Ministerie van Landbouw, Visserij,
Voedselzekerheid en Natuur

> Retouradres Postbus 20401 2500 EK Den Haag

De Voorzitter van de Eerste Kamer
der Staten Generaal
Kazernestraat 52
2514 CV DEN HAAG

Directoraat-generaal Agro

Bezoekadres
Bezuidenhoutseweg 73
2594 AC Den Haag

Postadres
Postbus 20401
2500 EK Den Haag

Overheidsidentificatienr
00000001858272854000

T 070 379 8911 (algemeen)
F 070 378 6100 (algemeen)
www.rijksoverheid.nl/lvvn

Ons kenmerk
DGA-PAV / 101909058

Uw kenmerk
178345

Bijlage(n)
2

Datum 12 november 2025
Betreft Beantwoording vervolgvragen Eerste Kamer over Raadsmandaat NGT-voorstel

Geachte Voorzitter,

Hierbij zend ik u de antwoorden op de vragen van de PvdD fractie en SP fractie van de Eerste Kamer, die zijn gesteld op 4 oktober 2025. Deze vragen gingen over het Commissievoorstel en het behaalde Raadsmandaat over Nieuwe Genomische Technieken (NGTs) en mijn beantwoording van eerdere vragen op 23 juni 2025.

Jean Rummenie
Staatssecretaris van Landbouw, Visserij, Voedselzekerheid en Natuur

Vragen en beantwoording

Beantwoording SO vragen Eerste Kamer over het NGT-voorstel en de Kamerbrief van 11 april 2025 (Kamerstuk 27428, nr. 409) getiteld "Akkoord algemene oriëntatie NGT dossier in de Raad" en de brief aan de Eerste Kamer van 23 juni 2025 (kenmerk: 177466) getiteld "Beantwoording vragen Eerste Kamer over NGT-voorstel en Raasmandaat".

1. U verwijst naar een aantal rapporten en publicaties waarin onderbouwing zou worden geleverd voor de bijdragen van NGTs aan de duurzaamheidsdoelen van de EU. De genoemde rapporten en publicaties leveren dit bewijs echter niet. De impact analyse van de Europese Commissie noemt twee voorbeelden van planten die gezonder zouden kunnen zijn, namelijk soja en tomaat. De overige voorbeelden in de rapporten leveren geen bewijs – het zijn allemaal potentiële bijdragen, geen bewezen bijdragen. Ook Schneider et al. leveren geen bewijs dat NGTs bijdragen aan duurzaamheidsbeleid; er wordt alleen gesproken over een potentieel. Santosh Kumar et al. omschrijven een wetenschappelijk experiment met rijst – ook weer potentieel en geen bewijs. Het lijkt erop dat dit voorstel voor een verordening is gebaseerd op beloftes in plaats van bewijs.
   Welk wetenschappelijk bewijs heeft u dat NGT categorie 1 planten bijdragen aan duurzame ontwikkeling?

Antwoord: Mijns inziens laten de rapporten uit mijn eerdere antwoord uitstekend zien dat NGT planten bijdragen aan duurzame ontwikkeling. Daarbij plaats ik wel een belangrijke kanttekening: Duurzaamheidsvoordelen hangen af van de landbouwpraktijk, dit bepaalt de duurzaamheid. Een plantenras is van zichzelf niet duurzaam of onduurzaam, maar de eigenschappen van het ras kunnen de teler ondersteunen bij een duurzame landbouwpraktijk, bijvoorbeeld door de ziektedruk laag te houden door gebruik te maken van resistentiegenen tegen ziekten en plagen.
Dit is ook de essentie van mijn eerdere antwoord: Mijn positie is dat NGTs van zichzelf niet duurzaam of onduurzaam zijn. Voor mij zijn NGTs niet de gouden oplossing voor alle duurzaamheidsvraagstukken, maar een gereedschap dat onderdeel is van de bredere gereedschapskist die ik de plantaardige sector wil bieden.

2. Naar aanleiding van het antwoord op onze eerdere vraag over bomen en bosproducten (vraag 3) concluderen deze leden dat inderdaad categorie 1 NGT bomen en bosproducten op grote schaal kunnen worden geplant waardoor op de langere termijn deze planten de natuur kunnen gaan domineren.
   Bent u het met deze leden eens dat dit ook het geval kan zijn voor tuinplanten?

Antwoord: Inderdaad kunnen binnen de voorgestelde NGT verordening ook NGTs worden gebruikt bij de veredeling van tuinplanten, waarmee tuinplanten NGT-1 of NGT-2 planten worden, conform de definitie in het wetsvoorstel van de Europese Commissie.

3. Hoe verhoudt het voorstel voor een verordening over NGTs zich tot internationale wet- en regelgeving, zoals het biodiversiteitsverdrag?

Antwoord: Het NGT voorstel is opgesteld met inachtneming van internationale wet- en regelgeving. Dit geldt ook voor het biodiversiteitsverdrag en bijbehorende protocollen. Zoals het Nagoya protocol dat geldt voor eigenschappen die met zowel conventionele veredelingstechnieken als NGT-technieken worden ingebracht in een plant. Het Nagoya protocol maakt geen onderscheid naar de methode van veredeling. Het Cartagena Protocol onder het Biodiversiteitsverdrag regelt grensoverschrijdende bewegingen en handelingen met genetisch gemodificeerde organismen, ook wel levende gemodificeerde organismen (LMO's) genoemd in het kader van dit protocol, en biedt richtlijnen voor veilige toepassingen van LMO's. Ook hieraan voldoet het NGT-voorstel met de verificatieprocedure voor NGT-1 en milieurisicobeoordeling voor NGT-2.

4. Wat als twee categorie 1 NGT planten zich op natuurlijke wijze met elkaar kruisen en hun nakomelingen zich ook kruisen met andere categorie 1 NGT planten van dezelfde soort? Dan kunnen de nakomelingen toch categorie 2 NGT planten zijn, gezien de grens tussen categorie 1 en 2 van 20 modificaties? Zodoende kan toch, met name op de langere termijn, de Europese natuur op grote schaal worden veranderd? Graag ontvangen deze leden uw reactie.

Antwoord: De nakomelingen van een categorie 1 NGT plant blijven een categorie 1 NGT plant, zolang er geen nieuwe modificaties worden gemaakt met NGTs. Als er wel nieuwe modificaties met NGTs worden gemaakt, en het totaal aan modificaties met NGTs meer dan 20 is, moet deze plant beoordeeld worden als categorie 2 NGT plant. In het NGT-voorstel van de Europese Commissie wordt dit beschreven in art. 3 lid 7 onder (b). De modificaties van NGT planten vallen binnen de soortgrens en zijn daarmee onderdeel van de 'breeders gene pool', deze modificaties kunnen ook bereikt worden met conventionele veredeling. Daarom zullen categorie 1 NGT planten dezelfde impact hebben als conventionele veredeling kan hebben op de Europese natuur.

5. Voorziet het voorstel in monitoring op de lange termijn, zodat de situatie zoals omschreven in de vorige vraag (op de langere termijn mogelijk maken van categorie 2 NGTs) kan worden vermeden? Zo nee, hoe verhoudt dit zich tot het voorzorgsprincipe?

Antwoord: Voor categorie 2 NGT planten voorziet het voorstel in monitoring. Bij de aanvraag voor een categorie 2 NGT plant moet een monitoringsplan worden ingediend. Categorie 1 NGT planten hebben een vergelijkbaar risicoprofiel met conventioneel veredelde planten, en de competente autoriteit verifieert dit voor markttoelating. Categorie 1 NGT planten worden vrijgesteld van de regelgeving voor genetisch gemodificeerde organismen (ggo-regelgeving) en hoeven, net als conventioneel veredelde planten, niet gemonitord te worden. Door de voorgestelde verificatieprocedure voor NGT-1 en de autorisatieprocedure van categorie 2 NGT voor markttoelating is het NGT voorstel in lijn met het voorzorgsprincipe.

6. Welke consequenties zou het hierboven omschreven scenario kunnen hebben op Europese in het wild levende dieren, en ecosystemen meer in het algemeen?

Antwoord: Aangezien categorie 1 NGT planten qua risicoprofiel equivalent zijn aan conventioneel veredelde planten, zijn er hiermee geen (nieuwe) milieurisico consequenties voor Europese in het wild levende dieren en ecosystemen. Categorie 1 NGT planten kunnen immers ook met conventionele veredeling worden gemaakt.

7. Voorziet de verordening er ook in dat categorie 1 NGT planten worden geïmporteerd van buiten de EU en in de Europese natuur worden geïntroduceerd? Zo ja, welke consequenties zou dit kunnen hebben voor de Europese natuur?

Antwoord: De NGT verordening wordt ook van toepassing op categorie 1 NGT planten als deze worden geïmporteerd van buiten de EU. Dat betekent dat deze planten voordat zij geïmporteerd kunnen worden in de EU de aanvraagprocedure zoals opgenomen in hoofdstuk II van het voorstel voor categorie 1 NGT moeten doorlopen. Hiermee worden de risico's voor mens en milieu op eenzelfde wijze beoordeeld als voor planten van binnen de EU, en zullen de consequenties voor de Europese natuur voor geïmporteerde planten vergelijkbaar zijn met die voor planten van binnen de EU.

8. Naar aanleiding van het antwoord op vraag 4 uit het vorig schriftelijk overleg, willen deze leden graag een toezegging krijgen dat als de Europese Commissie de criteria van vergelijkbaarheid update, de regering de Tweede en Eerste Kamer op tijd zal informeren zodat zij een rol kunnen spelen bij de besluitvorming. Bent u hiertoe bereid?

Antwoord: Vanwege de technische aard van de wijzigingen is deze wijziging belegd bij de Europese Commissie. De Eerste en Tweede Kamer hebben toegang tot het 'delegates portal' waar deze voorgestelde wijzigingen door de Europese Commissie worden gepubliceerd. Ik zal uw beide Kamers informeren over gedelegeerde besluiten van de Europese Commissie indien ik hier aanleiding voor zie.

9. Bent u bereid de Eerste Kamer te informeren over alle correspondentie over en rondom de NGT verordening (bijvoorbeeld over de studie over de impact van octrooieren van planteigenschappen) met de Tweede Kamer zodat de Eerste Kamer haar eigen rol in Europese wet- en regelgeving adequaat kan uitvoeren?

Antwoord: Ja, ik ben ook bereid uw Kamer mee te nemen in alle correspondentie over het NGT-dossier. Daarnaast zal ik uw Kamer een appreciatie sturen van het onderzoeksrapport van de Europese Commissie over impact van intellectueel eigendom op de plantenveredeling.

10. U geeft helaas geen volledig antwoord op vraag 11 uit het eerdere schriftelijk overleg. De vraag was welke wetenschappelijke basis er is voor de grens van 20 genetische modificaties als grens tussen NGT categorie 1 en 2. Graag ontvangen deze leden alsnog een volledige reactie. Het antwoord op vraag 12 uit het eerder schriftelijk overleg baart mijn fractie grote zorgen: 'dit maximum van 20 modificaties is uiteindelijk een beleidskeuze geweest'. Deze leden concluderen dat de EU met deze verordening een arbitraire grens vaststelt tussen categorie 1 en 2, met historische gevolgen voor de Europese en mondiale landbouw, voedsel, en natuur. Hoe rijmt dit met het voorzorgsprincipe?

