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Updated scientific opinion on plants developed through cisgenesis and intragenesis

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Abstract

In 2012, EFSA issued an opinion on plants developed through cisgenesis and intragenesis. With the development of New Genomic Techniques (NGTs) in the last decade, cisgenic and intragenic plants can now be obtained with the insertion of a desired sequence in a precise location of the genome. EFSA has been requested by European Commission to provide an updated scientific opinion on the safety and the risk assessment of plants developed through cisgenesis and intragenesis, in order to (i) identify potential risks, comparing them with those posed by plants obtained by conventional breeding and Established Genomic Techniques (EGTs) and (ii) to determine the applicability of current guidelines for the risk assessment of cisgenic and intragenic plants. The conclusions of the previous EFSA opinion were reviewed, taking into consideration the new guidelines and the recent literature. The GMO panel concludes that no new risks are identified in cisgenic and intragenic plants obtained with NGTs, as compared with those already considered for plants obtained with conventional breeding and EGTs. There are no new data since the publication of the 2012 EFSA opinion that would challenge the conclusions raised in that document. The conclusions of the EFSA 2012 Scientific Opinion remain valid. The EFSA GMO Panel reiterates from these conclusions that with respect to the source of DNA and the safety of the gene product, the hazards arising from the use of a related plant-derived gene by cisgenesis are similar to those from conventional plant breeding, whereas additional hazards may arise for intragenic plants. Furthermore, the EFSA GMO Panel considers that cisgenesis and intragenesis make use of the same transformation techniques as transgenesis, and therefore, with respect to the alterations to the host genome, cisgenic, intragenic and transgenic plants obtained by random insertion do not cause different hazards. Compared to that, the use of NGTs reduces the risks associated with potential unintended modifications of the host genome. Thus, fewer requirements may be needed for the assessment of cisgenic and intragenic plants obtained through NGTs, due to site-directed integration of the added genetic material. Moreover, the GMO panel concludes that the current guidelines are partially applicable and sufficient. On a case-by-case basis, a lesser amount of data might be needed for the risk assessment of cisgenic or intragenic plants obtained through NGTs.

Keywords: cisgenesis, intragenesis, plants, genome editing, new genomic techniques, site-directed nucleases, risk assessment

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Summary

In 2012, EFSA issued an opinion on plants developed through cisgenesis and intragenesis, in which the exogenous genetic material was always considered as the entire expression unit, comprising a promoter with other regulatory sequences, the coding region and a terminator. With the development of New Genomic Techniques (NGTs) in the last decade, the possibility to insert a desired sequence in a precise location of the genome allows the transfer of any genetic material, not necessarily an entire expression cassette. Since cisgenic or intragenic plants can be obtained via NGTs, the European Commission updated the definition of cisgenesis and intragenesis, which are now considered as '*genetic modifications involving genetic material obtained from outside the host organism and transferred to the host using various delivery strategies; the incorporated sequences contain an exact copy (cisgenesis) or a re-arranged copy (intragenesis) of sequences already present in the species or in a sexually compatible species*'.

EFSA was requested by European Commission to provide an updated scientific opinion on the safety and the risk assessment of plants developed through cisgenesis and intragenesis, considering the current state of the art and available knowledge on NGTs.

This Opinion addresses four requests from the European Commission as described in the terms of reference (ToR):

1) Identify potential risks that plants obtained by cisgenic and intragenic approaches could pose for humans, animals and the environment.
2) Compare the above-mentioned risks with those associated with plants obtained by conventional plant breeding techniques and plants obtained with EGTs.
3) Determine whether the existing guidelines for risk assessment are applicable, fully or partially, and sufficient to cisgenic and intragenic plants.
4) In case existing guidelines for risk assessment are considered not applicable, partially applicable or not sufficient, to identify on which aspects existing guidelines should be updated, adapted or complemented.

A protocol was developed according to 'Draft framework for protocol development for EFSA's scientific assessments' (EFSA, 2020). Each ToR was translated into scientifically answerable assessment questions. A literature search was conducted, with a focus on publications and patents reporting cisgenic/intragenic plants obtained with or without NGTs. The search only retrieved reports about cisgenic/intragenic plants and derived products obtained via established genomic techniques (EGTs).

Based on the review of the information retrieved by the literature search and the experts' knowledge, the GMO Panel concluded that:

- No new risks are identified in cisgenic and intragenic plants obtained with NGTs, as compared with those already considered for plants obtained with conventional breeding and EGTs. Therefore, the conclusions of the EFSA 2012 scientific opinion (EFSA GMO Panel, 2012a) remain valid. The EFSA GMO Panel reiterates from these conclusions that, with respect to the source of DNA and the safety of the gene product, the hazards arising from the use of a related plant-derived gene by cisgenesis are similar to those from conventional plant breeding, whereas additional hazards may arise for intragenic plants. Furthermore, the EFSA GMO Panel considers that cisgenesis and intragenesis make use of the same transformation techniques as transgenesis and, therefore, with respect to the alterations to the host genome, cisgenic, intragenic and transgenic plants obtained by random insertion do not cause different hazards.
- Fewer requirements may be needed for the assessment of cisgenic and intragenic plants obtained through NGTs, due to site-directed integration of the added genetic material.
- In the case where the donor plant has a history of safe use as food and feed, certain parts of the comparative analysis, toxicity, allergenicity or nutritional assessment may not be necessary.
- With respect to the environmental risk assessment, all elements described in the current guidelines can apply to cisgenic/intragenic plants.

Therefore, the GMO panel concludes that the current guidelines are partially applicable and sufficient. On a case-by-case basis, a lesser amount of data might be needed for the risk assessment of cisgenic or intragenic plants obtained through NGTs.

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1. Introduction

1.1. Background and Terms of Reference as provided by European Commission

Over the last 10 years, following the requests by the European Commission, the European Food Safety Authority (EFSA) has issued scientific opinions on plants obtained through certain new genomic techniques (NGTs). Among these, EFSA has published two opinions, one on site-directed nuclease (SDN)-1, SDN-2 and oligonucleotide directed mutagenesis (ODM),[1] and one on cisgenesis and intragenesis.[2] After the publication of the EFSA opinion on cisgenesis and intragenesis, an opinion on the safety assessment of plants developed through SDN-3 was also published.[3] In that document, EFSA was also envisaging the possibility to develop cisgenic and intragenic plants using SDN-3 techniques. These scientific opinions have focused on the potential risks associated to the new techniques, compared to conventional breeding techniques and established genomic techniques (EGTs),[4] and on the applicability of existing risk assessment guidance to plants produced with the NGTs under consideration.

The main conclusions of the abovementioned opinions, relevant to the present mandate, are the following:

- Plants produced by SDN-1, SDN-2 and ODM techniques have no new hazards compared to conventionally bred and transgenic plants.
- Similar hazards can be associated with cisgenic and conventionally bred plants, while novel hazards can be associated with intragenic and transgenic plants.
- The existing EFSA Guidance documents are sufficient and applicable in case of plants produced by cisgenesis and intragenesis, and sufficient and partially applicable in case of plants produced by SDN-1, SDN-2 and ODM techniques.
- There is a need for flexibility in the data requirements for the risk assessment, as on a case-by-case lesser amounts of data might be needed.
- SDN-3 opinion concludes that SDN-3 techniques can be used for cisgenesis/intragenesis.

While the scientific opinion on SDN-1, SDN-2 and ODM is very recent, dating from 2020, the cisgenesis/intragenesis and SDN-3 scientific opinions date from 2012. They take into account the techniques available at that time, notably *Agrobacterium*-mediated transformation and direct gene transfer, although several of the considerations therein are not linked to the use of a specific technique. Since 2012, several developments in terms of scientific knowledge and technologies have taken place. In particular, genome editing techniques, including SDN, can now also be used, alone or in combination with other techniques, to produce cisgenic and intragenic organisms, in addition to EGTs.

Against this background, the Commission would like EFSA to confirm whether the considerations and conclusions of EFSA scientific opinion on cisgenesis/intragenesis of 2012 are still applicable.

1.2. Background as provided by EFSA

Following a request from the European Commission on 11 June 2021, EFSA assigned the mandate to the ad hoc working group on Cisgenesis/Intragenesis of the GMO Panel[5] in September 2021.

1.3. Terms of Reference

Building on previous work of EFSA, notably the above-mentioned scientific opinions on SDN techniques and cisgenesis/intragenesis, the European Commission asks EFSA, in accordance with

[1] EFSA GMO Panel. Applicability of the EFSA Opinion on site-directed nucleases type 3 for the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide-directed mutagenesis. EFSA Journal 2020;18(11):6299, 14 pp. https://doi.org/10.2903/j.efsa.2020.6299

[2] 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.2012.2561

[3] EFSA GMO Panel. Scientific opinion addressing the safety assessment of plants developed using ZFN-3 and other SDNs with similar function. EFSA Journal 2012;10(10):2943, https://doi.org/10.2903/j.efsa.2012.2943

[4] For the purpose of this document, established genomic techniques (EGTs) are those genomic techniques developed prior to 2001, when the existing GMO legislation was adopted, and used to obtain the GMOs authorised in the EU so far. EGTs include techniques such as Agrobacterium-mediated transformation and direct gene transfer.

[5] https://www.efsa.europa.eu/en/science/scientific-committee-and-panels/gmo

Article 29 of Regulation (EC) No 178/2002, to provide an updated scientific opinion on the safety and the risk assessment of plants developed through cisgenesis and intragenesis.[6]

In particular, EFSA is requested to consider the current state of the art and available knowledge on NGTs and:

1) Identify potential risks that plants obtained by cisgenic and intragenic approaches could pose for humans, animals and the environment.
2) Compare the above-mentioned risks with those associated with plants obtained by conventional plant breeding techniques and plants obtained with EGTs.
3) Determine whether the existing guidelines for risk assessment are applicable, fully or partially, and sufficient[7] to cisgenic and intragenic plants.
4) In case existing guidelines for risk assessment are considered not applicable, partially applicable or not sufficient, to identify on which aspects existing guidelines should be updated, adapted or complemented.

