wheat and oats (6x), or strawberry (8x).[9]

To circumvent this problem, the same modification can be introduced simultaneously in multiple gene sequences in the genome by applying CRISPR-Cas, for example. Consequently, the number of 20 modifications could easily be reached and even exceeded.

Taking into account the aforementioned, COGEM sees no scientific justification for the restriction to 20 modifications. However, COGEM recognizes that it is justified to limit the number of modifications made in the sequence of the same gene, which theoretically could create a 'new gene' or a transgene. It is, therefore, proposed to arbitrarily limit the number of modifications in a single gene to (insertions, modifications and substitutions of) 20 nucleotides where the gene sequence in question may be present multiple times in the genome. Here, a gene refers to the transcribed sequence including promotor, coding and regulatory regions.

2.4 Crosses between NGT1 plants and modifications in different genes

COGEM points out that NGT1 plants have a similar risk profile to conventionally bred plants. It follows that NGT1 plants can be crossed with conventionally bred plants or other NGT1 plants and the progeny classified as NGT1. Indeed, in both cases, the progeny will fall in the range of the risk profile of conventionally bred plants because when plants with two different genes, alleles or traits are crossed, which have been assessed as 'equal to conventional', their progeny is so as well.

It also follows that if targeted modifications are made in a plant in two different genes, this also falls under NGT1, as the product is the same as that of a cross between two NGT1 plants. If modifications are made in a previously modified gene of an NGT1 plant, resulting in stacking of modifications in one gene, it will have to be verified whether the 20 modifications are not exceeded. If they are, the plant in question will have to be assessed as an NGT2 plant.

2.5 Targeted cisgenesis and targeted inversons?

All criteria in Annex I include the term 'targeted'. For criteria concerning targeted mutagenesis, this is logical, but not for cisgenesis or inversion of sequences. Cisgenesis, i.e. the introduction of genes from the breeder's gene pool, does not involve targeted insertion and that this is not currently possible either. In doing so, the criteria unintentionally exclude the possibility of applying cisgenesis under the proposed new regulations.

Inversion of sequences is a phenomenon that can occur both in classical mutagenesis and under natural conditions.[2] It is unclear why the criteria only allow 'targeted inversion of sequences'. It is possible that the term 'targeted' in the criteria has multiple meanings, but it leads to confusion and legal ambiguity.

3. Advice

In view of the above, COGEM arrives at the following proposal for amending Annex I:

Criteria of equivalence of NGT plants to conventional plants

Part 1: Targeted modifications

An NGT plant is considered equivalent to conventional plants when it differs from the recipient/parental plant by modifications of the types referred to in points 1 to 2:

1. Substitution, modification or insertion of no more than 20 nucleotides in a targeted gene (protein-coding and non-coding sequences), and its regulatory regions. The targeted sequence may occur multiple times in the genome due to gene and genome duplications;

2. Deletion, inversion or translocation of any number of nucleotides.

Part 2: Introduction of sequences from the breeder's gene pool

An NGT plant is considered equivalent to conventional plants when it differs from the recipient/parental plant by insertion of sequences present in the breeder's gene pool by:

1. Insertion of contiguous DNA sequences existing in the breeder's gene pool;
2. Any modification of any size, on the condition that the resulting contiguous DNA sequences already occur in a species from the breeders' gene pool.

References

1. COGEM (2023). Voorstel Europese Commissie voor nieuwe wetgeving voor planten verkregen met gerichte mutagenese, cisgenese en intragenese. COGEM advies CGM/230710-01
2. Technical paper by the Commission services (16 oktober 2023). Rationale for the equivalence criteria in Annex I to the proposal for a Regulation on plants obtained by certain new genomic techniques. https://data.consilium.europa.eu/doc/document/ST-14204-2023-INIT/EN/pdf
3. Zentrale Kommission für Biologische Sicherheit (oktober 2023). Position Statement of the ZKBS on the European Commission's proposal for a regulation on plants obtained by certain new genomic techniques and their food and feed, and amending Regulation (EU) 2017/625
4. van Loon J (2019). Door eendrachtige samenwerking - De geschiedenis van de aardappelveredeling in Nederland, van hobby tot industrie. 1888 - 2018. Thesis, Wageningen University, DOI: https://doi.org/10.18174/469088
5. Ossowski S *et al*. (2010). The rate and molecular spectrum of spontaneous mutations in Arabidopsis thaliana. Science 327: 92-94, https://doi.org/10.1126/science.1180677
6. Weng ML *et al*. (2019). Fine-grained analysis of spontaneous mutation spectrum and frequency in Arabidopsis thaliana. Genetics 211: 703-714, https://doi.org/10.1534/genetics.118.301721
7. Yang N *et al*. (2017). Contributions of Zea mays subspecies mexicana haplotypes to modern maize. Nat. Commun. 8: 1874, https://doi.org/10.1038/s41467-017-02063-5
8. Ma L *et al*. (2021). From classical radiation to modern radiation: past, present, and future of radiation mutation breeding. Front. Public Health 9: 768071, doi: 10.3389/fpubh.2021.768071
9. Akagi T *et al*. (2022). Polyploidy before and after domestication of crop species. Curr. Opin. Plant Biol. 69, https://doi.org/10.1016/j.pbi.2022.102255.