Antwoord: De grens van 20 modificaties is gesteld op basis van een wetenschappelijke literatuuranalyse van de Europese Commissie, ondersteund door de wetenschappelijke opinie van de EFSA en wetenschappelijke werk van het Joint Research Centre. In de wetenschappelijke literatuurstudie van de Commissie werden 90 wetenschappelijke studies meegenomen, over planten die conventioneel veredeld zijn en over genetische variaties verkregen via conventionele veredeling of op natuurlijke wijze. De Commissie concludeert dat het aantal willekeurige genetische modificaties per generatie van een plant gemiddeld ongeveer 30 tot 100 is. De Europese Commissie heeft op basis van haar studie de keuze gemaakt om het conservatieve aantal van 20 modificaties aan te houden om NGT's, waarvan het onwaarschijnlijk is dat die ook via conventionele veredeling te verkrijgen zijn, uit te sluiten. Daarmee is het NGT-voorstel in lijn met het voorzorgsprincipe.

11. Hoe kunt u zo stellig zeggen dat categorie 1 NGTs 'ook met conventionele veredeling kunnen worden gerealiseerd' (zie bijvoorbeeld antwoord op vraag 18 uit het eerder schriftelijk overleg) als de grens tussen categorie 1 en 2 uiteindelijk een beleidskeuze is – en dus arbitrair – oftewel een menselijke definitie over wat 'natuurlijk' is en niet? Op basis van welke wetenschappelijk bewijs doet u deze uitspraak?

Antwoord: De Nederlandse Commissie Genetische Modificatie (COGEM) gaf in haar eerdere adviezen[1] aan dat planten verkregen met behulp van NGT-1 (cisgenese en mutagenese) qua veiligheid vergelijkbaar zijn met conventioneel veredelde gewassen. Ook de EFSA heeft in verschillende wetenschappelijke studies en opinies aangegeven dat sommige toepassingen van NGTs ook met conventionele veredeling gerealiseerd kunnen worden. Onafhankelijk wetenschappelijk werk van de Joint Research Centre ondersteunt deze bevingen. De Europese Commissie heeft een technische publicatie[2] uitgebracht over de rationale voor de equivalentiecriteria in Annex I van het voorstel. De Europese Commissie heeft op basis van wetenschappelijke studies de grens zodanig ingesteld dat de planten verkregen met NGT in de categorie 1 klassen vallen, ook altijd via conventionele veredeling of op natuurlijke wijze te verkrijgen zijn.

[1] COGEM adviezen CGM/190321-02, CGM/091222-01, CGM/170308-01 & CGM/200731-01
[2] eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CONSIL:ST_14204_2023_INIT

12. Kunt u de literatuurstudie die ten grondslag ligt aan het besluit voor de grens van 20 modificaties delen met de Kamers?

Antwoord: De Europese Commissie heeft een technische 'paper' gepubliceerd om de rationale achter haar keuze voor de limiet van 20 modificatie uit te leggen. Deze 'paper' deel ik graag met de Kamer.[3]

13. Heeft u de COGEM om advies gevraagd over de huidige tekst in de algemene oriëntatie van de Raad? Zo nee, waarom niet? En bent u alsnog bereid zo een reactie van de COGEM te vragen? Zo ja, kunt u deze reactie van de COGEM delen met de Kamers?

Antwoord: Ja, het kabinet heeft de COGEM om advies gevraagd over de equivalentiecriteria van het Raadsakkoord en het akkoord van het Europees Parlement. De adviezen van de COGEM zijn openbaar en het advies in kwestie deel ik hierbij met u[4]*.*

[3] Regulation on new genomic techniques (NGT) – Technical paper on the rationale for the equivalence criteria in Annex I - Publications Office of the EU
[4] COGEM advies

Advice Differences in EU positions regarding Annex I of the NGT-legislation

COGEM-advies CGM/250516-01

1. Introduction

COGEM was asked by the Ministry of Infrastructure and Water Management to provide scientific advice on the differences between the European Parliament's (EP) position and the Council of the European Union's position regarding Annex I of the new legislation on plants produced using "new genomic techniques" (NGTs). Furthermore, COGEM was asked to assess whether the EP's position aligns with the European Commission's (EC) proposal, which is based on the premise that NGT1 plants - defined according to the criteria in Annex I - are plants that could occur naturally or are developed through conventional breeding techniques. Lastly, the COGEM was asked to what extent it considers the criteria unambiguously verifiable and widely applicable in implementation practice.

Since 2023, the EC published a proposal, and the EP and the Council both adopted a position on the legislation that allows for the deliberate release into the environment or placement on the market of NGT plants, within the European Union (EU). The techniques falling under the scope of NGTs include targeted mutagenesis and cisgenesis. The proposed legislation distinguishes between two categories of NGT plants. First, NGT1 refers to plants whose modifications are comparable to those achievable by conventional breeding methods, and these plants would therefore be exempt from the obligations of the GMO legislation. Second, NGT2 plants pertain to plants with genetic modifications that go beyond what is possible using conventional techniques. These plants would require a case-by-case risk assessment.

In this advice, COGEM examines the positions of the EP and the Council with respect to Annex I, evaluating their differences, scientific implications, and determining if they allow for modifications comparable to conventional breeding methods. Further assessment includes the verifiability and clarity of the criteria they propose.

2. Previous COGEM advice

In recent years, COGEM has repeatedly highlighted that current GMO regulations regarding GM plants are outdated and fail to align with advances in scientific and technological developments, necessitating updates.[1] COGEM has recommended exempting plants produced through targeted mutagenesis and cisgenesis, but not intragenesis, from the GMO regulation, as their safety profiles are comparable to conventionally bred crops.[1,2,3,4] Additionally, COGEM noted that enforcing the GMO regulation will become increasingly challenging with these new techniques, as detecting certain modifications is only possible if the specific changes are known in advance.[5,6]

COGEM was broadly positive about the European Commission's proposal and the European Food Safety Authority's (EFSA) justification for the criteria in Annex I.[7,8,9] COGEM did advise limiting the scope of the proposal to land plants only and further clarifying the text in Annexes I, II, and III.[7] At a

later point, COGEM offered specific recommendations for improving Annex I, including setting a limit on the number of modifications permissible in a gene, taking polyploidy of plants into account, and removing the term "targeted" in cisgenesis or inversions.[10]

3. The European Parliament's (EP) position and the Council of the European Union's position regarding Annex I

Several modifications are allowed in Annex I of the EC proposal for a plant to be considered equivalent to 'plants that could also occur naturally or be produced by conventional breeding techniques' (i.e., NGT1 plants). These modifications are (1) the insertion or substitution of up to 20 nucleotides; (2) the deletion of any number of nucleotides; (3) the insertion or substitution of a continuous DNA sequence existing in the breeder's gene pool and (4) the inversion of sequences. When this advice refers to modifications, it specifically refers to these four types. The annexes in the positions of the EP and the Council can be found below.

Annex I – European Parliament position
Criteria of equivalence of NGT plants to conventional plants

A NGT plant is considered equivalent to conventional plants if the following conditions referred to in points 1 and 1a are met:

(1) The number of the following genetic modifications, which can be combined with each other, does not exceed 3 per any protein-coding sequence taking into account that mutations in introns and regulatory sequences are excluded from this limit:
- (a) substitution or insertion of no more than 20 nucleotides;
- (b) deletion of any number of nucleotides;

(1a) The following genetic modifications, which can be combined with each other, do not create a chimeric protein that is not present in species from the gene pool for breeding purposes or does not interrupt an endogenous gene;
- (a) insertion of continuous DNA sequences existing in the gene pool for breeding purposes;
- (b) substitution of endogenous DNA sequences with continuous DNA sequences existing in the gene pool for breeding purposes;
- (c) inversion or translocation of continuous endogenous DNA sequences existing in the gene pool for breeding purposes.

Annex I – Council of the European Union position
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 4, in any DNA sequence sharing sequence similarity with the targeted site that can be predicted by bioinformatic tools.

Criteria specific to the use of targeted mutagenesis:

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

Criteria specific to 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) insertion of a continuous DNA sequence existing in the breeders's gene pool;
    (b) 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

4. Differences between the positions of the European Parliament and the Council of the European Union and equivalence of the described criteria to conventional breeding techniques

The annexes of the European Parliament (EP) and Council align on several criteria. However, there are differences on the exact wording of the criteria and the conditions of their use. In the following sections COGEM examines the annexes of the EP and the Council, assessing their differences, evaluating their scientific implications and determining if they allow for modifications comparable to conventional breeding methods. The differences in criteria and their implications will be addressed top-to-bottom of the Annex I in the subsequent sections.

4.1 Number of modifications allowed

COGEM notes that the exact scope of the number of modifications is up for interpretation, and further elaboration on the lack of clarity of the criteria can be found in paragraph 5.2. The interpretation of the positions given below is what the COGEM deems to be most likely reading of the text.

The EP's position appears to permit unlimited modifications across the genome, provided there are no more than three deletions or insertions/substitutions of 20 nucleotides within a single protein-coding sequence. This approach allows the possibility of large-scale changes, for example, a stacked combination of substitutions or insertions of up to 20 nucleotides in any non-protein-coding sequence. This includes any regulatory sequence or any sequence coding for a Non-Coding RNA.[11]

During random mutagenesis, dozens of mutations in genes are expected to occur, as mentioned in EFSA's technical paper.[9] However, the more modifications are clustered in a single region of the genome the more unlikely this is to occur through mutagenesis. Although in theory, this would be

possible with unlimited repeats of mutagenesis steps, it stretches the definition of equivalence to conventional breeding. Therefore, COGEM is of the opinion that unlimited or clustered modifications are not equivalent to what is realistically possible to achieve by conventional breeding.

The Council position sets a limit of 20 modifications across the entire plant genome, without restrictions on their locations. As mentioned earlier, modifications spread across the genome align with the EC's premise of equivalence, and the number of 20 modifications is in line with the conclusions of EFSA's technical paper.[9]

However, introducing these 20 modifications within a single protein coding sequence could result in alterations that are more in line with transgenesis than conventional breeding. Thus, the Council's position exceeds what is possible with conventional breeding in this regard.

As stated in COGEM's prior advice, it is possible to expect up to 20 nucleotides to be altered within a single gene, including both regulatory and coding regions, with conventional breeding techniques.[7,10] Both positions, with the EP's allowing for 3 modifications of 20 nucleotides to be changed in any protein coding sequences and the Council's allowing for 20 modifications of 20 nucleotides, exceed the limit of 20 nucleotides per gene as set out previously by COGEM. The EP's position which sets a limit on the number of modifications in a protein coding sequence more closely reflects COGEM's opinion on the matter.

4.2 Insertion of continuous DNA sequences

The Annex I in the EP position does not explicitly mention cisgenesis, but instead refers to the "insertion or substitution of continuous DNA sequences existing in the gene pool for breeding purposes". This is in line with how the Council defines cisgenesis and therefore does not result in a meaningful difference between the two.

The EP Annex I excludes the creation of "a chimeric protein that is not present in species from the breeding gene pool" when inserting or substituting continuous DNA sequences, or the disruption of endogenous genes. This position would allow for new combinations of DNA sequences as long as they do not result in a chimeric protein. This could theoretically result in new combinations that are not found in the breeders' gene pool, for example by combining regulatory sequences with coding sequences of other genes. Furthermore, chimeric proteins are defined in the EP position as joining two sequences that originally code for separate proteins.[a] This allows for the combination of a protein coding sequence with an initially non-coding region. By placing a protein-coding sequence upstream of a DNA region that typically doesn't produce a protein, the previously non-coding region can now be translated into a protein and fused with the newly added coding sequence.

The COGEM notes that creation of chimeric proteins also occurs naturally[12] and it would therefore not be needed to exclude the formation of such chimeric proteins to fulfil the criteria of equivalence. If

[a] Position of the European Parliament on adoption of the Regulation on plants obtained by certain new genomic techniques and their food and feed, and amending Regulation (EU) 2017/625 and Directive 98/44/EC. Page 41, section 15b - "Chimeric protein" means proteins created through the joining of two or more genes or parts of genes that originally coded for separate proteins. [Am. 29]

there are additional reasons for prohibiting the creation of fused proteins, specifying these reasons in the text would provide further clarification.