1.4. Interpretation of Terms of Reference

The EFSA Opinion on cisgenesis and intragenesis published in 2012 (EFSA GMO Panel, 2012a) addresses the safety of plants modified through cisgenesis and intragenesis as defined by a working group of EU Member States' experts on new techniques. In that opinion, cisgenesis is defined as '*. . .the genetic modification of a recipient organism with a gene from a crossable –sexually compatible – organism (same species or closely related species). This gene includes its introns and is flanked by its native promoter and terminator in the normal sense orientation'*, whereas intragenesis is defined as '*genetic modification of a recipient organism that leads to a combination of different gene fragments from donor organism(s) of the same or a sexually compatible species as the recipient. These may be arranged in a sense or antisense orientation compared to their orientation in the donor organism. Intragenesis involves the insertion of a reorganised, full or partial coding region of a gene frequently combined with another promoter and/or terminator from a gene of the same species or a crossable species.'* Therefore, the given definitions limited cisgenesis/intragenesis approaches to the introduction of (protein-coding) genes, similar to transgenesis, but with the difference that in cisgenesis/ intragenesis, the introduced sequences are from a crossable species. The new developments of site-directed modification of genomes offer the possibility of targeting the insertion of new sequences at specific loci in the genome. With a targeted insertion, any genetic sequence (i.e. promoters, terminators, introns, signal peptides, etc.) and not only protein-coding genes can potentially be transferred in a predetermined locus and maintain their original function in the host genome. The potential of site-directed modifications can be applied to cisgenesis/intragenesis approaches, when the introduced sequences are already present in a crossable species. Therefore, EFSA was requested to develop the current opinion using a newer definition for cisgenesis/intragenesis. According to the new definition provided by European Commission, *'cisgenesis and intragenesis are genetic modifications involving genetic material obtained from outside the host organism and transferred to the host using various delivery strategies; the incorporated sequences contain an exact copy (cisgenesis) or a re-arranged copy (intragenesis) of sequences already present in the species or in a sexually compatible species'*. Because these two definitions cover different plants and derived products that can raise different type of risks, in delivering its opinion EFSA chose to address separately cisgenesis/ intragenesis products already covered in the EFSA 2012 Opinion (EFSA, GMO Panel 2012a) (i.e. that aim to introduce a protein-coding gene from a crossable species into a plant) and new potential cisgenesis/intragenesis products not covered in that opinion (i.e. that aim to introduce sequences from a crossable species other than complete protein-coding genes) separately. Therefore, for terms of

[6] For the purpose of this mandate, the following definitions apply: Cisgenesis and intragenesis are genetic modifications involving genetic material obtained from outside the host organism and transferred to the host using various delivery strategies; the incorporated sequences contain an exact copy (cisgenesis) or a re-arranged copy (intragenesis) of sequences already present in the species or in a sexually compatible species. (Adapted from 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 978–92–76-24,696-1, https://doi.org/10.2760/710056), JRC121847.

[7] In the context of this mandate, 'applicable' means 'that can be used for the purpose', 'fully applicable' means 'that can be used in full', 'partially applicable' means 'that can be used only in part' and 'sufficient' means 'that does not need to be complemented'.

reference (ToRs) 1, 2 and 3, specific questions for each of the two types of products were answered (the same assessment questions are reported in the protocol – Appendix A).

ToRs 1 and 2: Identify potential risks that plants obtained by cisgenic and intragenic approaches could pose for humans, animals and the environment; compare the above-mentioned risks with those associated with plants obtained by conventional plant breeding techniques and plants obtained with EGTs.

For cisgenesis/intragenesis plants and derived products already covered in the EFSA 2012 opinion, the following questions were addressed:

AQ1. What are the risks that cisgenic/intragenic plants could pose to humans, animals and the environment, that were identified in the 2012 cisgenesis Opinion?
AQ2. Is there new information available that could impact on the risk assessment of the plants and derived products included in the EFSA 2012 Opinion?
AQ3. Are there new techniques/approaches developed since 2012 that could be used to obtain cisgenic/intragenic plants as defined in the 2012 Opinion?
AQ4. If there are new techniques/approaches, what are the potential risks that may arise compared with those already covered in the 2012 Opinion?

For cisgenesis/intragenesis plants and derived products not covered in the EFSA 2012 Opinion, the following questions were addressed:

AQ1. What are the new plants and derived products that could be obtained using new approaches, in particular with the use of SDNs, that could give rise to cisgenic/intragenic plants according to the definition given in the framework of this mandate[6]?
AQ2. What could be the risks that those plants and derived products could pose to humans, animals and the environment, compared with the risks associated with plants obtained by conventional plant breeding techniques and plants obtained with EGTs?

ToR 3: Determine whether the existing guidelines for risk assessment are applicable, fully or partially, and sufficient to cisgenic and intragenic plants.

For cisgenesis/intragenesis plants and derived products already covered in the EFSA 2012 Opinion, the following question was addressed:

AQ1. Are the conclusions of the EFSA 2012 Opinion on the applicability of the existing guidelines still valid, taking into account the new guidelines published and the information made available since the publication of this opinion?

For cisgenesis/intragenesis plants and derived products not covered in the EFSA 2012 opinion, the following question was addressed:

AQ1. Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient for these new plants and derived products?

ToR 4: In case existing guidelines for risk assessment are considered not applicable, partially applicable or not sufficient, to identify on which aspects existing guidelines should be updated, adapted or complemented.

AQ1. Which aspect (if any) of existing guidelines should be updated, adapted or complemented?

For clarity purposes, the GMO Panel deemed it appropriate to address ToR1 and ToR2 together, as both required an analysis of the potential risks posed by cisgenic/intragenic plants.

2. Data and methodologies

2.1. Existing guidelines and ad hoc expert Working Group

EFSA established an ad hoc expert Working Group of the GMO Panel to provide an updated scientific opinion on the molecular characterisation (MC), Food and Feed (FF) and Environmental Risk Assessment (ERA) of plants developed through cisgenesis and intragenesis. The ad hoc Working Group met regularly to address the mandate of the European Commission.[8] In delivering its Scientific Opinion, the GMO Panel, together with the ad hoc expert Working Group, considered the current GMO

[8] https://www.efsa.europa.eu/sites/default/files/2021-10/wg-plants-cisgenesis-intragenesis-minutes.pdf

legislation and corresponding EFSA guidelines. The guidelines that are relevant for MC, FF and ERA of plants developed through cisgenesis and intragenesis are presented in Table 1.

Table 1: Existing guidelines and regulatory framework for MC, FF and ERA

References	Title
Directive 2001/18/EC	Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms[(a)]
Regulation No. 1829/2003	Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed[(b)]
EFSA GMO Panel (2010)	Guidance on the environmental risk assessment of genetically modified plants
EFSA GMO Panel (2011a)	Guidance for risk assessment of food and feed from genetically modified plants
EFSA GMO Panel (2011b)	Guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants
EC Regulation No. 503/2013	Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006[(c)]
EFSA GMO Panel (2015)	Guidance on the agronomic and phenotypic characterisation of genetically modified plants (ERA)
EFSA (2018) EFSA-Q-2013-00738	Guidance on Uncertainty Analysis in Scientific Assessments
EFSA GMO Panel (2017)	Guidance on allergenicity assessment of genetically modified plants
Commission Directive 2018/350	Commission Directive (EU) 2018/350 of 8 March 2018 amending Directive 2001/18/EC of the European Parliament and of the Council as regards the environmental risk assessment of genetically modified organisms[(d)]

(a): Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC - Commission Declaration. OJ L 106, 17.4.2001, p. 1–39.
(b): Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed.
(c): Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L 157, 8.6.2013, pp. 1–48.
(d): Commission Directive (EU) 2018/350 of 8 March 2018 amending Directive 2001/18/EC of the European Parliament and of the Council as regards the environmental risk assessment of genetically modified organisms.

To address the four ToRs, the ad hoc working group took into considerations the guidelines listed above, focusing on the ones issued after 2011.

For this mandate, EFSA has been requested to consider the current state of the art and available knowledge on NGTs. The GMO Panel has considered the available information on NGTs published in EFSA opinions. Table 2 reports the opinions that have been considered regarding the NGTs.

Table 2: EFSA opinions addressing new genomic techniques

References	Title
EFSA, GMO Panel, 2012a	EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2012a. Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis. EFSA Journal 2012;10(2):2561, 33 pp.
EFSA, GMO Panel, 2012b	EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2012b. Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function. EFSA Journal 2012;10(10): 2943, 31 pp.
EFSA, GMO Panel, 2020	EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2020. Applicability of the EFSA Opinion on site directed nucleases type 3 for the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide-directed mutagenesis. EFSA Journal 2020;18(11):6299, 14 pp.

References	Title
EFSA, 2021	EFSA (European Food Safety Authority), Paraskevopoulos, K and Federici, S, 2021. Overview of EFSA and European national authorities' scientific opinions on the risk assessment of plants developed through New Genomic Techniques. EFSA Journal 2021; 19(4):6314, 43 pp.

2.2. EFSA Opinion on cisgenesis and intragenesis

In 2007, a New Techniques Working Group (NTWG) was established to analyse a non-exhaustive list of techniques for which it was unclear whether they would result in a genetically modified organism, or a genetically modified micro-organism as defined under Directive 2001/18/EC or Directive 2009/41/EC[9], respectively. An initial list of eight techniques, including cisgenesis, was proposed for consideration. In 2012, EFSA issued a scientific opinion on plants developed through cisgenesis and intragenesis (EFSA GMO Panel, 2012a). For this opinion, the GMO Panel was asked: (1) to determine whether there was a need for new guidance or whether the existing guidance on risk assessment should be updated or further elaborated, in anticipation of the placing of products on the market through the application of the listed techniques; and (2) to determine the risks in terms of impact on humans, animals and the environment that the eight techniques listed could pose and compare plants obtained by these new techniques with plants obtained by conventional plant breeding techniques and secondly with plants obtained with currently used genetic modification techniques.

The EFSA Opinion (EFSA GMO Panel, 2012a) addressed the risks on humans, animals and environment by comparing plants obtained through cisgenesis and intragenesis with plants obtained by conventional breeding and by transgenesis. In order to identify the risks, a list of potential hazards was considered, including the source of the DNA and the safety of the gene products; alterations to the host genome at the insertion site and elsewhere; the potential presence of non-plant sequences in the insert; the expression of the trait.

In order to assess the applicability of the current guidelines, the opinion focused on the guidance for risk assessment of food and feed from genetically modified plants (EFSA GMO Panel, 2011a) and the guidance on the environmental risk assessment of genetically modified plants (EFSA GMO Panel, 2010).