The Council's position also similarly restricts insertion or substitution of continuous DNA sequences, but instead of excluding the formation of chimeric proteins, it states that the resulting combination may not interrupt an endogenous gene or result in a DNA sequence that is not already present in the breeders' gene pool.

By excluding any formation of new DNA sequences not existing in the breeder's gene pool, the Council does not allow for intragenesis. The Council's position even goes further by explicitly mentioning intragenesis as not being allowed in the main body of the text.

Previously the COGEM has stated that complex forms of intragenesis using and combining multiple genetic elements should not be considered equivalent to conventional breeding techniques.[13] As the EP's position can be interpreted as allowing intragenesis, it is not in line with the COGEM's opinion on equivalence to conventional breeding techniques.

4.3 Inversions

In the EP's position, inversions or translocations are only allowed for continuous endogenous DNA sequences and are not allowed to disrupt endogenous genes.

In contrast, the Council's position is that an inversion of any number of nucleotides is allowed with no further caveats. The Council's position thus allows for the disruption of endogenous genes and the creation of DNA sequences not already present in the breeders' gene pool via inversions. This strategy may also be applied to silence endogenous genes.

The Council's position makes no explicit mention of translocations, but as translocations can be considered as a form of cisgenesis COGEM sees no functional difference between the positions in this regard. Furthermore, the Council's position allows for the disruption of endogenous genes, whereas the EP's position does not. In nature, and when induced by conventional techniques, inversions may occur at any position in the genome. Thus, by not allowing the possible disruption of endogenous genes the EP's position does not align with the premise of equivalence to conventional techniques outlined in the EC proposal, whereas the Council's position does.

5. Verifiability, and clarity of Annex I

In the previous paragraphs COGEM assessed the differences in the EP's and Councils positions regarding Annex I, evaluating their scientific implications and determining if the allowed modifications are comparable to what can be obtained by conventional breeding methods. Additionally, the COGEM was asked to what extend it considers the criteria unambiguously verifiable and widely applicable in implementation practice. Significant challenges remain in ensuring both verifiability and traceability throughout the breeding and production pipeline and in the clarity of Annex I, which will be discussed below.

5.1 *Verifiability*

It is possible to confirm whether a NGT1 plant contains specific intentional modifications as reported by the applicant. While testing for the presence of these changes is feasible, it can be costly, especially if a plant contains a large number of modifications, and the volume of plants needing testing increases as NGTs become widely adopted. In contrast, ensuring that no unintended or additional modifications are present is significantly more challenging. Proving the absence of unintentional changes would require whole-genome sequencing combined with extensive bioinformatics analysis, which introduces significant logistical and financial hurdles. Without such comprehensive testing, it would be a significant hurdle to confirm that a plant does not contain modifications falling under the classification of NGT2 or even be transgenic.

In the current positions, it is clearly stated that when any seed is sold or made available in any way, it should be made clear whether the plant's reproductive material belongs to a NGT category. Furthermore, it is stated that the production chains that wish to remain free of NGTs can do so to safeguard consumer trust. However, traceability becomes increasingly complex if adoption grows, despite documentation providing a mechanism for tracking these plants. For instance, if NGT1 crops are grown alongside conventional varieties, the risks of unintentional mixing or outcrossing increase significantly. This challenge becomes even more pronounced when farmers save and reuse seeds. In such scenarios, untracked NGT1 plants - derived from saved or crossed seeds - could proliferate unchecked. Such practices could undermine labeling and traceability throughout the pipeline, reducing the practicality of their use.

COGEM notes that the risks associated with NGT1 plants, if they fulfil the criteria for equivalence, are comparable to those of conventionally bred plants, and that there are no additional environmental risks associated with their use, even when not tracked.

5.2 *Clarity of the annexes*

While the current positions outline important criteria for plant breeding, the annexes could benefit from greater clarity to ensure consistent understanding and application. Some elements of the text leave room for different interpretations, particularly regarding the scale and scope of permissible modifications. Enhancing clarity and explicitly defining the intent of specific criteria would make the Annex I more accessible and practical.

For example, in the Council's position, Criterion 3 of Annex I refers to the disruption of endogenous genes, but does not define what constitutes a gene, leaving room for different interpretations. In

contrast, the Council's position - unlike the EP's position - explicitly mentions intragenesis as undesirable in the text. By identifying intragenesis[b,14] as undesirable, it indicates that "endogenous genes" in this context include both regulatory and coding sequences. Otherwise, insertions combining regulatory elements (e.g., promoters) with coding sequences could potentially lead to intragenesis. Providing a similar level of specificity in other key areas would help stakeholders to better understand which types of modifications align with the NGT1 classification.

Clarity is especially important for cases where the result of a modification might be possible in one criterion but is specifically excluded in another one. For example, criterion 3 of the Council's position states that no new combinations of DNA sequences can be created and criterion 1a of the EP's positions states that no chimeric proteins can be created. However, in both cases such results can be achieved through the deletion of intervening sequences between two protein-coding sequences, a natural process that is also found in conventional breeding. This inconsistency may cause confusion, which could be avoided by clearly specifying which outcomes are considered undesirable in the text.

Additionally, the wording concerning the size and number of permissible modifications needs to be further refined. For example, in the EP's position, it is unclear whether three separate insertions of up to 20 nucleotides each are allowed within a single coding sequence, or whether the total insertions and substitutions must not exceed 20 nucleotides in total, spread across a maximum of three locations. Similarly, the Council's proposal leaves room for different interpretations regarding whether "20 nucleotide modifications in total" are allowed, or whether it permits 20 individual modifications, each composed of up to 20 nucleotides. Clear use of language could support uniform interpretation and implementation of the criteria.

In summary, COGEM concludes that both the Annex of the EP and the EC I require clarity on some aspects to avoid ambiguity in interpretations. Specifying which outcomes are considered undesirable in the text and using more precise wording in the definition of the criteria would ensure the legislation is clearly defined and easier to apply.

6. Conclusion

In summary, COGEM identifies key differences between Annex I of the European Parliament (EP) and that of the Council of the European Union.

Firstly, the EP's position permits far more modifications than that of the Council. COGEM is of the opinion that setting no limit on the number of modifications, as well as allowing for the occurrence of

[b] Position of the European Parliament on adoption of the Regulation on plants obtained by certain new genomic techniques and their food and feed, and amending Regulation (EU) 2017/625 and Directive 98/44/EC. Page 7, section 2
- Intragenic plants result from the use of intragenesis techniques, but can be also obtained by through cisgenesis techniques in the strict sense. In the latter case, new developments of site-directed modification also offer the possibility to target the insertion of continuous DNA sequences other than complete genes (for example promoters or regulatory sequences), from the breeders' gene pool at specific loci in the genome. When the insertion of such fragments occurs within an endogenous gene, interrupting it, this leads to the formation of a rearranged gene in the recipient plant and, as such, the plant should also be considered intragenic, except in those particular cases in which the resulting DNA sequences in the recipient plant already occur in species from the breeder´s gene pool.

multiple clustered modifications, does not align with the initial premise of equivalence to conventional breeding techniques set out by the European Commission.

Secondly, both positions differ in their wording concerning combinations of new sequences. For example, the EP specifies only that the formation of chimeric proteins is prohibited, allowing for intragenesis by combining regulatory and protein-coding sequences, whereas the Council's position does not allow for such results. COGEM is of the opinion that plants produced by intragenesis should not be deemed equivalent to plants derived by conventional breeding techniques.

Lastly, the EP's position on inversions is more stringent than that of the Council, specifying that no genes may be interrupted. The EP's position does not align with the premise of equivalence, as inversions can naturally occur at any location within the genome.

To maintain the possibility of NGT1 free production chains would require tracing of NGT1 plants. This tracing may present challenges if these plants become widely adopted, particularly if testing is intended to verify for instance that they do not mix with organic food products.

For both Annexes, certain terms used throughout the text would benefit from further clarification, either within the Annex itself or in other relevant sections of the text. Specifically, the text needs better definitions of the types of intended modifications that are permitted and also explicitly state those that are not permitted to avoid ambiguity and misinterpretation.

Table 1. Summary of the comparison between Annex 1 in the EP and the Council position and their equivalence to conventional breeding techniques according to COGEM.

Subject	EP position	Council position	Difference	Equivalence to conventional techniques
Number of modifications allowed	Unlimited, except for 3 deletions, insertions, or substitutions in protein-coding sequences.	Limited to 20 modifications, with no restriction on location.	The EP position allows far more modifications but limits changes in protein-coding sequences.	The EP's unlimited modification allowance is not equivalent to conventional techniques. Both allow closely stacked modifications, which is not equivalent to conventional techniques.
Insertion of continuous DNA sequences	Allowed if it does not interrupt an endogenous gene or creates chimeric proteins.	Allowed if it does not interrupt an endogenous gene or create DNA sequences not present in the breeder's pool.	The EP position does not allow chimeric protein formation but permits intragenesis by combining regulatory and coding sequences.	Extensive recombination of regulatory and coding sequences, as allowed by the EP, is not equivalent to conventional techniques.
Inversions	Allowed if they target continuous endogenous sequences and do not disrupt endogenous genes.	Allowed if they target endogenous sequences.	The Council position allows disruption of endogenous genes, while the EP position does not.	Disruption of endogenous genes occurs naturally and with conventional techniques. Thus, the Council's position, not the EP's, is equivalent to conventional techniques.

Referenties

1. COGEM (2019). Proposal for Amending the Exemption in the GMO Legislation: Additional criteria for exempting GM plants. COGEM advice CGM/190321-02 [available in Dutch and English]
2. COGEM (2009). Adviserende brief onderzoeksrapport 'nieuwe veredelingstechnieken'. COGEM advice CGM/091222-01 [in Dutch]
3. COGEM (2017). Advies CRISPR-Cas en gerichte mutagenese bij planten. COGEM advice CGM/170308-01 [in Dutch]
4. COGEM (2020). Signalerende aanbiedingsbrief bij onderzoeksrapport Natuurlijke genoomvariatie. COGEM advisory-report CGM/200731-01 [in Dutch]
5. COGEM (2009), Geen roos zonder doornen. Implicaties van een product-georiënteerde regelgeving voor gg-gewassen in Europa. COGEM advisory-report CGM/191010-01 [in Dutch]
6. COGEM & Gezondheidsraad (2023). Trendanalyse biotechnologie 2023. Tijd voor een integrale visie. COGEM, Bilthoven. [available in Dutch and English]
7. COGEM (2023). Voorstel Europese Commissie voor nieuwe wetgeving voor planten verkregen met gerichte mutagenese, cisgenese en intragenese. COGEM advice CGM/230710-01 [in Dutch]
8. European Commission (2023). Proposal for a regulation of the European parliament and of the council on plants obtained by certain new genomic techniques and their food and feed, and amending Regulation (EU) 2017/625.