2.3. EFSA Opinion on SDN-3

Similarly, for the SDN-3 opinion (EFSA, GMO Panel 2012b), the GMO Panel was asked (1) to determine the risks in terms of impact on humans, animals and the environment that the eight techniques listed could pose and compare plants obtained by these new techniques with plants obtained by conventional plant breeding techniques and, secondly, with plants obtained with currently used genetic modification techniques, and (2) to determine whether there was a need for new guidance or whether the existing guidance on risk assessment should be updated or further elaborated, in anticipation of the placing of products on the market through the application of the listed techniques.

To address the above-mentioned ToRs, the GMO Panel initially focused on plants developed by the zinc finger nuclease 3 technique (ZFN-3). The opinion was developed to include all site-directed nuclease techniques (SDNs) that deliver the genetic modifications associated with the ZFN-3 technique, referring to these generically as the SDN-3 technique: '*The SDN-3 technique targets DNA insertion into a predefined genomic locus. This locus may or may not have extensive similarity to the DNA to be inserted as the purpose of SDN-3 technique is to allow insertions or exchanges of entire genes or any other DNA sequence at a specific locus. Thus, SDN-3 technique can be used for transgenesis as well as for cisgenesis and intragenesis. The induction of a DSB* [double-strand break] *at a particular locus with an SDN greatly increases the targeted integration of DNA, which otherwise would integrate randomly into naturally induced chromosome breaks. Therefore, the use of SDNs makes it possible to insert DNA at a specific locus in the plant genome. The integration of the DNA can be mediated by HR* [homologous recombination] *or by NHEJ* [non-homologous end-joining] *(the latter is designated SDN-3–NHEJ technique), depending on the presence or not of sequence similarity between the DNA to be inserted and the target locus.*'

[9] Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms.

When considering hazards related to plants developed by SDN-3 approaches compared with transgenic and conventionally bred plants, the major considerations made by the GMO Panel included: the source of the DNA and the safety of the gene products; alterations to the host genome at the insertion site and elsewhere (also including the potential presence of non-plant sequences in the insert and the expression of the trait/modification of gene expression and its potential wider applications).

In order to assess the applicability of the current guidelines, the opinion focused on the Guidance for risk assessment of food and feed from genetically modified plants (EFSA GMO Panel, 2011a) and the Guidance on the environmental risk assessment of genetically modified plants (EFSA GMO Panel, 2010).

2.4. Methodologies

2.4.1. Problem formulation

A detailed description of the problem formulation is reported in the methodological protocol that is included in Appendix A. The problem formulation has been made operational by translating the ToRs into assessment questions, in line with the EFSA draft document on protocol development for non-application mandates (Step 1.1 and Step 1.2; EFSA, 2020).

2.4.2. Literature search

The GMO Panel decided to focus on publications reporting cisgenic/intragenic plants and derived products obtained with or without the use of NGTs. Based on a literature search described in Appendix A, 650 publications were found and subjected to a two-step screen by two independent reviewers, using the Distiller SR software. The first screening was based on titles and abstracts; 73 documents were selected, according to the exclusion criteria listed in the protocol (Appendix A). The second stage included full-text screening. None of the selected publications reported cisgenic or intragenic plants and derived products developed through the use of NGTs. The experts included at a later stage additional reports that, although not including the terms 'cisgenesis/intragenesis', were considered relevant for the assessment.

A patent search was carried out using two databases, Google Patents and Espacenet, following the criteria listed in the protocol (Appendix A). In total, 174 patents were selected from a first screening based on the presence of the keywords (cisgenesis, cisgenic, intragenesis and intragenic) in the title and claims. The search was limited to the patents published after 2010. The second step of the screen was based on full-text reading by an independent reviewer. In total, 164 patents were excluded because not related to cisgenic/intragenic plants and derived products, despite the presence of the searched keywords. The remaining 10 patents were all relative to cisgenic/intragenic plants and derived products achieved through EGTs. The list of publications and patents retrieved from the search is reported in an Annex that will be published together with the final Scientific Opinion.

2.4.3. Consultation

In line with its policy on openness and transparency, EFSA consulted EU Member States and its stakeholders by an online public consultation. Between May 2022 and June 2022, all stakeholders were invited to submit their comments on the draft GMO Panel Opinion.[10] Following this consultation process, the document has been revised by the GMO Panel and the experts of its ad hoc Working Group on Cisgenesis/Intragenesis and comments received have been incorporated whenever appropriate.

The outcome of the online public consultation is reported in an Annex that will be published on EFSA's website together with the final Scientific Opinion.

3. Assessment

3.1. Introduction

3.1.1. Established genomic techniques (EGTs)

With advances in biotechnology starting from the end of the 1970s, conventional breeding techniques, used to hybridise parent plants with the possibility to overcome sexual compatibility

[10] Published at: https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0l7U0000011Zb2/pc0176

barriers, have started to evolve, with the use of novel sophisticated methodologies able to introduce genetic changes. The term 'established genomic techniques' (EGTs) refers to those genomic techniques developed before 2001.

Random mutagenesis, for example, has been extensively used to introduce genetic changes by applying physical or chemical mutagens, either *in vivo* or *in vitro*, to cells, tissues or entire plants (EFSA GMO Panel, 2021).

While the term 'EGT' is broad and includes techniques that cause genetic alterations similar to the ones that occur in nature, here we refer to genetic techniques that involve the transfer of genetic material to the host organism, using various strategies, such as *Agrobacterium*-mediated transformation, biolistic transformation or microinjection. Typically, the inserted nucleic acid contains the sequence coding for the desired trait, including the elements necessary to its expression in the host cell. The exogenous DNA integrates stably in the genome of the host cell; plant cells carrying the genetic alteration are regenerated into fertile plants, which are then screened for the presence of the desired trait (JRC, 2021).

With all the above-mentioned EGTs, the exogenous sequence integrates randomly at one or several positions in the genome, with potential consequences on the expression patterns.

3.1.2. New genomic techniques (NGTs)

The scientific advances witnessed since 2001 have allowed the development of new methodologies with different features, compared with EGTs. The term 'new genomic techniques' (NGTs) refers to relatively new methodologies that are able to cause either subtle changes in the genomes, such as point mutations, or larger deletions/insertions or sequence replacements, all with a distinctive characteristic: target specificity within the host genome.

The components that cause the genome alterations are delivered to the plant cells using a variety of methods, most of which are also used for EGTs. The most common delivery systems are T-DNA insertion from *Agrobacterium tumefaciens* and the biolistic approach by direct acceleration of microparticles coated with DNA. Another common way is the direct uptake by protoplasts of the desired DNA, using electroporation or chemical agents, such as polyethylene glycol (PEG). Once the components for the NGTs have been delivered and have completed their function, they are no longer needed, and the transgenes encoding these components are usually segregated out via sexual crossing or through specific recombinases.

Other methods have been developed that do not involve the stable insertion of recombinant DNA. The components of the NGTs can be expressed transiently in the plant cell, via *Agrobacterium tumefaciens* transformation or via biolistics, avoiding stable integration into the host genome and the consequent need for segregation.

More recently, alternative transient methods have facilitated the direct delivery of either the protein responsible for the genetic alteration, the mRNA expressing it or the ribonucleoprotein complex (Metje-Sprink et al., 2019).

3.1.3. NGTs relevant for this mandate

The JRC report (JRC, 2021) divides the NGTs into four main groups:

Group 1 is composed of NGTs that create a DSB in the DNA. This group includes the so-called 'site-directed nuclease (SDN)' techniques.

Group 2 is composed of NGTs that create a single-strand break (SSB) or no break at all in the genome.

Group 3 includes techniques that affect the epigenome, with alterations that affect the way the DNA is expressed.

Group 4 includes NGTs that act directly on the RNA, rather than the DNA.

The techniques that can be used to produce cisgenic/intragenic plants, and therefore relevant for the current mandate, belong to Group 1 and 2.

Group 1 includes techniques that rely on the induction of a DSB, followed by the attempt of the cell to repair it. The range of molecular tools able to cause the DSB is named SDNs and includes enzymes that recognise the target DNA through protein–DNA interactions, such as homing endonucleases, zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENs), and nucleases that rely on targeted RNA–DNA interaction (CRISPR/Cas9 system).

Among Group 1, considering the new definition of cisgenesis/intragenesis provided for this mandate (Section 1.4), the GMO panel considered SDN-3 as the most relevant technique for this mandate.

Group 2 contains genome editing mechanisms that induce SSBs, instead of DSBs. Oligo-directed mutagenesis, base editing and prime editing belong to this group, but the latter is the only one relevant to this mandate, as it has been shown to allow insertions of long exogenous sequences (< 100 bp), in the targeted location (Anzalone et al., 2022).

Prime editing (PE) requires an impaired Cas protein, capable of producing an SSB, paired with an engineered reverse transcriptase (RT) and a prime editing guide RNA (pegRNA) that recognises the target site. The RT copies the RNA of the guide into DNA, inserting the desired modification (Anzalone et al., 2019). A relatively new variation of PE has been reported, named TwinPE, which utilises two pegRNAs, each targeting a different DNA strand. Upon the action of the RT, each pegRNA creates a 3' flap, complementary to each other, creating an intermediate with 3' overhangs that replaces the original sequences. The advantage of this technique is the possibility to insert much larger exogenous sequences, compared with the insertion achievable with the conventional PE (Anzalone et al., 2022).

The above-mentioned strategies are considered relevant to this mandate, but do not constitute an exhaustive list, since current methodologies are in continuous evolution and new techniques could be developed.

3.2. Addressing ToR1 and ToR2: To identify potential risks that plants obtained by cisgenic and intragenic approaches could pose for humans, animals and the environment and to compare the above-mentioned risks with those associated with plants obtained by conventional plant breeding techniques and plants obtained with EGTs

3.2.1. For cisgenesis/intragenesis plants and derived products already covered in the EFSA 2012 Opinion, the following assessment questions were addressed

3.2.1.1. What are the risks that cisgenic/intragenic plants could pose to humans, animals and the environment, that were identified in the 2012 cisgenesis Opinion?

The EFSA scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis (EFSA GMO Panel, 2012a) describes cisgenesis as the introduction in a plant of 'specific alleles/genes present in the breeders' gene pool,[11] without any change to the DNA sequence'. In this definition, the cisgene corresponds to the native gene including introns, 5' and 3' UTRs, and flanking native promoter and terminator in the normal sense orientation. The EFSA 2012 opinion describes intragenesis as the introduction in a given plant of genetic elements *'created by recombining genetic elements such as promoters, coding sequences and terminators of different genes within the breeder's gene pool'* for that particular plant species. It is important to note that cisgenesis and intragenesis differ from transgenesis in the source of the genetic elements introduced, which in the case of transgenesis consists of '*new combinations of genetic material from outside the breeders' gene pool*'. In addition, this document also states that '*cisgenesis and intragenesis make use of the same transformation techniques as transgenesis*'.