9. Technical paper by the Commission services (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
10. COGEM (2023). Advies over aanpassing van de Annex I in het NGT-voorstel van de EC. COGEM advice CGM/231124-01
11. Yadav A *et al*. (2024). The Emerging Role of Non-Coding RNAs (ncRNAs) in Plant Growth, Development, and Stress Response Signaling. Noncoding RNA. 7:13
12. Zhou, Y & Zhang, C (2019) Evolutionary patterns of chimeric retrogenes in *Oryza* species. Sci Rep 9: 17733
13. COGEM (2020). Signalerende aanbiedingsbrief bij onderzoeksrapport Natuurlijke genoomvariatie. COGEM advisory-report CGM/200731-01 [in Dutch]
14. EFSA GMO Panel. Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis.EFSA Journal 2012;10(2):2561, 33 pp. https://doi.org/10.2903/j.efsa.2012.25613

Council of the European Union

Brussels, 16 October 2023
(OR. en)

14204/23

Interinstitutional File: 2023/0226(COD)

AGRI 610
AGRILEG 235
ENV 1122
CODEC 1884

NOTE

From:	General Secretariat of the Council
To:	Delegations
Subject:	Regulation on new genomic techniques (NGT) – Technical paper on the rationale for the equivalence criteria in Annex I

Delegations will find in annex a technical paper prepared by the Commission services on the rationale for the equivalence criteria in Annex I.

ANNEX

Rationale for the equivalence criteria in Annex I to the proposal for a Regulation on plants obtained by certain new genomic techniques

Technical paper by the Commission services

This technical paper provides detailed explanation on the rationale for the criteria in Annex I of the proposal for a regulation on plants obtained by certain new genomic techniques (NGTs) and their food and feed (in the following "the NGT proposal")[1], and presents information on the relevant scientific literature.

The criteria were developed to define type and number of mutations introduced by targeted mutagenesis and cisgenesis that could also be obtained by conventional breeding methods or could occur spontaneously. They were developed on the basis of a literature analysis of 90 scientific, peer-reviewed original studies and reviews (see Annex) on plants obtained by conventional breeding methods[2] and on genetic variations in plants. The objective of the analysis was to explore:

- Which type of mutations occur due to natural mutation or application of conventional breeding methods.
- What size ranges these mutations span.

[1] COM(2023) 411 final

[2] For a description of conventional breeding techniques in plants, see e.g.:

- EFSA Panel on Genetically Modified Organisms (GMO). (2012). Scientific opinion addressing the safety assessment of plants developed using zinc finger nuclease 3 and other site- directed nucleases with similar function. EFSA Journal, 10(10), 2943. https://doi.org/10.2903/j.efsa.2012.2943
- European Commission, Directorate-General for Research and Innovation, New techniques in agricultural biotechnology, Publications Office, 2017, https://data.europa.eu/doi/10.2777/574498.

- How many of these mutations do typically occur in a single plant.

Also relevant considerations in scientific opinions issued by the European Food Safety Authority (EFSA)[3] and scientific work of the Joint Research Centre (JRC)[4] were taken into account in defining the criteria.

Similar genetic modifications obtained by different techniques are not expected to present different risks. Therefore, this analysis was not meant to assess the effects of genetic variations or genetic modifications introduced by conventional breeding methods.

1. Nature of the criteria

The criteria are based on the modifications resulting from the technique(s), i.e., on molecular characteristics. Furthermore, if certain type and number of mutations can be introduced by both conventional breeding techniques and NGTs, also the type of traits associated to these mutations would not be different between the techniques. Therefore, for the purpose of assessing equivalence, the analysis of type and number of mutations is considered sufficient.

[3] EFSA Panel on Genetically Modified Organisms, 2012. Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis. EFSA Journal 2012;10(2):2561.
EFSA Panel on Genetically Modified Organisms, 'Applicability of the EFSA Opinion on SDNs type 3 for the safety assessment of plants developed using SDNs type 1 and 2 and oligonucleotide-directed mutagenesis', EFSA Journal 2020;18(11):6299. https://doi.org/10.2903/j.efsa.2020.6299
EFSA Panel on Genetically Modified Organisms, 2022. Updated scientific opinion on plants developed through cisgenesis and intragenesis. EFSA Journal 20(10):7621, 33 pp. https://doi.org/10.2903/j.efsa.2022.7621.
EFSA Panel on Genetically Modified Organisms, 2022. Statement on criteria for risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis. EFSA Journal 20(10):7618, 12 pp. https://doi.org/10.2903/j.efsa.2022.7618

[4] Broothaerts, W., Jacchia, S., Angers, A., Petrillo, M., Querci, M., Savini, C., Van den Eede, G. and Emons, H., New Genomic Techniques: State-of-the-Art Review, EUR 30430 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-24696-1, doi:10.2760/710056, JRC121847

2. Justification of the type of genetic modifications included in the criteria

The literature analysis as described in the Annex showed that targeted mutagenesis and cisgenesis techniques can lead to genetic modifications that are similar to mutations occurring spontaneously in nature or as a result of conventional breeding techniques, including random mutagenesis techniques using chemicals or various types of irradiation. These mutations include substitutions, insertions (including duplications, translocations and inversions) and deletions of nucleotides in the DNA. Furthermore, insertions of cisgenes[5] or parts of cisgenes are also possible through crossing or conventional breeding. These types of mutations are observed also in combination.

In view of these results and EFSA's conclusion that targeted mutagenesis and cisgenesis (with the exclusion of intragenesis) do not *per se* generate specific hazards different to those from conventional breeding methods, targeted substitutions, insertions and deletions (criteria 1 and 2 of Annex I of the NGT proposal), targeted insertions and substitutions of cisgenes (criterion 3) as well as targeted inversions (criterion 4) were included among the criteria of equivalence. Criterion 5 was included to consider possible outcomes (DNA sequences) that might be shown to occur in a species from the breeders' gene pool[6] but that might not be covered by the previous criteria. This criterion provides a derogation only from criterion 3 and from the condition that the genetic modification does not interrupt an endogenous gene.

Based on EFSA's conclusion that intragenic plants[7] may entail additional hazards compared to conventionally bred plants, intragenesis was excluded from the criteria by setting, under criterion 3, the two conditions of (i) no interruption of an endogenous gene[8] and (ii) insertion of (criterion 3a) or substitution with (criterion 3b) a contiguous DNA sequence. The random insertion of a cisgene was also excluded to take into account EFSA's opinion that an interruption of an endogenous gene by a cisgene may give rise to additional hazards that would require assessment. As regards criteria 1 and 2, they also only cover targeted modifications, since random mutagenesis is already exempted from the application of the GMO legislation and is not in the scope of the NGT proposal.

[5] A gene originating from the same or a crossable species.
[6] For the definition of breeders' gene pool see COM(2023) 411 final, Article 3(6).
[7] Plants containing a rearranged copy of genetic material originating from the same or a crossable species.
[8] An endogenous gene is a gene present in the target organism.

3. Justification of the size limits of individual genetic modifications included in the criteria

On the basis of the literature research, it appears that substitutions occurring after application of conventional methods typically affect one or few adjacent nucleotides. Insertions resulting from these methods can span up to several million nucleotides in cases of structural rearrangements like duplications or translocations of sequences already present elsewhere in the genome. Insertions of more random sequences are typically of a length of less than ten nucleotides but have been observed to extend to approximately fifty nucleotides. Furthermore, although smaller insertions so far have been reported to occur more frequently than larger rearrangements, the improvement of detection methods (i.e. long-read sequencing) has started to unveil higher rates of large insertions than previously estimated. Deletions of less than fifty nucleotides seem to be the most common, but deletions affecting large regions of plant genomes spanning up to several hundred or thousand nucleotides have also been observed. Finally, as with other structural rearrangements, inversions of several million nucleotides have been reported to occur after use of conventional methods.

Considering these findings in the literature, no thresholds for the lengths of admissible deletions and inversions were set in criterion 2 and 4, respectively.

In contrast, a threshold of twenty nucleotides in criterion 1 for substitutions and insertions was set since it fits with the sizes observed in the scientific analysis. The described very large insertions as part of structural rearrangements should be considered insertions of a cisgene, which are covered by criterion 3. Insertions of random sequences were reported to be much smaller. Furthermore, when considering genome diversity, the JRC calculated that the theoretical probability that a random sequence is unique in the genome of various crops boils down to a consistent relatively narrow size range between 19 and 21 bases[4]. This means that a modified sequence smaller than this size may already occur elsewhere in the genome and may therefore be already part of the natural genetic diversity.

As regards the threshold for the length of substitutions, the same threshold as for insertions of random sequences has been applied since substitutions can be considered as a combination of deletion and insertion. Any deviation between the threshold applicable to insertions and the one applicable to substitutions would thus have created an inconsistency.

4. Justification of the numerical limit of individual genetic modifications per plant included in the criteria

As regards the total number of modifications in an individual plant introduced by conventional breeding techniques, the literature illustrated variability depending on various factors, in particular the organism and the method used. In general, higher doses of chemicals or radiation and longer exposure times increased the number of genetic modifications in individual plants. Additionally, polyploid plants[9] tended to exhibit greater numbers of genetic modifications compared to monoploid plants. Typically, from the literature analysis, the total number of genetic modifications in individual viable plants ranged from thirty to one hundred. The mutation frequency after using random mutagenesis was higher compared to natural mutation rates. It remained nevertheless below the total number of accumulated single nucleotide polymorphisms[10] naturally occurring between different cultivars or the number of genetic mutations resulting from conventional methods using tissue culture, clonal propagation or protoplast regeneration.

Through conventional breeding, new or improved cultivars are obtained by stacking genes or making new genomic combinations. However, these techniques are more successful for some crops and genes than for others. While in general stacking of multiple desirable modifications is possible with conventional methods, there are several examples where conventional breeding is not effective in this respect, due to a number of factors. The probability to achieve specific, potentially more extensive, combinations of modifications as a result of the application of conventional methods may be low[11]. Based on this, a limit to the total number of individual modifications per plant was set to twenty in Annex I of the NGT proposal. By this threshold, a demarcation is drawn so to exclude from category 1 of the proposal NGT plants with complex modifications unlikely to be obtainable by conventional breeding methods.

The increasing precision of certain NGTs compared to conventional breeding approaches is well recognised in the scientific community[12]. However, to consider in the verification of equivalence all possible modifications introduced by the use of the new techniques, the limit of 20 to the total number of individual modifications per plant is set to cover not only the on-target genetic modifications but also possible off-target modifications occurring in DNA sequences sharing sequence similarity with the targeted site that can be predicted by bioinformatic tools.

9 Plants containing more than two homologous chromosomes.

10 Substitutions at a single position in the genome.

11 For example, traditional mutagenesis and plant breeding have not been effective in obtaining low gluten wheat varieties for patients with coeliac disease due to the significant number of specific mutations required to obtain such a trait. Targeted mutagenesis has been instead used to precisely and efficiently reduce the amount of gluten in wheat seed kernels (Sanchez-Leon et al., 2018).

12 SWD(2023) 412 final, section 1.1 and Annex 6.

Annex

Analysis of the scientific literature on mutations occurring naturally or obtained by conventional breeding techniques

1. Introduction

The Commission's services analysed scientific, peer-reviewed literature regarding the type, size and occurrence of mutations, as well as the number of mutated genes, which mainly focuses on random mutagenesis techniques such as irradiation and the application of EMS. The mutations induced by random mutagenesis techniques (e.g. using ethyl methanesulfonate (EMS), gamma ray irradiation, fast-neutron (FN) irradiation) include **nucleotide substitutions, insertions and deletions** of various sizes. Mutations introduced by these techniques are comparable to mutations derived from certain NGTs in which breaks are induced in the DNA and edits result from imperfections in the natural DNA repair mechanism of plants (EFSA GMO Panel, 2012; Pacher and Puchta, 2017; Holme, Gregersen & Brinch-Pedersen, 2019; EFSA GMO Panel, 2020). According to the consulted literature, random mutagenesis techniques lead to a lower number of mutations compared to e.g. *in vitro* breeding techniques such as tissue culture and clonal propagation (Zhang *et al.,* 2014; Adamek *et al.*, 2022). Also, the natural variation found in existing cultivars, generated over time by natural and breeding processes, is much larger than the number of mutations induced by random mutagenesis (Anderson *et al*., 2016).