Against this background, the EFSA scientific opinion (EFSA GMO Panel, 2012a) considers hazards related to cisgenic and intragenic plants compared with transgenic and conventionally bred plants in relation to *'the source of the DNA and the safety of gene products'*, *'alterations to the host genome at the insertion site and elsewhere'*, *'the potential presence of non-plant sequences in the insert'* and *'the expression of the trait and its potential wider implications'*.

With respect to the source of DNA and the safety of the gene product, the EFSA scientific opinion (EFSA GMO Panel, 2012a) concluded that *'the hazards arising from the use of a related plant-derived*

[11] In the EFSA 2012 opinon, the breeders' gene pool is defined as follows: *'The sources of genes available for conventional plant breeding are referred to as the 'breeders' gene pool'. Breeders distinguish between primary, secondary and tertiary gene pools. Each primary gene pool comprises one cultivated species together with other taxonomic species with which it can interbreed freely. The secondary gene pool includes species that can be cross-bred only with difficulty with a member of the primary gene pool but which produce at least some fertile hybrids. The tertiary gene pool comprises those species that are more distantly related to a member of the primary gene pool, but which can be cross-bred only using advanced techniques such as embryo rescue, induced polyploidy and bridge crosses.'*

gene by cisgenesis are similar to those from conventional plant breeding, as similar traits are expressed by the gene'. Therefore, the capacity to generate new toxins or allergens or to alter the composition of cisgenic plants will not be different from that of conventional breeding plants. However, *'when a related plant-derived gene is used in intragenesis, some new combinations of genetic elements may arise that are not found in cisgenic and conventionally bred plants and these may present novel traits with novel hazards. Hazards can be identified which are specific for transgenic plants as the transgenes and their gene products can be obtained from any source including non-plant'*.

With respect to the alterations to the host genome, the EFSA scientific opinion (EFSA GMO Panel, 2012a) indicates that *'cisgenesis and intragenesis make use of the same transformation techniques as transgenesis'*, i.e. random insertion of a DNA fragment in the genome. Therefore, cisgenic, intragenic and transgenic plants do not cause different hazards with respect to, for example, the interruption of endogenous genes or the creation of new DNA junctions with the host genome. In this respect, the opinion (EFSA GMO Panel, 2012a) also reminds that *'the potential for 'random' changes to the genome caused by the insertion event is not limited to transgenesis, cisgenesis and intragenesis. Insertional mutagenesis is known to occur naturally through the random movement of the numerous mobile genetic elements such as transposons and retrotransposons, which are present in all plant genomes with varying prevalence'*. The EFSA scientific opinion (EFSA GMO Panel, 2012a) concludes that *'In summary, unintentional changes to the genome can arise during transgenesis, intragenesis and cisgenesis and result in a safety issue. However, the same mechanisms and types of unintentional genome changes occur during conventional breeding as it is well known that the plant genome is not a fixed entity'*.

With respect to the potential presence of non-plant DNA sequences, the opinion (EFSA GMO Panel, 2012a) indicates that *'by definition, cisgenic and intragenic plants must not contain vector backbone sequences of bacterial origin'*. It also specifies that *'cisgenesis with T-DNA borders only differs from cisgenesis by the presence of short T-DNA border sequences'* and that *'similar sequences can be found in different plant species'*.

With respect to the modification of the expression of the genes of the recipient, the EFSA opinion (EFSA GMO Panel, 2012a) describes that *'given the known plasticity of the plant genome, conventional breeding is expected to result in changes in genome-wide gene expression patterns in the progeny compared with the parental lines'*. With respect to the introduced sequences in cisgenesis, intragenesis and transgenesis, the EFSA scientific opinion (EFSA GMO Panel, 2012a) analyses the position effect of the insert, and states that *'the random integration of the cis/intra/transgene in plant genomes can influence the expression of genes or affect the functionality of regulatory elements around the site of integration. The inserted DNA may also have an enhancing or silencing effect on the expression of genes of the recipient'*. The opinion (EFSA GMO Panel, 2012a) analyses the issue of promoter functionality and explains that *'the promoter (its core, proximal and distal elements) is the major factor determining the level of gene expression'*, but also recognises that *'promoters of plant genes can have regulatory elements that are positioned several kilobases away from the transcriptional start site or located downstream or within the transcribed region'* and that *'the prediction of the gene expression levels is therefore currently not possible on theoretical grounds'*.

3.2.1.2. Is there new information available that could impact on the risks assessment of the plants and derived products included in the EFSA 2012 Opinion?

Although no applications for commercialisation of products derived from cisgenic or intragenic plants have been assessed by EFSA since the publication of the EFSA scientific opinion (EFSA GMO Panel, 2012a), the analysis of few cisgenic and intragenic plants, as defined in the EFSA 2012 Opinion, has been reported in scientific publications. For example, Holme and colleagues (Holme et al., 2012) reported the analysis of barley plants containing an extra copy of the barley phytase gene, including its own promoter and terminator sequences, which resulted in a 2.6–2.8-fold increase in phytase activity, a potentially interesting agronomical trait. The inserted cis-gene was flanked by 36 synthetic and 19 T-DNA border nucleotides, which were the only nucleotides from non-barley origin in the final plant. For this reason, the authors proposed to consider this plant cisgenic. A follow-up of this study (Holme et al., 2020) confirmed a 2.2-fold increase of phytase activity in grains under field conditions. This study also showed the potential of stacking different phytase cisgenes for the increase of phytase activity.

With respect to intragenesis, Miroshnichenko and colleagues (Miroshnichenko et al., 2020) reported the analysis of plants transformed with a hairpin construct consisting of an inverted repeat of a

fragment of potato *eIF4E1* gene separated by a functional intron from the potato *rbsc1* gene, under the control of potato *Lhca3* promoter and terminator sequences. This plant resulted in the partial silencing of the endogenous *eIF4E1* and *eIF4E2* genes and a strong level of viral resistance. The absence of plasmid backbone was demonstrated and the authors proposed to consider this plant intragenic. However, as this plant was obtained by conventional *Agrobacterium*-mediated transformation, the insert is flanked by T-DNA sequences. In order to avoid the insertion of T-DNA sequences, Almeraya and Sánchez-de-Jiménez (Almeraya and Sánchez-de-Jiménez, 2016) followed a biolistic transformation approach of the intragene construct only. In this case, the intragenic construct consisted of the coding sequence of the maize RuBisCo activase protein gene under the control of the RuBisCo promoter and terminator, which resulted in plants overexpressing the RuBisCo activase protein. Although the full characterisation of these plants was not reported, they should in principle only contain sequences of maize origin.

The data included in reports on the characterisation of cisgenic and intragenic plants published since 2012, including the ones discussed above, do not indicate additional hazards compared with those already identified in the 2012 EFSA Opinion (EFSA GMO Panel, 2012a). Moreover, no new data have been made available since the publication of the EFSA Opinion (EFSA GMO Panel, 2012a) that would challenge the conclusions raised in that document.

3.2.1.3. Are there new techniques/approaches developed since 2012 that could be used to obtain cisgenic/intragenic plants as defined in the 2012 Opinion?

The 2012 EFSA Opinion (EFSA GMO Panel, 2012a) specifies that *'cisgenesis and intragenesis make use of the same transformation techniques as transgenesis. Commonly used methods will result in random integration of the gene in the plant genome'* but also points out that *'in the future site-directed integration might become more widely available'*. Since 2012, the use of SDNs, and in particular of CRISPR approaches, has become widely adopted in public and private research laboratories. One of the possible uses of site-directed nucleases is the targeted integration of sequences, as anticipated in the EFSA scientific opinion (EFSA GMO Panel, 2012a), a use referred to as SDN-3. SDN-3 could be used to introduce a cisgene/intragene to a particular location of the genome to ensure, for example, its expression or to minimise the impact in the recipient genome, as already discussed in EFSA scientific opinion on SDN-3 (EFSA GMO Panel, 2012b). One of the most common techniques used in plant breeding by plant breeders is to introgress a trait (often controlled by a single gene, e.g. resistance to a pathogen) from a compatible wild species into a cultivated one by crossing and then successively back-crossing with the cultivated variety. The newly obtained variety carries the introgressed gene but also regions of DNA from the wild species on either side of the introgressed gene (linkage drag). Sometimes these regions carry genes that have negative effects on the phenotype of the new variety. It is then very difficult for the breeder to eliminate these genes which are genetically linked to the gene of interest. NGTs allow, by cisgenesis, to introgress only the gene of interest, without the adjacent regions, either in replacement of the orthologous gene, which could be considered an 'SDN-2-like' approach, or through an SDN-3 approach in a predefined region (landing pad) in the genome of the cultivated variety. Breeders may use this technique in the years to come rather than classical introgression by crossing and then back-crossing.

While no new applications for commercialisation of products derived from cisgenic or intragenic plants obtained through SDN-2 or SDN-3 techniques have been proposed since 2012, the very rapid development of NGTs in the recent years (Huang and Puchta, 2019) should facilitate the production of such plants. As already proposed in the 2012 Opinion (EFSA GMO Panel, 2012a), targeted insertion of the cisgenes or intragenes should facilitate their risk assessment.

3.2.1.4. If there are new techniques/approaches, what are the potential risks that may arise compared with those already covered in the 2012 opinion?

The EFSA GMO Panel published in 2012 its 'Scientific Opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function' (EFSA GMO Panel, 2012b), which concludes that *'the SDN- 3 technique can optimise the genomic environment for gene expression and minimize hazards associated with the disruption of genes and/or regulatory elements in the recipient genome'* that may arise when a cisgene, intragene or transgene is integrated into the genome. In addition, in 2020, the GMO Panel did not identify new hazards specifically linked to the genomic modification produced via SDN-2 compared with both SDN-3 and conventional breeding (EFSA GMO Panel, 2020). Therefore, the production of cisgenic plants by 'SDN-2-like' or SDN-3 approaches could minimise the hazards related to the introduced DNA and trait, as

these already exist in the gene pool of the breeder and, on this aspect, would be similar to plants obtained through classical breeding. In addition, use of SDN-2 like or SDN-3 approaches to produce cisgenic or intragenic plants would both minimise the potential alterations to the host genome observed during random integration through EGTs and avoid the possible linkage drag effect when using classical breeding techniques of gene introgression.