The types of mutations described below have been observed in the literature analysis. Combination of these types of mutations were also observed (Belfield *et al*., 2012; Hase *et al*., 2023; Li *et al*., 2016b; Weng *et al*., 2019).

2. Type and size of mutations caused by random mutagenesis techniques

2.1. Substitutions

Single base substitutions (SBSs), i.e. the replacement of a single nucleotide or a few adjacent nucleotides in the DNA, were the most common group of edits when using FN irradiation in rice (52.6% of observed mutations) (Li *et al*., 2016a). The majority of mutations in carbon ion-irradiated *Arabidopsis thaliana* also constituted SBSs (38%-43% in dry seed and 59%-62% in seedlings) (Hase *et al*., 2018). SBSs were also four times more frequent compared to short insertions or deletions (indels) in six gamma-irradiated rice lines, where they were randomly distributed over the genome (Li *et al*., 2016b). In addition, the application of EMS for random mutagenesis in *Arabidopsis*, soybean and rice led predominantly to SBSs (e.g. more than 99% of mutations are G/C to A/T transitions in *Arabidopsis*) (Greene *et al.,* 2003; Cooper *et al*., 2008; Henry *et al*., 2014). These results indicate that SBSs are commonly observed as a result of random mutagenesis.

2.2. Deletions

Although SBSs were the most abundant mutations overall in FN-irradiated rice, the largest fraction of mutated genes (71.5%) harboured deletions. Of these, small deletions were the most abundant, with 26.3% comprising a single base pair (bp). Nevertheless, several deletions exceeding a size of 1 kilobase pair (kbp) were also observed and the largest deletions spanned several hundred kbp (Li *et al*., 2016a). The most frequent mutation type in 24 rice plants with a mutant phenotype after gamma ray irradiation were small deletions of up to 16 bp (62.5%), but larger deletions ranging in size between 9 and 130 kbp were also noted (16.9%) (Morita *et al*., 2009). Large deletions were also observed in 264 FN-irradiated soybean lines, where on average two to three homozygous deletions of more than 500 bp were detected per line (Bolon *et al*., 2014). In five FN-irradiated common bean plants, large deletions were found to range in size from 40 bp to 43 kbp (O´Rourke *et al*., 2013). The majority of the deletions in carbon ion-irradiated dry seed and seedlings of *Arabidopsis* were below 50 bp in size (95% in dry seed and 91.5% in seedlings), but larger deletions of more than 1 kbp were also observed (Hase *et al*., 2018). A recent study of the same group comparing carbon ion and gamma ray irradiation of dry seed and seedlings of *Arabidopsis* produced similar results with mainly deletions of less than 10 bp but also several instances of large deletions of more than 100 bp particularly when using carbon ion irradiation. The largest deletion found had a size of 380 kbp (Hase, Satoh & Kitamura, 2023). Likewise, in FN-irradiated *Arabidopsis* most of the deletions (97%) were smaller than 56 bp in length, with single base deletions being the most frequently observed (36%). However, a larger deletion of 7.2 kbp was also identified (Belfield *et al*., 2012). In a similar study, Li *et al*. (2001) found that in FN-irradiated *Arabidopsis* lines most of the deletions were of a size of up to 4 kbp (58.3%). However, deletions as large as 12 kbp were also found (Li *et al*., 2001). Large deletions of up to 35 kbp resulting from FN irradiation of *Arabidopsis* were also reported in other studies (summarised in Li & Zhang, 2002).

In conclusion, although small deletions seem to be more abundant when using random mutagenesis techniques, large deletions of several kbp also frequently occur.

2.3. Insertions

Although insertions are less frequently reported in comparison to deletions, they do occur. For instance, in FN-irradiated rice most insertions identified were 1 bp long (69%). Insertions of 2-6 bp were also observed (27%), while insertions of more than 10 bp were rare (Li *et al.,* 2016a). In carbon ion-irradiated *Arabidopsis*, single base insertions were most frequent (18 out of 35 insertion events), seven events were insertions of 2 bp and the remainder ranged between 3 and 47 bp (Hase *et al*., 2018). However, larger insertions of more than 50 bp also occur, which are classified as several subtypes of Structural Variations (SVs), for instance translocations, inversions and duplications (Saxena, Edwards & Varshney, 2014; Huang & Rieseberg, 2020; Zanini *et al.,* 2021). For instance, in 264 FN-irradiated soybean plants on average one segmental duplication with an average size of more than 2 megabase pairs (Mbp) was identified per mutant line (Bolon *et al*., 2014). Similarly, two studies using carbon ion or gamma ray irradiation on *Arabidopsis* revealed several inversions and translocations of various sizes (Hase *et al.,* 2018; Hase, Satoh & Kitamura, 2023).

Recent reports suggest that the prevalence of short-read sequencing techniques as the standard detection method for genetic mutations may have led to an underrepresentation of the number and size of larger SVs in the genomes of plants subjected to random mutagenesis techniques (Sedlazeck *et al*., 2018; De Coster & Van Broeckhoven, 2019; Ho, Urban & Mills, 2020; Zanini *et al.,* 2021; Lemay *et al*., 2022; Zhang *et al*., 2022). Also, it was suggested that the combination of multiple algorithms for data analysis may improve the detection rate of SVs (Hase, Satoh & Kitamura, 2023). Indeed, several recent studies reported a much higher than predicted occurrence of SVs across conventionally bred and wild varieties of agricultural crops, including maize, rice, grapevine, rapeseed and tomato (Chia *et al*., 2012; Wang *et al*., 2018; Zhou *et al*., 2019; Fuentes *et al*., 2019; Alonge *et al*., 2020; Chawla *et al*., 2020; Huang & Rieseberg, 2020; Orantes-Bonilla *et al*., 2022; Yildiz *et al*., 2023).

In conclusion, although predominantly smaller insertions are detected after use of random mutagenesis techniques, larger structural rearrangements involving insertions are also not uncommon.

3. Number of mutations introduced by random mutagenesis techniques

The number of mutations and mutated genes observed is dependent, amongst various factors, on the random mutagenesis technique used. In FN-irradiated rice, the average number of mutated genes per line was 31 (varying between seven and 147) and the number of mutations per line was on average 59 (varying between 28 and 78) (Li *et al.,* 2016a). In *Arabidopsis*, six FN-irradiated mutation lines were reported to display a number of mutations ranging between eight and 32 per line (Belfield *et al.,* 2012). Between 41 and 76 homozygous substitutions were found in 10 lines with a mutant phenotype in FN-irradiated soybean (Anderson *et al.,* 2016). As mentioned above, 1216 duplications and deletions were induced by FN irradiation in a total of 264 soybean plants (averaging one segmental duplication, two to three homozygous deletions and one hemizygous deletion per individual) (Bolon *et al.,* 2014). In EMS-treated rice, per plant an average of 37 mutations that were deleterious for the gene's function was observed in a population of 72 individuals (the total number of mutations was more than 2700) (Henry *et al.,* 2014). On average, higher mutation densities are seen in polyploid species when using random mutagenesis techniques (Kurowska *et al.,* 2011).

4. Mutations as a result of other conventional breeding techniques

4.1. *In vitro* plant tissue/cell culture and clonal propagation

Genetic variation may result from stress factors during *in vitro* plant tissue culture, cell culture propagation (somaclonal variation) or from clonal propagation. This variation can be the basis for the development of new and improved cultivars, but it is not always desirable, e.g. in cases of *in vitro* cloning or germplasm preservation (Krishna *et al*., 2016).

In *in vitro* propagated rice, somaclonal variation in the form of Single Nucleotide Polymorphisms (SNPs) (substitutions) and indels was observed. The mutation rate of these regenerated rice lines was estimated at 1.74×10^{-6} base substitutions per site per generation (Miyao *et al*., 2012), which is higher than the estimated natural mutation rate in rice of $\sim 5.4 \times 10^{-8}$ per site per diploid genome per generation (Tang *et al*., 2018). Zhang *et al*. (2014) also identified extensive inheritable somaclonal genomic variation in rice tissue culture and estimated a mutation rate of 5×10^{-5} base substitutions per site.

Non-heritable somatic mutations can accumulate in clonal propagation of micropropagated crops (e.g. strawberry, banana, potato and coffee). More than 1 million Single Nucleotide Variants (SNVs, a single nucleotide change in the DNA) were found in a clonally propagated cannabis line, with variation seen between different tissues (Adamek *et al*., 2022). Larger structural genomic variations are also possible. For example, all analysed potatoes within a set regenerated from protoplasts displayed aneuploidy or structural chromosomal changes (Fossi *et al*., 2019). In addition, gene duplications and insertions of variable sizes can occur through transposon activity (Cerbin & Jiang, 2018).

The above genetic changes are sometimes intentionally induced: the chemical mutagen colchicine is commonly applied in conventional breeding for polyploidisation *in vitro* (Alemanno & Guiderdoni, 1994; Eng & Ho, 2019). In addition, several commercial varieties belonging to various species have been derived from somaclonal variation (Bhojwani & Dantu, 2013; Krishna *et al*., 2016).

4.2. Natural mutation rate and inter-cultivar variation

Ossowski *et al*. (2010) observed a natural mutation rate of 7.1 x 10^{-9} per site per generation in *A. thaliana*. A more recent study with this plant came to a similar conclusion with 6.95 x 10^{-9} single nucleotide mutations per site per generation for lines that went through 25 generations. The rate of occurrence of indels was lower at 1.30 x 10^{-9} per site per generation and deletions were more frequent and larger (excluding the seven deletions greater than 100 bp, the mean was 6.5 bp) than insertions (mean 3.9 bp) (Weng *et al*., 2019). In maize, the natural mutation rate was estimated at 2.17-3.87 x 10^{-8} per site per generation (Yang *et al*., 2017) and in rice ~5.4 x 10^{-8} per site per diploid genome per generation (Tang *et al*., 2018). Analyses of SNPs and indels showed that these were common in twelve analysed maize lines: SNPs and indels occurred on average every 73 and 309 bp, respectively (Vroh Bi *et al*., 2005).

Genomic structural variation is also found in polyploid crops and can take the form of presence-absence variation, copy-number variation and homoeologous exchanges (Schiessl *et al*., 2018). Although the natural mutation rate is lower than the rate obtained through induced random mutagenesis, the amount of inter-cultivar variation already available is extensive. Anderson *et al*. (2016) examined the genomic variation in soybean cultivars and mutagenized plants. The inter-cultivar variation extending to over 1 million SNPs was far greater than the variation seen amongst FN and *Agrobacterium*-transformed plants, which led to less than 100 single nucleotide substitutions genome-wide. Other examples include variation among elite maize inbred lines (Lai *et al*., 2010), structural variation in rice (Fuentes *et al*., 2019) and variation in US wheat varieties (Sthapit *et al*., 2022).

5. **Gene introgression**

Whole Genome Sequencing (WGS) can be used to identify the genetic diversity present in a species' gene pool which then can be utilized to introgress genetic regions of interest into elite cultivars by crop improvement programs (Tao *et al*., 2019).

5.1. Resistance breeding

Resistance breeding is used for the development of new cultivars resistant to pathogens by introgressing *Resistance* genes (*R* genes) from wild germplasm into agricultural varieties (Dangl *et al*., 2013). This is often a time-consuming task, further complicated by the lack of knowledge on a large proportion of plant genetic diversity that, to date, remains uncharacterized. Next-generation sequencing (NGS) and high-throughput genotyping technologies (HTGT) can contribute to unveiling new *R* genes (Sánchez-Martín *et al.*, 2019). Introgressing *R* genes into elite cultivars can be time consuming for some crops such as those that are usually vegetatively propagated (e.g. potato and banana) or trees (e.g. apple and citrus). In potato, it can take up to 50 years to introgress resistance into a new variety (Haverkort *et al*., 2009). Furthermore, to prevent resistance breaking in the field, multiple *R* genes would need to be introgressed (Dangl *et al*., 2013). The stacking of *R* genes through resistance breeding ("gene pyramiding") has been demonstrated in sexually propagated crops such as tomato, wheat, and pepper (Fuchs, 2017). For example, marker assisted breeding was used to cross two grapevine cultivars carrying one *R* gene each to generate a new cultivar with two *R* genes (Eibach *et al*., 2007). Similar successful attempts have been undertaken, *inter alia*, in rice for bacterial leaf blight resistance (Suh *et al*., 2013), in maize for different virus resistances (Zambrano *et al*., 2014), in barley for resistance against various pathogens (Friedt & Ordon 2007) and in wheat for powdery mildew (Liu *et al*., 2008) and stem rust resistance (Liu *et al*., 2020).

5.2. Examples of introgression of other traits

Hybridization and mutation breeding are two techniques that can be used for breeding soybean varieties with high protein content (Guo *et al*., 2022). Several introgression lines carrying quantitative trait loci (QTLs) for productivity, biotic and abiotic stress tolerance can be used for breeding programs (Ashikari & Matsuoka, 2006; Lippman *et al*., 2007). Abiotic tolerance traits have also been introgressed in commercial varieties, for example in sunflower by hybridising two North American species (Whitney *et al*., 2010). Traits related to drought tolerance have also been introgressed in cereal crops such as rice (Dharmappa *et al*., 2019), wheat (Placido *et al*., 2013) and chickpea (Bharadwaj *et al*., 2021).

6. Modifications that are difficult to obtain by conventional breeding techniques

Through conventional breeding, new or improved cultivars are made by stacking genes or making new genomic combinations (Prohens, 2011; Bradshaw, 2017). Random mutagenesis has further advanced conventional breeding, by inducing mutations in agronomical interesting crops, which can be later crossbred into elite cultivars. However, these techniques are more successful for some crops and genes than for others. Several examples are given below where conventional breeding is slower and/or less efficient compared to certain NGTs.

Gene stacking in vegetatively propagated crops is relatively difficult using conventional breeding techniques despite advances such as speed breeding, genotyping, marker-assisted selection and high-throughput phenotyping (Hickey *et al*., 2019; Hasan *et al*., 2021). Another factor making stacking of desirable traits less efficient is the possibility of linkage drag: the association and carry-over of undesirable traits with desirable ones (Wolter *et al*., 2019; Lee & Wang, 2020). Furthermore, the targeting of specific genes is not possible when using conventional mutagenesis techniques. When using these techniques large progeny populations and extensive screening are thus required. Nonaka *et al*. (2017) reported that, in an attempt to mutate the C-terminus of *GAD3* to increase the level of γ-aminobutyric acid (GABA) in tomato fruits, no such mutation was found among ~4500 lines resulting from EMS mutagenesis. Likewise, the targeting of multiple homologous genes or of genes responsible for polygenic traits, especially in polyploid species, can be difficult using conventional breeding techniques (Liu *et al*., 2022; Martínez-Fortún *et al*. 2022). For example, *MLO* is a well-known pathogen *susceptibility* gene that leads to enhanced resistance to powdery mildew when knocked out (Jørgensen, 1992). *Mlo* mutants have since also been naturally found or induced through random mutagenesis in crops such as cucumber, pea and tomato (Kusch & Panstruga 2017). CRISPR/Cas was used to engineer the same trait in bread wheat, since no spontaneous or induced *mlo* mutants had been reported, probably due to the presence of three different *MLO* homoeoalleles (Wang *et al*., 2014). Since then, the same results were obtained by Targeting Induced Lesions IN Genomes (TILLING) and combining the mutations by crosses (Acevedo-Garcia *et al*., 2017). Conventional breeding techniques can therefore be used to obtain similar results as can be achieved by the application of certain NGTs. However, on average the conventional breeding techniques are less efficient, require more resources and take longer than targeted mutagenesis techniques. Even more so when a large number of genes need to be altered to achieve the desired trait.

An example of multiple targeted gene knockouts is the low-gluten wheat that was obtained by CRISPR-Cas editing of several homologs in the α-gliadin gene family (Sánchez-León *et al*., 2018). Low-gluten products have been obtained through conventional breeding, such as low-gluten barley and wheat by combining recessive alleles or deletion lines (Tanner *et al*., 2016; Van den Broeck *et al*., 2009). Compared to conventional breeding however, the use of NGTs for multiplex targeted gene editing appears to be more efficient and requires less back-crosses (Nogué *et al*., 2016).

7. Conclusions

In this analysis, the most frequently reported mutations resulting from random mutagenesis techniques are SBSs, followed by deletions and lastly insertions. Although the mutation frequency when using random mutagenesis is higher compared to natural mutation rates, it is lower than the total accumulated number of SNPs naturally occurring between different cultivars (Anderson *et al*., 2016) or the number of genetic mutations resulting from tissue culture (Zhang *et al*., 2014), clonal propagation (Adamek *et al*., 2022) or protoplast regeneration (Fossi *et al*., 2019).

Larger deletions, translocations, inversions and genome duplications also occur naturally; these mutations depend on a variety of biological processes (e.g. homologous vs. non homologous DNA repair, or transposon activity (Xiao *et al*., 2008; Anderson *et al*., 2019)). So far, they have been reported to occur less frequently than smaller mutations, but the improvement of detection methods (i.e. long-read sequencing) has started to unveil higher rates than previously estimated (Chia *et al*., 2012; Wang *et al*., 2018; Zhou *et al*., 2019; Fuentes *et al*., 2019; Alonge *et al*., 2020; Chawla *et al*., 2020; Yildiz *et al*., 2023; Lemay *et al*., 2022; Zhang *et al*., 2022). The occurrence of larger genome modifications can be enhanced using conventional breeding techniques (Custers *et al*. 2019; Martínez-Fortún *et al*. 2022). Introgression of cisgenes is also possible and broadly performed by conventional breeding methods. Finally, the average number of mutations per gene and the number of mutated lines observed with conventional breeding methods is variable and dependent on the technique and the reproductive capability of the plant material.

In certain cases, NGTs can produce genetic modifications that are difficult to obtain by conventional breeding techniques: 1) "Gene pyramiding" is common for sexually propagated crops, but it is more challenging for crops with a low regenerative potential or a long generation time (e.g. trees). Cisgenesis using NGTs can greatly improve efficiency and reduce breeding time compared to conventional "gene pyramiding". 2) Targeting of multiple homologous genes is efficient using NGTs, but it is impractical or extremely difficult using random mutagenesis, as it involves screening of large progeny populations.

References:

1. Acevedo-Garcia, J., Spencer, D., Thieron, H., Reinstädler, A., Hammond-Kosack, K., Phillips, A. L. & Panstruga, R. (2017). Mlo-based powdery mildew resistance in hexaploidy bread wheat generated by a non-transgenic TILLING approach. *Plant biotechnology journal*, *15*(3), 367–378. https://doi.org/10.1111/pbi.12631
2. Adamek, K., Jones, A. M. P., & Torkamaneh, D. (2022). Accumulation of somatic mutations leads to genetic mosaicism in cannabis. *The plant genome*, *15*(1), e20169. https://doi.org/10.1002/tpg2.20169
3. Alemanno, L. & Guiderdoni, E. (1994). Increased doubled haploid plant regeneration from rice (Oryza sativa L.) anthers cultured on colchicine-supplemented media. *Plant cell reports*, *13*(8), 432–436. https://doi.org/10.1007/BF00231961
4. Alonge, M., Wang, X., Benoit, M., Soyk, S., Pereira, L., Zhang, L., … & Lippman, Z. B. (2020). Major impacts of widespread structural variation on gene expression and crop improvement in tomato. *Cell*, *182*(1), 145-161. https://doi.org/10.1016/j.cell.2020.05.021
5. Anderson, J. E., Michno, J. M., Kono, T. J., Stec, A. O., Campbell, B. W., Curtin, S. J., & Stupar, R. M. (2016). Genomic variation and DNA repair associated with soybean transgenesis: a comparison to cultivars and mutagenized plants. *BMC biotechnology*, *16*(1), 41. https://doi.org/10.1186/s12896-016-0271-z
6. Anderson, S. N., Stitzer, M. C., Brohammer, A. B., Zhou, P., Noshay, J. M., O'Connor, C. H., … & Springer, N. M. (2019). Transposable elements contribute to dynamic genome content in maize. *The Plant journal: for cell and molecular biology*, *100*(5), 1052–1065. https://doi.org/10.1111/tpj.14489
7. Ashikari, M. & Matsuoka, M. (2006). Identification, isolation and pyramiding of quantitative trait loci for rice breeding. *Trends in plant science*, *11*(7), 344–350. https://doi.org/10.1016/j.tplants.2006.05.008
8. Belfield, E. J., Gan, X., Mithani, A., Brown, C., Jiang, C., Franklin, K., … & Harberd, N. P. (2012). Genome-wide analysis of mutations in mutant lineages selected following fast-neutron irradiation mutagenesis of Arabidopsis thaliana. *Genome research*, *22*(7), 1306–1315. https://doi.org/10.1101/gr.131474.111
9. Bharadwaj, C., Tripathi, S., Soren, K. R., Thudi, M., Singh, R. K., Sheoran, S., … & Varshney, R. K. (2021). Introgression of "QTL- hotspot" region enhances drought tolerance and grain yield in three elite chickpea cultivars. *The Plant Genome*, *14*(1), e20076. https://doi.org/10.1002/tpg2.20076