3.2.2. For cisgenesis/intragenesis plants and derived products not covered in the EFSA 2012 Opinion, the following assessment questions were addressed

3.2.2.1. What are the new plants and derived products that could be obtained using new approaches, in particular with the use of SDNs, that could give rise to cisgenic/ intragenic plants according to the definition given in the framework of this mandate[6]?

The new developments of site-directed modification of genomes offer the possibility to target the insertion of new sequences at specific loci in the genome. The introduction of sequences belonging to the gene pool of the species, other than a complete gene, was not envisaged and assessed in the 2012 EFSA Opinion (EFSA GMO Panel, 2012a), and is considered here for the first time. A fragment of genomic DNA that originates from a crossable species can be introduced in a plant as a single intact and continuous sequence. In addition, fragments of genomic DNA that originate from one or more crossable species can be combined and introduced in a plant.

When the insertion of such fragments occurs within a host gene, the end result leads to the formation of a rearranged gene and, as such, should be considered intragenic.

The possibility of targeting the insertion of such sequences or re-arranged sequences originating from one or multiple crossable species at specific loci opens up the possibility of modifying regulatory sequences, promoters, introns, terminators or coding regions, to alter the pattern/level of expression of a given gene, or the sequence, the characteristics (allergenicity, toxicity, nutritional values, etc.), the function, the stability or the subcellular localisation of the corresponding protein.

At this time, the GMO Panel is not aware of any cisgenic or intragenic plants and derived product achieved through NGTs that are close to commercialisation in the EU. For the new plants and derived products, as defined earlier, one publication reports a sequence inserted in a crossable species through NGT (Shi et al., 2017). The authors described a CRISPR/Cas-mediated approach to insert the maize GOS2 constitutive promoter in the 5′ UTR region of ARGOS8, a natural maize negative ethylene regulator. In another experiment, the authors replaced the native promoter with the GOS2 constitutive promoter. With both gene-edited variants, the *ARGOS8* transcript levels were significantly higher than those observed in wild-type plants, with consequent improved grain yield under drought stress conditions. In both circumstances, the GOS2 promoter was integrated by CRISPR/Cas, via homology-directed DNA repair (HDR), using one sgRNA in the promoter insertion, and two sgRNAs in the promoter swap. The report shows a clear example of introducing a sequence present in a crossable species to create allelic variation for enhancing crop drought tolerance.

3.2.2.2. What could be the risks that those plants and derived products could pose to humans, animals and the environment, compared with the risks associated with plants obtained by conventional plant breeding techniques and plants obtained with EGTs?

As introduced in Section 3.2.2.1, the range of cisgenic/intragenic potential plants and derived products covers different types of modifications, which may raise different potential hazards. The original or re-arranged sequences could be targeted to promoters or regulatory sequences of endogenous genes. In this case, the cisgenic/intragenic plant will not produce a newly expressed protein with respect to its conventional counterpart. The main potential hazard foreseen would be related to the modifications of the pattern and/or level of expression of the endogenous protein. The original or re-arranged sequences could also be targeted to the coding sequence of an endogenous gene with the aim of altering the produced protein. In this case, and depending on the modification introduced, the expressed protein could be considered as a newly expressed protein (NEP), and the potential hazards related to its expression will have to be evaluated on a case-by-case basis.

All these cisgenic and intragenic plants and derived products will be produced by targeted insertion/modification (e.g. via SDN3), which further minimises the potential hazards associated with the disruption of other genes and/or regulatory elements in the recipient genome.

3.3. Addressing ToR3: To determine whether the existing guidelines for risk assessment are applicable, fully or partially, and sufficient to cisgenic and intragenic plants

3.3.1. For cisgenesis/intragenesis plants and derived products already covered in the EFSA 2012 Opinion, the following assessment question was addressed

3.3.1.1. Are the conclusions of the EFSA 2012 opinion on the applicability of the existing guidelines still valid, taking into account the new guidelines published and the information made available since the publication of this opinion?

With respect to the MC aspects, the EFSA 2012 Opinion concluded that*'Considering that cisgenes are derived from the breeders' gene pool and contain their own promoter and terminator, the rationale for some elements of the molecular characterisation should be reconsidered (e.g. ORF searches within the insert are not needed as no new internal junctions are present)'*. Consequently, it concluded that *'an update of the existing guidance on risk assessment should be considered to introduce additional flexibility'*. As already discussed (in Section 3.2.1.2), no new data have been made available since the publication of the EFSA scientific opinion (EFSA GMO Panel, 2012a) that would challenge the conclusions raised in that document, and therefore, the conclusions on the applicability of the existing guidelines to the cisgenic/intragenic plants and derived products covered in the EFSA 2012 Opinion remain valid. Moreover, although the case-by-case principle is still present in the additional guidance and regulatory documents published since 2012, the additional flexibility recommended in the EFSA 2012 Opinion has not been introduced, and therefore, this recommendation also remains valid.

As anticipated in the 2012 Opinion, site-directed integration can now be achieved. In these cases, as discussed in the EFSA SDN-3 opinion, the risk associated with the integration in the genome can be minimised and fewer requirements may be needed for the assessment of these plants. Therefore, when these techniques are used to produce cisgenic or intragenic plants, the need for flexibility in the assessment would be even more justified.

With respect to the food and feed risk assessment, the EFSA scientific opinion (EFSA GMO Panel, 2012a) concludes that *'the general approach and all elements described in the guidance for risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011) is, at the present time, sufficient for the evaluation of cisgenic/intragenic plants and derived food and feed. However, for the assessment of food and feed products derived from cisgenic plants and intragenic plants it can be envisaged that, on a case-by-case basis, lesser amounts of event-specific data are needed'*. Subsequently, all elements of this food and feed safety assessment guidance have been implemented in Regulation 503/2013, together with additional requirements such as, for example, a mandatory 90-day feeding study in rats. Also, the assessment of potential allergenicity of newly expressed proteins and of the whole GM plant has been further developed (EFSA GMO Panel, 2017). In the case of cisgenic/transgenic plants where it is well documented that the donor plant has a history of safe consumption as food and feed, certain parts of the comparative analysis, toxicity, allergenicity or nutritional assessment may not be necessary. Thus, the GMO Panel confirms that the general approach and all elements described in the aforementioned guidance documents and implementing regulation are, at present, sufficient for the evaluation of cisgenic/intragenic plants and derived food and feed. The GMO Panel also considers that the conclusions of the EFSA 2012 Opinion for the assessment of food and feed products derived from cisgenic and intragenic plants remain unchanged.

With respect to the environmental risk assessment, the EFSA scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis (EFSA GMO Panel, 2012a) confirmed that all elements described in its guidance on the environmental risk assessment of GM plants (EFSA GMO Panel, 2010) can apply to cisgenic/intragenic plants, and the relevance of applying specific elements of the guidance is defined on a case-by-case basis. The GMO Panel confirms that the conclusions of the EFSA Scientific Opinion (EFSA GMO Panel, 2012a) remain valid. Moreover, the GMO Panel has published guidance on the agronomic and phenotypic characterisation of GMPs (EFSA GMO Panel, 2015) that are applicable for the parts of the comparative assessment that on a case-by-case basis will be necessary for the assessment of cisgenic and intragenic plants.

3.3.2. For cisgenesis/intragenesis plants and derived products not covered in the EFSA 2012 Opinion, the following assessment question was addressed

3.3.2.1. Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient for these new plants and derived products?

As discussed in Section 3.2.2.1, the use of NGTs to introduce original or re-arranged sequences of a crossable species at specific *loci* opens up the possibility to modify regulatory sequences, promoters, introns, terminators or coding regions to alter the pattern/level of expression of a given gene, or the sequence, the characteristics (allergenicity, toxicity, nutritional values, etc.), the function, the stability or the cellular compartmentation of the corresponding protein. Therefore, the range of plant traits and plant-derived products that can be obtained is wider than the one proposed in the 2012 Opinion, and the requirements needed for their risk assessment may vary case by case. The need for flexibility in the risk assessment would be particularly relevant.

If the original or re-arranged sequence of a crossable species is targeted to promoters or regulatory sequences of endogenous genes, the plant will not produce a newly expressed protein with respect to its conventional counterpart and, therefore, the requirements that aim to assess any potential hazards may not be relevant. However, a main potential hazard foreseen in these cases will be related to the modifications of the expression pattern of the endogenous protein. The functions of the endogenous protein and the fact that the modification of the expression remains in the range or is outside what exists in the conventional varieties will be considered to determine the possible need for a specific risk assessment of this potential hazard.

In cases in which the original or re-arranged sequence of a crossable species is targeted to the coding sequence of an endogenous gene with the aim of altering the produced protein, depending on the differences introduced and consequences on its initial function (e.g. change of enzymatic activity, substrate specificity, stability of the protein), the expressed protein could be considered as a newly expressed protein, and the hazards related to its expression will have to be evaluated on a case-by-case basis.

A particular case would be the use of the targeted introduction/modification of a gene to obtain an allele already existing within the species. As explained in Section 3.2.1.3, this introgression of alleles is typically done by conventional breeding but has limitations that could be solved by the targeted insertion of a sequence by cisgenesis (or by targeted modification using, e.g. SDN-2 or PE). These plants would not present new hazards compared with conventional plants, and therefore most, if not all, risk assessment requirements would not be relevant.

As explained in Section 3.2.2.2, all these cisgene and intragene plants and derived products will be produced by targeted insertion/modification (e.g. via SDN-3) to the endogenous gene to be modified. Therefore, they will not present hazards associated with the disruption of other genes and/or regulatory elements in the recipient genome, and the requirements of the Regulation (EU) No 503/2013[12] and guidelines that aim at assessing these unintended effects will not be relevant. In addition, as an original sequence from a crossable species will not present internal new ORFs, only the new ORF at the junctions of the integration should be assessed.

The environmental risk assessment of cisgene/intragene products will, as per Regulation (EU) No 503/2013,[12] be assessed on a case-by-case basis. Considering the examples above (e.g. a sequence inserted in a promoter/UTR or in the coding sequence), the GMO Panel considers that the existing guidelines are applicable and sufficient. On a case-by-case basis, a lesser amount of data might be needed for the environmental risk assessment of cisgenic or intragenic plants obtained through NGTs.