10. Bhojwani, S. & Dantu, P. (2013). Plant Tissue Culture: An Introductory Text. https://doi.org/10.1007/978-81-322-1026-9
11. Bolon, Y. T., Stec, A. O., Michno, J. M., Roessler, J., Bhaskar, P. B., Ries, L., … & Stupar, R. M. (2014). Genome resilience and prevalence of segmental duplications following fast neutron irradiation of soybean. *Genetics*, *198*(3), 967–981. https://doi.org/10.1534/genetics.114.170340
12. Bradshaw, J. E. (2017). Plant breeding: past, present and future. *Euphytica*. *213*, 60. https://doi.org/10.1007/s10681-016-1815-y
13. Cerbin, S. & Jiang, N. (2018). Duplication of host genes by transposable elements. *Current opinion in genetics & development*, *49*, 63–69. https://doi.org/10.1016/j.gde.2018.03.005
14. Chia, JM., Song, C., Bradbury, P., Costich, D., de Leon, N., Doebley, J., … & Ware, D. (2012). Maize HapMap2 identifies extant variation from a genome in flux. *Nature Genetics 44*, 803–807. https://doi.org/10.1038/ng.2313
15. Chawla, H. S., Lee, H., Gabur, I., Vollrath, P., Tamilselvan- Nattar- Amutha, S., Obermeier, C., ... & Snowdon, R. J. (2021). Long- read sequencing reveals widespread intragenic structural variants in a recent allopolyploid crop plant. *Plant biotechnology journal*, *19*(2), 240-250. https://doi.org/10.1111/pbi.13456
16. Cooper, J. L., Till, B. J., Laport, R. G., Darlow, M. C., Kleffner, J. M., Jamai, A., … & Henikoff, S. (2008). TILLING to detect induced mutations in soybean. *BMC plant biology*, *8*, 9. https://doi.org/10.1186/1471-2229-8-9
17. Custers, R., Casacuberta, J. M., Eriksson, D., Sági, L. & Schiemann, J. (2019). Genetic alterations that do or do not occur naturally; Consequences for genome edited organisms in the context of regulatory oversight. *Frontiers in bioengineering and biotechnology*, *6*, 213. https://doi.org/10.3389/fbioe.2018.00213
18. Dangl, J. L., Horvath, D. M. & Staskawicz, B. J. (2013). Pivoting the plant immune system from dissection to deployment. *Science*, *341*(6147), 746–751. https://doi.org/10.1126/science.1236011
19. De Coster, W. & Van Broeckhoven, C. (2019). Newest methods for detecting structural variations. *Trends in biotechnology*, 37(9), 973-982. https://doi.org/10.1016/j.tibtech.2019.02.003
20. Dharmappa, P. M., Doddaraju, P., Malagondanahalli, M. V., Rangappa, R. B., Mallikarjuna, N. M., Rajendrareddy, S. H., … & Sheshshayee, S. M. (2019). Introgression of root and water use efficiency traits enhances water productivity: An Evidence for physiological breeding in rice (Oryza sativa L.). *Rice*, *12*(1), 14. https://doi.org/10.1186/s12284-019-0268-z
21. EFSA Panel on Genetically Modified Organisms (GMO). (2012). Scientific opinion addressing the safety assessment of plants developed using zinc finger nuclease 3 and other site- directed nucleases with similar function. *EFSA Journal*, *10*(10), 2943. https://doi.org/10.2903/j.efsa.2012.2943
22. EFSA Panel on Genetically Modified Organisms (EFSA GMO Panel), Naegeli, H., Bresson, J. L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., ... & Rostoks, N. (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*, *18*(11), e06299. https://doi.org/10.2903/j.efsa.2020.6299
23. Eibach, R., Zyprian, E., Welter, L. & Topfer, R. (2007). The use of molecular markers for pyramiding resistance genes in grapevine breeding. *Vitis 46*(3), 120-124. https://doi.org/10.5073/vitis.2007.46.120-124
24. Eng, W.H. & Ho, W. (2019). Polyploidization using colchicine in horticultural plants: A review. *Scientia Horticulturae*. *246*, 604-617. https://doi.org/10.1016/j.scienta.2018.11.010
25. Fossi, M., Amundson, K., Kuppu, S., Britt, A. & Comai, L. (2019). Regeneration of *Solanum tuberosum* plants from protoplasts induces widespread genome instability. *Plant physiology*, *180*(1), 78–86. https://doi.org/10.1104/pp.18.00906
26. Friedt, W. & Ordon, F. (2007). Molecular Markers for Gene Pyramiding and Disease Resistance Breeding in Barley. In: Varshney, R.K., Tuberosa, R. (eds) Genomics-Assisted Crop Improvement. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-6297-1_4
27. Fuchs, M. (2017). Pyramiding resistance-conferring gene sequences in crops. *Current opinion in virology*, *26*, 36–42. https://doi.org/10.1016/j.coviro.2017.07.004
28. Fuentes, R. R., Chebotarov, D., Duitama, J., Smith, S., De la Hoz, J. F., Mohiyuddin, M., … & Alexandrov, N. (2019). Structural variants in 3000 rice genomes. *Genome research*, *29*(5), 870–880. https://doi.org/10.1101/gr.241240.118
29. Greene, E. A., Codomo, C. A., Taylor, N. E., Henikoff, J. G., Till, B. J., Reynolds, S. H., … & Henikoff, S. (2003). Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in Arabidopsis. *Genetics*, *164*(2), 731–740. https://doi.org/10.1093/genetics/164.2.731
30. Guo, B., Sun, L., Jiang, S., Ren, H., Sun, R., Wei, Z., … & Qiu, L. J. (2022). Soybean genetic resources contributing to sustainable protein production. *Theoretical and applied genetics. Theoretische und angewandte Genetik*, *135*(11), 4095–4121. https://doi.org/10.1007/s00122-022-04222-9
31. Hasan, N., Choudhary, S., Naaz, N., Sharma, N., Laskar, R. A. (2021). Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. *Journal genetic engineering & biotechnology, 19*, 128. https://doi.org/10.1186/s43141-021-00231-1
32. Hase, Y., Satoh, K., Kitamura, S. & Oono, Y. (2018). Physiological status of plant tissue affects the frequency and types of mutations induced by carbon-ion irradiation in Arabidopsis. *Scientific reports*, *8*(1), 1394. https://doi.org/10.1038/s41598-018-19278-1

33. Hase, Y., Satoh, K. & Kitamura, S. (2023). Comparative analysis of seed and seedling irradiation with gamma rays and carbon ions for mutation induction in Arabidopsis. *Frontiers in plant science*, *14*, 1149083. https://doi.org/10.3389/fpls.2023.1149083

34. Haverkort, A. J., Struik, P. C., Visser, R. G. F. & Jacobsen, E. (2009). Applied biotechnology to combat late blight in potato caused by *Phytophthora Infestans*. *Potato research. 52*, 249–264. https://doi.org/10.1007/s11540-009-9136-3

35. Henry, I. M., Nagalakshmi, U., Lieberman, M. C., Ngo, K. J., Krasileva, K. V., Vasquez-Gross, H., … & Comai, L. (2014). Efficient genome-wide detection and cataloging of EMS-induced mutations using exome capture and Next-Generation Sequencing. *The Plant cell*, *26*(4), 1382–1397. https://doi.org/10.1105/tpc.113.121590

36. Hickey, L. T., N Hafeez, A., Robinson, H., Jackson, S. A., Leal-Bertioli, S. C. M., Tester, M., … & Wulff, B. B. H. (2019). Breeding crops to feed 10 billion. *Nature biotechnology*, *37*(7), 744–754. https://doi.org/10.1038/s41587-019-0152-9

37. Ho, S.S., Urban, A.E. & Mills, R.E. (2020). Structural variation in the sequencing era. *Nature reviews genetics, 21*, 171–189. https://doi.org/10.1038/s41576-019-0180-9

38. Holme, I. B., Gregersen, P. L. & Brinch-Pedersen, H. (2019). Induced genetic variation in crop plants by random or targeted mutagenesis: Convergence and differences. *Frontiers in plant science, 10*, 1468. https://doi.org/10.3389/fpls.2019.01468

39. Huang, K. & Rieseberg, L. H. (2020). Frequency, origins, and evolutionary role of chromosomal inversions in plants. *Frontiers in plant science*, *11*, 296. https://doi.org/10.3389/fpls.2020.00296

40. Jørgensen, I. H. (1992). Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. *Euphytica 63*, 141–152. https://doi.org/10.1007/BF00023919

41. Krishna, H., Alizadeh, M., Singh, D., Singh, U., Chauhan, N., Eftekhari, M. & Sadh, R. K. (2016). Somaclonal variations and their applications in horticultural crops improvement. *3 Biotech*, *6*(1), 54. https://doi.org/10.1007/s13205-016-0389-7

42. Kurowska, M., Daszkowska-Golec, A., Gruszka, D., Marzec, M., Szurman, M., Szarejko, I. & Maluszynski, M. (2011). TILLING: a shortcut in functional genomics. *Journal of applied genetics*, *52*(4), 371–390. https://doi.org/10.1007/s13353-011-0061-1

43. Kusch, S. & Panstruga, R. (2017). Mlo-based resistance: an apparently universal "weapon" to defeat powdery mildew disease. *Molecular plant-microbe interactions*, *30*(3), 179–189. https://doi.org/10.1094/MPMI-12-16-0255-CR

44. Lai, J., Li, R., Xu, X., Jin, W., Xu, M., Zhao, H., … & Wang, J. (2010). Genome-wide patterns of genetic variation among elite maize inbred lines. *Nature genetics*, *42*(11), 1027–1030. https://doi.org/10.1038/ng.684

45. Lee, K. & Wang, K. (2020). Level up to chromosome restructuring. *Nature plants*, *6*(6), 600–601. https://doi.org/10.1038/s41477-020-0669-4

46. Lemay, M. A., Sibbesen, J. A., Torkamaneh, D., Hamel, J., Levesque, R. C. & Belzile, F. (2022). Combined use of Oxford Nanopore and Illumina sequencing yields insights into soybean structural variation biology. *BMC biology*, 20(1), 1-24. https://doi.org/10.1186/s12915-022-01255-w

47. Li, X., Song, Y., Century, K., Straight, S., Ronald, P., Dong, X., Lassner, M. & Zhang, Y. (2001). A fast neutron deletion mutagenesis-based reverse genetics system for plants. *The plant journal: for cell and molecular biology*, *27*(3), 235–242. https://doi.org/10.1046/j.1365-313x.2001.01084.x

48. Li, X. & Zhang, Y. (2002). Reverse genetics by fast neutron mutagenesis in higher plants. *Functional & integrative genomics*, *2*(6), 254–258. https://doi.org/10.1007/s10142-002-0076-0

49. Li, G., Chern, M., Jain, R., Martin, J. A., Schackwitz, W. S., Jiang, L., … & Ronald, P. C. (2016a). Genome-wide sequencing of 41 rice (*Oryza sativa* L.) mutated lines reveals diverse mutations induced by fast-neutron irradiation. *Molecular plant*, *9*(7), 1078–1081. https://doi.org/10.1016/j.molp.2016.03.009

50. Li, S., Zheng, Y. C., Cui, H. R., Fu, H. W., Shu, Q. Y. & Huang, J. Z. (2016b). Frequency and type of inheritable mutations induced by γ rays in rice as revealed by whole genome sequencing. *Journal of Zhejiang University. Science. B*, *17*(12), 905–915. https://doi.org/10.1631/jzus.B1600125

51. Lippman, Z. B., Semel, Y. & Zamir, D. (2007). An integrated view of quantitative trait variation using tomato interspecific introgression lines. *Current opinion in genetics & development*, *17*(6), 545–552. https://doi.org/10.1016/j.gde.2007.07.007

52. Liu, J., Liu, D., Tao, W., Li, W., Wang, S., Chen, P., Cheng, S. & Gao, D. (2008), Molecular marker-facilitated pyramiding of different genes for powdery mildew resistance in wheat. *Plant Breeding*, *119*, 21-24. https://doi.org/10.1046/j.1439-0523.2000.00431.x

53. Liu, T., Fedak, G., Zhang, L., Zhou, R., Chi, D., Fetch, T., … & Zhang, B. (2020). Molecular marker based design for breeding wheat lines with multiple resistance and superior quality. *Plant disease*, *104*(10), 2658–2664. https://doi.org/10.1094/PDIS-02-20-0420-RE

54. Liu, X., Zhang, S., Jiang, Y., Yan, T., Fang, C., Hou, Q., … & Wan, X. (2022). Use of CRISPR/Cas9-based gene editing to simultaneously mutate multiple homologous genes required for pollen development and male fertility in maize. *Cells*, *11*(3), 439. https://doi.org/10.3390/cells11030439

55. Martínez-Fortún, J., Phillips, D. W. & Jones, H. D. (2022). Natural and artificial sources of genetic variation used in crop breeding: A baseline comparator for genome editing. *Frontiers in genome editing*, *4*, 937853. https://doi.org/10.3389/fgeed.2022.937853

56. Miyao, A., Nakagome, M., Ohnuma, T., Yamagata, H., Kanamori, H., Katayose, Y., … & Hirochika, H. (2012). Molecular spectrum of somaclonal variation in regenerated rice revealed by whole-genome sequencing. *Plant & cell physiology*, *53*(1), 256–264. https://doi.org/10.1093/pcp/pcr172

57. Morita, R., Kusaba, M., Iida, S., Yamaguchi, H., Nishio, T. & Nishimura, M. (2009). Molecular characterization of mutations induced by gamma irradiation in rice. *Genes and Genetic Systems*, *84*(5), 361-370. https://doi.org/10.1266/ggs.84.361