For the three areas of risk assessment, the existing guidelines will be partially applicable and the parts of the guidance not applicable will differ on a case-by-case basis. Therefore, flexibility may be needed in the risk assessment to adapt it to each particular plant and product under assessment. The EFSA GMO Panel is of the opinion that the potential hazards of cisgenesis/intragenesis plants produced in the future would be addressed by the present guidance. Therefore, the present guidelines could be considered as sufficient and partially applicable for the assessment of cisgenesis/intragenesis plants and products obtained through NGTs.

[12] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L 157, 8.6.2013, pp. 1–48.

3.4. Addressing ToR 4: In case existing guidelines for risk assessment are considered not applicable, partially applicable or not sufficient, to identify on which aspects existing guidelines should be updated, adapted or complemented

3.4.1. Which aspect (if any) of existing guidelines should be updated, adapted or complemented?

As mentioned in Section 3.3.1.1, for cisgenic/transgenic plants for which it is well documented that the donor plant has a history of safe consumption as food and feed, certain parts of the comparative analysis, toxicity, allergenicity or nutritional assessment may not be necessary. Also, it can be expected that the increased target precision of novel methods in achieving intended effects compared with established genetic techniques will progressively allow more flexibility in the need for data aimed at the identification of unintended effects. In this context, for example, some mandatory requirements such as the 90-day feeding studies in the absence of any hazard/risk hypothesis may be revisited.

As explained in sections addressing ToR3, due to the possibility to target the integration of a DNA sequence, the range of cisgenic and intragenic plants and derived products might increase noticeably. On the one hand, plants could be produced in which the cisgene, corresponding to an already existing allele in the genetic pool of the breeder, would be targeted to the corresponding endogenous gene (through an SDN2-like strategy). Such plants could be considered as safe as plants where the gene of interest would have been introgressed via classical breeding. On the other hand, hazards associated with an intragenic plant, where the targeted integration of an intragenic sequence could significantly modify the function and expression of an endogenous gene, would potentially be comparable with the ones associated with a transgenic plant obtained through SDN3.

Between these two opposite cases there is a continuum, and the requirements needed for the risk assessment of these plants may vary. Section 3.3.2.1 provides examples of plants and products for which particular risk assessment requirements will not be relevant.

4. Conclusions

Following a request of the European Commission, EFSA evaluated whether the conclusions of the EFSA scientific opinion (EFSA GMO Panel, 2012a) on cisgenesis and intragenesis remain applicable, in light of the development of NGTs. The EFSA GMO Panel was asked to (1) identify potential risks that plants obtained by cisgenic and intragenic approaches could pose for humans, animals and the environment; (2) compare the above-mentioned risks with those associated with plants obtained by conventional plant breeding techniques and plants obtained with EGTs; (3) determine whether the existing guidelines for risk assessment are applicable, fully or partially, and sufficient to cisgenic and intragenic plants; (4) in case existing guidelines for risk assessment are considered not applicable, partially applicable or not sufficient, to identify on which aspects existing guidelines should be updated, adapted or complemented.

The EFSA GMO Panel concludes that:

- There are no new data since the publication of the 2012 EFSA opinion that would challenge the conclusions raised in that document (EFSA GMO Panel, 2012). The conclusions of the EFSA 2012 scientific opinion remain valid. The EFSA GMO Panel reiterates that with respect to the source of DNA and the safety of the gene product, *'the hazards arising from the use of a related plant-derived gene by cisgenesis are similar to those from conventional plant breeding, whereas additional hazards may arise for intragenic plants'*. Furthermore, the EFSA GMO Panel considers that *'cisgenesis and intragenesis make use of the same transformation techniques as transgenesis'*, and therefore, with respect to the alterations to the host genome, cisgenic, intragenic and transgenic plants obtained by random insertion do not cause different hazards. NGTs can allow, by cisgenesis, the introgression of a gene of interest without its adjacent regions (linkage drag), either in replacement of the orthologous gene ('SDN-2-like' approach), or in a predefined region in the genome of the cultivated variety (SDN-3 approach). Cisgenic/intragenic plants by 'SDN-2-like' or SDN-3 approaches could:
    - minimise the hazards related to the introduced DNA and trait, as these already exist in the gene pool of the breeder and, on this aspect, would be similar to plants obtained through classical breeding,

- minimise the potential alterations to the host genome observed during random integration through EGTs (i.e. disruption of other genes and/or regulatory elements; formation of new ORFs),
- avoid the possible linkage drag effect when using classical breeding techniques of gene introgression.

The use of NGTs allows the generation of new cisgenic/intragenic plants and derived products not covered in the EFSA 2012 scientific opinion. For these plants and derived products, the EFSA GMO Panel concludes that:

- If the cisgenic/intragenic sequence is targeted to promoters or regulatory sequences of endogenous genes, the cisgenic/intragenic plant will not produce a newly expressed protein with respect to its conventional counterpart and the main potential hazard would be related to the modifications of the pattern and/or level of expression of the endogenous protein.
- If the cisgenic/intragenic sequence is targeted to the coding sequence of an endogenous gene, the expressed protein could be considered as a newly expressed protein (NEP), depending on the modification introduced, and the potential hazards related to its expression will have to be evaluated on a case-by-case basis.
- With respect to the environmental risk assessment, all elements described in the current guidelines are sufficient for cisgenic/intragenic plants.
- The existing guidelines will be partially applicable, as on a case-by-case basis some requirements may not be needed for the risk assessment. Therefore, flexibility may be needed in the risk assessment to adapt it to each particular plant and product under assessment.
- The present guidelines could be considered as sufficient and partially applicable for the assessment of cisgenesis/intragenesis plants and products obtained through NGTs.

Documentation as provided to EFSA

- Request for an updated scientific opinion on plants developed through cisgenesis and intragenesis. June 2021. Submitted by the European Commission (Directorate-General for Health and Food Safety);
- Acknowledgement of receipt of the mandate. June 2021. Submitted by the European Food Safety Authority.

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Abbreviations

CRISPR	clustered regularly interspaced short palindromic repeats
DSB	double-strand break
EGT	established genomic techniques
ERA	environmental risk assessment
FF	food and feed
GM	genetically modified
GMO	genetically modified organism
HDR	homology-directed repair
MC	molecular characterisation
NGT	new genomic techniques
NEP	newly expressed protein
NHEJ	non-homologous end-joining
ODM	oligonucleotide-directed mutagenesis
ORF	open reading frame
PE	prime editing
PEG	polyethylene glycol
RT	reverse transcriptase
SSB	single-strand break
SDN	site-directed nuclease
TALEN	transcription activator-like effector nuclease
ZFN	zinc finger nuclease

Glossary

Breeder's gene pool	The sources of genes available for conventional plant breeding
Cisgenesis	Genetic modifications involving genetic material obtained from outside the host organism and transferred to the host using various delivery strategies; the incorporated sequences contain an exact copy of sequences already present in the species or in a sexually compatible species
CRISPR	Clustered regularly interspaced short palindromic repeats, a component of bacterial immunity used to recognise and protect against viruses. It is commonly used as a shorthand for the CRISPR/Cas9 system
Double-strand break (DSB)	The mechanical, chemical or enzymatical cleavage of both strands of the DNA
Exogenous DNA	DNA originating outside the plant being modified which can be introduced naturally or by technological intervention
Genome	The haploid set of chromosomes of a given organism which contains all the genetic information necessary for its maintenance
Genomic mutation	Permanent change of the nucleotide sequence in the genome of a given organism
Homology-directed repair (HDR)	A molecular mechanism which allows the repair of DNA double-strand breaks using a homologous sequence of DNA as template
Intragenesis	Genetic modifications involving genetic material obtained from outside the host organism and transferred to the host using various delivery strategies; the incorporated sequences contain a re-arranged copy of sequences already present in the species or in a sexually compatible species
Non-homologous end joining (NHEJ)	A molecular mechanism which allows the repair of DNA double-strand breaks when a homologous sequence of DNA is not available. In some cases, NHEJ results in genomic mutations, usually insertion or deletion of fragments of DNA
Oligonucleotide	A stretch of nucleic acid consisting of a relatively low number of nucleotides
Ribonucleoprotein	A macromolecule complex composed of protein and RNA polymers
Sequence	Usually refers to the linear order of nucleotides in DNA and RNA or amino acids in proteins
Site-directed mutagenesis	A molecular biology method that is used to make specific and intentional changes (insertions, deletions and substitutions) to a genomic locus
Site-directed nuclease (SDN)	An enzyme which recognises a specific sequence and cleaves the DNA usually creating a double-strand break
Transformation	The process by which a prokaryotic or eukaryotic cell takes up exogenous DNA
Transgenesis	The process of introducing gene(s) from a different, sexually incompatible, species into the genome of a given cell and the propagation of such gene (s) thereafter

Appendix A – Protocol supporting the updated scientific opinion on plants developed through cisgenesis and intragenesis

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Approved by:	Nils Rostoks (Chair of the ad hoc WG on Cisgenesis/Intragenesis and GMO Panel member)

A.1. Introduction

A.1.1. Background

Over the last 10 years, following the requests by the European Commission, the European Food Safety Authority (EFSA) has issued scientific opinions on plants obtained through certain new genomic techniques (NGTs). Among these, EFSA has published two opinions, one on site-directed nuclease (SDN)-1, SDN-2 and oligonucleotide directed mutagenesis (ODM),[13] and another on cisgenesis and intragenesis,[14] After the publication of the EFSA opinion on cisgenesis and intragenesis, an opinion on the safety assessment of plants developed through SDN-3 was also published.[15] In that document, EFSA was also envisaging the possibility to develop cisgenic and intragenic plants using SDN-3 techniques. These scientific opinions have focused on the potential risks associated with the new techniques, compared to conventional breeding techniques and established genomic techniques (EGTs),[16] and on the applicability of existing risk assessment guidance to plants produced with the NGTs under consideration.

The main conclusions of the above-mentioned opinions, relevant to the present mandate, are the following:

- Plants produced by SDN-1, SDN-2 and ODM techniques have no new hazards compared to conventionally bred and transgenic plants.
- Similar hazards can be associated with cisgenic and conventionally bred plants, while novel hazards can be associated with intragenic and transgenic plants.
- The existing EFSA Guidance documents are sufficient and applicable in case of plants produced by cisgenesis and intragenesis, and sufficient and partially applicable in case of plants produced by SDN-1, SDN-2 and ODM techniques.
- There is a need for flexibility in the data requirements for the risk assessment, as on a case-by-case lesser amounts of data might be needed.
- The SDN-3 opinion concludes that SDN-3 techniques can be used for cisgenesis/intragenesis.

While the scientific opinion on SDN-1, SDN-2 and ODM is very recent, dating from 2020, the cisgenesis/intragenesis and SDN-3 scientific opinions date from 2012. They take into account the techniques available at that time, notably *Agrobacterium*-mediated transformation and direct gene transfer, although several of the considerations therein are not linked to the use of a specific technique. Since 2012, several developments in terms of scientific knowledge and technologies have taken place. In particular, genome editing techniques, including SDN, can now also be used, alone or in combination with other techniques, to produce cisgenic and intragenic organisms, in addition to EGTs.

Against this background, the Commission would like EFSA to confirm whether the considerations and conclusions of EFSA scientific opinion on cisgenesis/intragenesis of 2012 are still applicable.

[13] EFSA GMO Panel. Applicability of the EFSA Opinion on site-directed nucleases type 3 for the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide-directed mutagenesis. EFSA Journal 2020;18(11):6299, 14 pp. https://doi.org/10.2903/j.efsa.2020.6299

[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.2012.2561

[15] EFSA GMO Panel. Scientific opinion addressing the safety assessment of plants developed using ZFN-3 and other SDNs with similar function. EFSA Journal 2012;10(10):2943, 31 pp. https://doi.org/10.2903/j.efsa.2012.2943

[16] For the purpose of this document, established genomic techniques (EGTs) are those genomic techniques developed prior to 2001, when the existing GMO legislation was adopted, and used to obtain the GMOs authorised in the EU so far. EGTs include techniques such as Agrobacterium-mediated transformation and direct gene transfer.

A.1.2. Terms of Reference of the mandate as provided by the requestor

Building on previous work of EFSA, notably the above-mentioned scientific opinions on SDN techniques and cisgenesis/intragenesis, the European Commission asks EFSA, in accordance with Article 29 of Regulation (EC) No 178/2002, to provide an updated scientific opinion on the safety and the risk assessment of plants developed through cisgenesis and intragenesis.[17] In particular, EFSA is requested to consider the current state of the art and available knowledge on NGTs and:

1) Identify potential risks that plants obtained by cisgenic and intragenic approaches could pose for humans, animals and the environment.
2) Compare the above-mentioned risks with those associated with plants obtained by conventional plant breeding techniques and plants obtained with EGTs.
3) Determine whether the existing guidelines for risk assessment are applicable, fully or partially, and sufficient[18] to cisgenic and intragenic plants.
4) In case existing guidelines for risk assessment are considered not applicable, partially applicable or not sufficient, to identify on which aspects existing guidelines should be updated, adapted or complemented.

A.1.3. Scope of this protocol

This document represents the protocol for the scientific assessment of new evidence on plants developed through cisgenesis and intragenesis, which will be used to clarify the current state of the art and available knowledge on New Genomic Techniques (NGTs) and to update, if needed, the GMO Panel conclusions of EFSA (EFSA, 2012). This protocol has been developed with the aim of defining the methods for collecting data, appraising the relevant evidence and analysing and integrating the evidence in light of the identified uncertainties.

The protocol is based on the recommendations for protocol development for non-application mandates given by a Working Group of EFSA's Scientific Committee (EFSA, 2020).

In line with EFSA (EFSA, 2020) on protocol development for non-application mandates, EFSA developed this protocol to clarify the interpretation of the ToRs of the mandate.

The protocol consists of:

1) A problem formulation that outlines what the assessment aims to address and thus the objectives of the assessment.
2) An analysis plan that outlines which methods will be used to address the problem (i.e. how the assessment will be carried out).

A.2. Problem formulation

The ToRs were translated into scientifically answerable assessment questions in line with the EFSA draft document on protocol development for non-application mandates (Step 1.1 and Step 1.2; EFSA, 2020).

The EFSA opinion on cisgenesis and intragenesis (EFSA, 2012) addressed the safety of plants modified through cisgenesis and intragenesis as defined by a working group of EU Member States' experts on new techniques. This definition[19] limited cisgenesis/transgenesis approaches to the introduction of (protein-coding) genes from a crossable species. The new developments of site-

[17] For the purpose of this mandate, the following definitions apply: cisgenesis and intragenesis are genetic modifications involving genetic material obtained from outside the host organism and transferred to the host using various delivery strategies; the incorporated sequences contain an exact copy (cisgenesis) or a re-arranged copy (intragenesis) of sequences already present in the species or in a sexually compatible species. (Adapted from 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 978–92–76-24,696-1, https://doi.org/10.2760/710056), JRC121847.

[18] In the context of this mandate, 'applicable' means 'that can be used for the purpose', 'fully applicable' means 'that can be used in full', 'partially applicable' means 'that can be used only in part' and 'sufficient' means 'that does not need to be complemented'.

[19] 'Cisgenesis is the genetic modification of a recipient organism with a gene from a crossable –sexually compatible – organism (same species or closely related species). This gene includes its introns and is flanked by its native promoter and terminator in the normal sense orientation…'; '…Intragenesis is a genetic modification of a recipient organism that leads to a combination of different gene fragments from donor organism(s) of the same or a sexually compatible species as the recipient. These may be arranged in a sense or antisense orientation compared to their orientation in the donor organism. Intragenesis involves the insertion of a reorganised, full or partial coding region of a gene frequently combined with another promoter and/or terminator from a gene of the same species or a crossable species.' (as reported in EFSA, 2012).

directed modification of genomes offer the possibility to target the insertion of new sequences or introduce changes at specific loci in the genome. When these sequences/changes are already present in a crossable species, this could also be considered as cisgenic/intragenic modification in light of the definition given in the framework of this mandate.[17] Because these two definitions cover different products that can raise different type of risks, in delivering its opinion EFSA will address cisgenesis/intragenesis products already covered in the EFSA 2012 opinion (EFSA, 2012) (i.e. that aim at introducing new protein-coding genes into plants) and new potential cisgenesis/intragenesis products not covered in that opinion (i.e. that aim at introducing sequences not limited to complete protein-coding genes) separately. Therefore, Terms of Reference 1, 2 and 3 were translated into assessment question(s) specific for the two types of products.

A.2.1. Assessment questions

ToR 1: Identify potential risks that plants obtained by cisgenic and intragenic approaches could pose for humans, animals and the environment.

Tor 2: Compare the above-mentioned risks with those associated with plants obtained by conventional plant breeding techniques and plants obtained with EGTs.

1- For cisgenesis/intragenesis plants and derived products already covered in the EFSA 2012 opinion, the following questions were addressed:

AQ1. What are the risks that cisgenic/intragenic plants could pose to humans, animals and the environment, that were identified in the 2012 cisgenesis Opinion?

AQ2. Is there new information available that could impact on the risk assessment of the plants and derived products included in the EFSA 2012 Opinion?

AQ3. Are there new techniques/approaches developed since 2012 that could be used to obtain cisgenic/intragenic plants as defined in the 2012 Opinion?

AQ4. If there are new techniques/approaches, what are the potential risks that may arise compared with those already covered in the 2012 Opinion?

2- For cisgenesis/intragenesis plants and derived products not covered in the EFSA 2012 Opinion, the following questions were addressed:

AQ1. What are the new plants and derived products that could be obtained using new approaches, in particular with the use of SDNs, that could give rise to cisgenic/intragenic plants according to the definition given in the framework of this mandate[17]?

AQ2. What could be the risks that those plants and derived products could pose to humans, animals and the environment, compared with the risks associated with plants obtained by conventional plant breeding techniques and plants obtained with EGTs?

ToR 3: Determine whether the existing guidelines for risk assessment are applicable, fully or partially, and sufficient to cisgenic and intragenic plants.

1- For cisgenesis/intragenesis plants and derived products already covered in the EFSA 2012 Opinion, the following question was addressed:

AQ1. Are the conclusions of the EFSA 2012 Opinion on the applicability of the existing guidelines still valid, taking into account the new guidelines published and the information made available since the publication of this opinion?

2- For cisgenesis/intragenesis plants and derived products not covered in the EFSA 2012 opinion, the following question was addressed:

AQ1. Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient for these new plants and derived products?

ToR 4: In case existing guidelines for risk assessment are considered not applicable, partially applicable or not sufficient, to identify on which aspects existing guidelines should be updated, adapted or complemented.

AQ1. Which aspect (if any) of existing guidelines should be updated, adapted or complemented?

A.3. Methods foreseen for undertaking the assessment

The assessment will be based on the evidence retrieved from the literature via a literature search and from the expert judgement (Table A.1).

Table A.1: Approaches foreseen for answering the assessment questions

Assessment questions(1)	Approaches foreseen for answering the subquestion
AQs for ToR1 and ToR2; AQ1 for ToR3	Using evidence from the scientific literature and directly submitted to EFSA
AQ2 for ToR3; AQ1 for ToR4	Using expert judgement

(1): Already defined in the problem formulation section above.

A.3.1. Using evidence from the scientific literature

A.3.1.1. Eligibility criteria for study selection

Eligibility criteria will be used to assess the relevance of evidence for inclusion in the review.

Table A.2: Eligibility criteria to establish the relevance of evidence pertaining to study characteristics

Key elements/concepts		Eligibility criteria
Study design/type	In	Publications about genetically modified plants, obtained with the techniques of cisgenesis, intragenesis
Exposure/Intervention	In	Transfer of genetic material already present in the species or in a sexually compatible species, for Agro food feed applications
	Out	Chemical, medical, biofuel applications
Population (study subjects)/Receptor	In	Plants, major crops used for feed and food
	Out	Humans, animals, microorganisms
Outcome	In	Genetic modification obtained with cisgenesis, intragenesis
	Out	Genetic modification obtained with other technologies
Study location	In	All

Table A.3: Eligibility criteria to establish the relevance of evidence pertaining to record characteristics

Key elements/ concepts	Eligibility criteria	
Time	In	Studies published since 2011
	Out	Studies published before 2011
Language	In	Studies in English
	Out	Studies in other languages
Reporting format	In	The evidence presents original/primary data, or reports on a relevant case–specific problem formulation,
Publication type	In	– Primary research studies (i.e. studies generating new data) – Reviews – Conference abstracts or posters if they contain primary data – Patents
	Out	– Letters to the editor and editorials – Expert opinions – PhD theses and dissertations as primary data are expected to have been published

A.3.1.2. Sources of evidence

For the review of scientific literature, electronic bibliographic databases listed in Table A.4 and Table A.5 will be searched to identify relevant studies.