58. Nogué, F., Mara, K., Collonnier, C. & Casacuberta, J. M. (2016). Genome engineering and plant breeding: impact on trait discovery and development. *Plant cell reports*, *35*(7), 1475–1486. https://doi.org/10.1007/s00299-016-1993-z

59. Nonaka, S., Arai, C., Takayama, M., Matsukura, C. & Ezura, H. (2017). Efficient increase of ɣ-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. *Scientific reports*, *7*(1), 7057. https://doi.org/10.1038/s41598-017-06400-y

60. O'Rourke, J. A., Iniguez, L. P., Bucciarelli, B., Roessler, J., Schmutz, J., McClean, P. E., … & Vance, C. P. (2013). A re-sequencing based assessment of genomic heterogeneity and fast neutron-induced deletions in a common bean cultivar. *Frontiers in plant science*, *4*, 210. https://doi.org/10.3389/fpls.2013.00210

61. Orantes-Bonilla, M., Makhoul, M., Lee, H., Chawla, H. S., Vollrath, P., Langstroff, A., … & Snowdon, R. J. (2022). Frequent spontaneous structural rearrangements promote rapid genome diversification in a *Brassica napus* F1 generation. *Frontiers in plant science, 13*. 1057953. https://doi.org/10.3389/fpls.2022.1057953

62. Ossowski, S., Schneeberger, K., Lucas-Lledó, J. I., Warthmann, N., Clark, R. M., Shaw, R. G., Weigel, D. & Lynch, M. (2010). The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. *Science*, *327*(5961), 92–94. https://doi.org/10.1126/science.1180677

63. Pacher, M. & Puchta, H. (2017). From classical mutagenesis to nuclease-based breeding – directing natural DNA repair for a natural end-product. *The Plant journal: for cell and molecular biology*, *90*(4), 819–833. https://doi.org/10.1111/tpj.13469

64. Placido, D. F., Campbell, M. T., Folsom, J. J., Cui, X., Kruger, G. R., Baenziger, P. S. & Walia, H. (2013). Introgression of novel traits from a wild wheat relative improves drought adaptation in wheat. *Plant physiology*, *161*(4), 1806–1819. https://doi.org/10.1104/pp.113.214262

65. Prohens J. (2011). Plant Breeding: A Success Story to be Continued Thanks to the Advances in Genomics. *Frontiers in plant science*, *2*, 51. https://doi.org/10.3389/fpls.2011.00051

66. Sánchez-León, S., Gil-Humanes, J., Ozuna, C. V., Giménez, M. J., Sousa, C., Voytas, D. F. & Barro, F. (2018). Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. *Plant biotechnology journal*, *16*(4), 902–910. https://doi.org/10.1111/pbi.12837

67. Sánchez-Martín, J. & Keller, B. (2019). Contribution of recent technological advances to future resistance breeding. *Theoretical and applied genetics. Theoretische und angewandte Genetik*, *132*(3), 713–732. https://doi.org/10.1007/s00122-019-03297-1

68. Saxena, R. K., Edwards, D. & Varshney, R. K. (2014). Structural variations in plant genomes. *Briefings in functional genomics*, *13*(4), 296-307. https://doi.org/10.1093/bfgp/elu016

69. Schiessl, S., Katche, E., Ihien, E., Chawla, H. S. & Harmeet, Mason, A. (2018). The role of genomic structural variation in the genetic improvement of polyploid crops. *The Crop Journal*. *7*(2), 127-140. https://doi.org/10.1016/j.cj.2018.07.006

70. Sedlazeck, F. J., Rescheneder, P., Smolka, M., Fang, H., Nattestad, M., Von Haeseler, A. & Schatz, M. C. (2018). Accurate detection of complex structural variations using single-molecule sequencing. *Nature methods*, *15*(6), 461-468. https://doi.org/10.1038/s41592-018-0001-7

71. Sthapit, S. R., Ruff, T. M., Hooker, M. A. & See, D. R. (2022). Population structure and genetic diversity of U.S. wheat varieties. *The plant genome*, *15*(2), e20196. https://doi.org/10.1002/tpg2.20196

72. Suh, J. P., Jeung, J. U., Noh, T. H., Cho, Y. C., Park, S. H., Park, H. S., … & Jena, K. K. (2013). Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. *Rice*, *6*(1), 5. https://doi.org/10.1186/1939-8433-6-5

73. Tang, X., Liu, G., Zhou, J., Ren, Q., You, Q., Tian, L., … & Zhang, Y. (2018). A large-scale whole-genome sequencing analysis reveals highly specific genome editing by both Cas9 and Cpf1 (Cas12a) nucleases in rice. *Genome biology*, *19*(1), 84. https://doi.org/10.1186/s13059-018-1458-5

74. Tanner, G. J., Blundell, M. J., Colgrave, M. L. & Howitt, C. A. (2016). Creation of the first ultra-low gluten barley (Hordeum vulgare L.) for coeliac and gluten-intolerant populations. *Plant biotechnology journal*, *14*(4), 1139–1150. https://doi.org/10.1111/pbi.12482

75. Tao, Y., Zhao, X., Mace, E., Henry, R. & Jordan, D. (2019). Exploring and Exploiting Pan-genomics for Crop Improvement. *Molecular plant*, *12*(2), 156–169. https://doi.org/10.1016/j.molp.2018.12.016

76. Van den Broeck, H. C., van Herpen, T. W., Schuit, C., Salentijn, E. M., Dekking, L., Bosch, D., … & van der Meer, I. M. (2009). Removing celiac disease-related gluten proteins from bread wheat while retaining technological properties: a study with Chinese Spring deletion lines. *BMC plant biology*, *9*, 41. https://doi.org/10.1186/1471-2229-9-41

77. Vroh Bi, I., McMullen, M. D., Sanchez- Villeda, H., Schroeder, S., Gardiner, J., Polacco, M. ... & Coe, E. H. (2006). Single nucleotide polymorphisms and insertion–deletions for genetic markers and anchoring the maize fingerprint contig physical map. *Crop Science*, *46*(1), 12-21. https://doi.org/10.2135/cropsci2004.0706

78. 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

79. Wang, W., Mauleon, R., Hu, Z., Chebotarov, D., Tai, S., Wu, Z., … & Leung, H. (2018). Genomic variation in 3,010 diverse accessions of Asian cultivated rice. *Nature 557*, 43–49. https://doi.org/10.1038/s41586-018-0063-9

80. Weng, M. L., Becker, C., Hildebrandt, J., Neumann, M., Rutter, M. T., Shaw, R. G., Weigel, D. & Fenster, C. B. (2019). Fine-grained analysis of spontaneous mutation spectrum and frequency in *Arabidopsis thaliana*. *Genetics*, *211*(2), 703-714. https://doi.org/10.1534/genetics.118.301721

81. Whitney, K. D., Randell, R. A. & Rieseberg, L. H. (2010). Adaptive introgression of abiotic tolerance traits in the sunflower *Helianthus annuus*. *The new phytologist*, *187*(1), 230–239. https://doi.org/10.1111/j.1469-8137.2010.03234.x

82. Wolter, F., Schindele, P. & Puchta, H. (2019). Plant breeding at the speed of light: the power of CRISPR/Cas to generate directed genetic diversity at multiple sites. *BMC plant biology*, *19*(1), 176. https://doi.org/10.1186/s12870-019-1775-1

83. Xiao, H., Jiang, N., Schaffner, E., Stockinger, E. J. & Van Der Knaap, E. (2008). A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. *Science*, *319*(5869), 1527-1530. https://doi.org/10.1126/science.1153040

84. Yang, N., Xu, X. W., Wang, R. R., Peng, W. L., Cai, L., Song, J. M., … & Yan, J. (2017). Contributions of *Zea mays* subspecies mexicana haplotypes to modern maize. *Nature communications*, *8*(1), 1874. https://doi.org/10.1038/s41467-017-02063-5

85. Yildiz, G., Zanini, S. F., Afsharyan, N. P., Obermeier, C., Snowdon, R. J. & Golicz, A. A. (2023). Benchmarking Oxford Nanopore read alignment- based insertion and deletion detection in crop plant genomes. *The Plant Genome*, e20314. https://doi.org/10.1002/tpg2.20314

86. Zambrano, J. L., Jones, M. W., Brenner, E., Francis, D. M., Tomas, A. & Redinbaugh, M. G. (2014). Genetic analysis of resistance to six virus diseases in a multiple virus-resistant maize inbred line. *Theoretical and applied genetics. Theoretische und angewandte Genetik*, *127*(4), 867–880. https://doi.org/10.1007/s00122-014-2263-5

87. Zanini, S. F., Bayer, P. E., Wells, R., Snowdon, R. J., Batley, J., Varshney, R. K., ... & Golicz, A. A. (2022). Pangenomics in crop improvement—From coding structural variations to finding regulatory variants with pangenome graphs. *The Plant Genome*, *15*(1), e20177. https://doi.org/10.1002/tpg2.20177

88. Zhang, D., Wang, Z., Wang, N., Gao, Y., Liu, Y., Wu, Y., … & Liu, B. (2014). Tissue culture-induced heritable genomic variation in rice, and their phenotypic implications. *PloS one*, *9*(5), e96879. https://doi.org/10.1371/journal.pone.0096879

89. Zhang, F., Xue, H., Dong, X., Li, M., Zheng, X., Li, Z., ... & Wei, C. (2022). Long-read sequencing of 111 rice genomes reveals significantly larger pan-genomes. *Genome Research*, *32*(5), 853-863. https://doi.org/10.1101/gr.276015.121

90. Zhou, Y., Minio, A., Massonnet, M., Solares, E., Lv, Y., Beridze, T., Cantu, D. & Gaut, B. S. (2019). The population genetics of structural variants in grapevine domestication. *Nature plants 5*, 965–979. https://doi.org/10.1038/s41477-019-0507-8

Ministerie van Landbouw, Visserij,
Voedselzekerheid en Natuur

Directoraat-generaal Agro

Auteur
[redacted]

Aan de Staatssecretaris van Landbouw, Visserij, Voedselzekerheid en Natuur

TER BESLISSING
Datum
20 oktober 2025

Kenmerk
DGA / 101852001

nota
TER BESLISSING

Kopie aan

Bijlage(n)

Parafenroute

[redacted]

Aanleiding
De leden van de Eerste Kamer van de PvdD en de SP hebben u vragen gesteld naar aanleiding van de beantwoording over het Europese Raadsakkoord voor het NGT-voorstel.

Geadviseerd besluit
U kunt de begeleidende brief ondertekenen en samen met de antwoorden op de vragen sturen aan de leden van de Eerste Kamer.

Kernpunten
- Op 14 maart 2025 is het raadsakkoord gesloten voor het NGT-voorstel. Naar aanleiding van dit raadsakkoord heeft u op 11 april 2025 een Kamerbrief gestuurd met uw duiding van het raadsakkoord (Kamerstuk 27428 nr. 409).
- De PvdD fractie heeft naar aanleiding van deze brief vragen gesteld. U heeft op 23 juni 2025 de vragen van de PvdD fractie beantwoord (Kamerstukken I 2024/25, 27428, G).
- Naar aanleiding van uw beantwoording stellen de fracties van de PvdD en SP vervolgvragen aan u. Met deze Kamerbrief beantwoordt u de vragen van deze fracties.
- Een aantal vragen ziet toe op de verificatieprocedure voor equivalentie van NGT-1 planten met conventioneel veredelde planten. Deze vragen vallen onder de beleidsverantwoordelijkheid van de Staatssecretaris van IenW en zijn ook aan uw collega daar voorgelegd.