Table A.4: Electronic bibliographic databases that will be used to search for relevant publications

Database	Platform
Scopus	Scopus.com
BIOSIS	Web of Science
CAB Abstracts	

Database	Platform
Current Contents Connect	
FSTA	
MEDLINE	
Web of Science Core Collection	

Table A.5: Electronic bibliographic databases that will be used to search for relevant patents

Database	URL
ESPACENET	https://worldwide.espacenet.com/patent/
Google patents	https://patents.google.com/

A.3.1.3. Search strategy

The Cisgenesis WG, with the support of an EFSA information specialist, designed literature searches to identify relevant publications and patents.

The search strings to retrieve relevant studies are reported in Tables A.6–A.7. They are structured as a combination of four or five searches, using Boolean operators. Keywords have been selected with the help of the members of the WG, consulting thesaurus (e.g. CAB Thesaurus) and dictionaries.

Table A.6: Search strings. Scopus

Set	Query	Results
#5	#3 AND #4	407
#4	PUBYEAR >2010	32,650,756
#3	#1 AND #2	681
#2	TITLE-ABS-KEY(cisgenes* OR cisgenic OR intragenes* OR intragenic)	6,749
#1	TITLE-ABS-KEY (crop OR crops OR plant OR plants OR oilseed* OR grain OR grains OR fruit OR fruits OR vegetable* OR cereal* OR maize OR "zea mays" OR "z mays" OR corn OR sweetcorn* OR barley OR "Hordeum vulgare" OR "H vulgare" OR rye OR oat OR oats OR avena OR rice OR oryza OR "O sativa" OR rye OR "Secale cereale" OR sorghum OR triticale OR wheat OR "Triticum aestivum" OR buckwheat OR soyabean* OR soybean* OR "Glycine max" OR "G max" OR soja OR soya OR spelt OR spelta OR bean OR beans OR faba* OR chickpea* OR "Cicer arietrinum" OR "C arietrinum" OR legume* OR lentil* OR "Lens culinaris" OR "L culinaris" OR pea OR peas OR "Pisum sativum" OR "P sativum" OR alfalfa OR "Medicago sativa" OR "M sativa" OR apple OR apples OR "malus domestica" OR "m domestica" OR pear OR pears OR "Pyrus communis" OR "P communis" OR quince OR quinces OR "Cydonia oblonga" OR "C oblonga" OR peach OR peaches OR prunus OR nectarine OR nectarines OR apricot OR apricots OR cherry OR cherries OR plum OR plums OR sloe OR sloes OR medlar* OR "Mespilus germanica" OR "M germanica" OR fig. OR figs. OR "Ficus carica" OR "F carica" OR kiwi OR kiwis OR kiwifruit* OR "Actinidia chinensis" OR "Actinidia deliciosa" OR "A chinensis" OR "A deliciosa" OR avocado OR avocados OR "Persea americana" OR "P americana" OR banana OR bananas OR musa OR orange OR oranges OR citrus OR satsuma OR satsumas OR clementine OR clementines OR mandarin OR mandarins OR lemon OR lemons OR lime OR limes OR grapefruit* OR grape OR grapes OR grapevine OR "Vitis vinifera" OR "v vinifera" OR strawberr* OR "Fragaria ananassa" OR "F ananassa" OR curcubit OR melon OR melons OR cantaloupe* OR "cucumis melo" OR "C melo" OR watermelon OR watermelons OR "Citrullus lanatus" OR "C lanatus" OR olive* OR "Olea europaea" OR "O europaea" OR aubergine* OR eggplant* OR "solanum melongena" OR "S melongena" OR celeriac* OR apium OR cucurbit* OR cucumber* OR pumpkin* OR squash* OR beetroot* OR beet OR beets OR "Red Beet*" OR carrot* OR "Daucus carota" OR "D carota" OR pepper* OR "Capsicum annuum" OR "C annuum" OR potato* OR "Solanum tuberosum" OR "S tuberosum" OR tomato* OR "Solanum lycopersicum" OR "S lycopersicum" OR brassica OR "Brussels sprout*" OR broccoli OR cabbage* OR cauliflower* OR kale OR lettuce* OR "Lactuca sativa" OR "L sativa" OR spinach* OR "Spinacia oleracea" OR "S oleracea" OR turnip* OR rape OR canola OR colza OR sunflower* OR "Helianthus annuus" OR "H annuus" OR sugarbeet* OR "sugar beet*" OR "beta vulgaris" OR sugarcane* OR "Saccharum officinarum" OR "S officinarum" OR squash*)	4,809,355

Date of the search: 08-09-2021.

Table A.7: Search strings. Web of Science Platform
Collections = BCI, CABI, CCC, FSTA, MEDLINE, WOS

Set	Query	Results
#4	(#1 AND #2) AND PY = (2011 OR 2012 OR 2013 OR 2014 OR 2015 OR 2016 OR 2017 OR 2018 OR 2019 OR 2020 OR 2021)	625
#3	#1 AND #2	1,700
#2	TS = (cisgenes* OR cisgenic OR intragenes* OR intragenic)	8,506
#1	TS = (Crop OR Crops OR Plant OR Plants OR Oilseed* OR Grain OR Grains OR Fruit OR Fruits OR Vegetable* OR cereal* OR maize OR "zea mays" OR "z mays" OR Corn OR Sweetcorn* OR barley OR "Hordeum vulgare" OR "H vulgare" OR rye OR oat OR oats OR Avena OR Rice OR oryza OR "O sativa" OR Rye OR "Secale cereale" OR sorghum OR Triticale OR Wheat OR "Triticum aestivum" OR buckwheat OR soyabean* OR soybean* OR "Glycine max" OR "G max" OR Soja OR Soya OR Spelt OR Spelta OR Bean OR Beans OR Faba* OR Chickpea* OR "Cicer arietrinum" OR "C arietrinum" OR Legume* OR Lentil* OR "Lens culinaris" OR "L culinaris" OR Pea OR Peas OR "Pisum sativum" OR "P sativum" OR Alfalfa OR "Medicago sativa" OR "M sativa" OR Apple OR Apples OR "malus domestica" OR "m domestica" OR Pear OR Pears OR "Pyrus communis" OR "P communis" OR Quince OR Quinces OR "Cydonia oblonga" OR "C oblonga" OR Peach OR Peaches OR Prunus OR Nectarine OR Nectarines OR Apricot OR Apricots OR Cherry OR Cherries OR Plum OR Plums OR Sloe OR Sloes OR Medlar* OR "Mespilus germanica" OR "M germanica" OR Fig. OR Figs. OR "Ficus carica" OR "F carica" OR Kiwi OR Kiwis OR Kiwifruit* OR "Actinidia chinensis" OR "Actinidia deliciosa" OR "A chinensis" OR "A deliciosa" OR Avocado OR Avocados OR "Persea americana" OR "P americana" OR Banana OR Bananas OR Musa OR Orange OR Oranges OR Citrus OR Satsuma OR Satsumas OR Clementine OR Clementines OR Mandarin OR Mandarins OR Lemon OR Lemons OR Lime OR Limes OR Grapefruit* OR Grape OR Grapes OR grapevine OR "Vitis vinifera" OR "v vinifera" OR Strawberr* OR "Fragaria ananassa" OR "F ananassa" OR Curcubit OR Melon OR Melons OR Cantaloupe* OR "Cucumis melo" OR "C melo" OR Watermelon OR Watermelons OR "Citrullus lanatus" OR "C lanatus" OR Olive* OR "Olea europaea" OR "O europaea" OR aubergine* OR eggplant* OR "solanum melongena" OR "S melongena" OR Celeriac* OR Apium OR Cucurbit* OR Cucumber* OR Pumpkin* OR Squash* OR Beetroot* OR Beet OR Beets OR "Red Beet*" OR Carrot* OR "Daucus carota" OR "D carota" OR Pepper* OR "Capsicum annuum" OR "C annuum" OR Potato* OR "Solanum tuberosum" OR "S tuberosum" OR Tomato* OR "Solanum lycopersicum" OR "S lycopersicum" OR Brassica OR "Brussels sprout*" OR Broccoli OR Cabbage* OR Cauliflower* OR Kale OR Lettuce* OR "Lactuca sativa" OR "L sativa" OR Spinach* OR "Spinacia oleracea" OR "S oleracea" OR Turnip* OR Rape OR Canola OR Colza OR sunflower* OR "Helianthus annuus" OR "H annuus" OR Sugarbeet* OR "sugar beet*" OR "beta vulgaris" OR Sugarcane* OR "Saccharum officinarum" OR "S officinarum" OR Squash*)	11,891,457

Date of the search 08-09-2021.

Tables A.8 and A.9 report the searches for patents, using Espacenet and Google patents databases, respectively.

Table A.8: Search Strings. ESPACENET

Search string	Results
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "crop?" OR ctxt any "plant?" OR ctxt any "oilseed*" OR ctxt any "grain?" OR ctxt any "fruit?" OR ctxt any "vegetable*")	53
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "cereal*" OR ctxt any "maize" OR ctxt all "zea mays" OR ctxt all "z mays" OR ctxt any "corn" OR ctxt any "sweetcorn*")	9
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "barley" OR ctxt all "Hordeum vulgare" OR ctxt all "H vulgare" OR ctxt any "rye" OR ctxt any "oat?" OR Ctxt any "avena")	6
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic")AND (ctxt any "rice" OR ctxt any "oryza" OR ctxt all "O sativa" OR ctxt any "rye" OR ctxt all "Secale cereale" OR ctxt any "sorghum")	7

Search string	Results
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "triticale" OR ctxt any "wheat" OR ctxt all "Triticum aestivum" OR ctxt any "buckwheat" OR ctxt any "soyabean*" OR ctxt any "soybean*")	15
(ctxt all "cisgenes*" OR ctxt all "cisgenic" OR ctxt all "intragenes*" OR ctxt all "intragenic") AND (ctxt all "Glycine max" OR ctxt all "G max" OR ctxt any "soja" OR ctxt any "soya" OR ctxt any "spelt?" OR ctxt any "bean?")	5
(ctxt all "cisgenes*" OR ctxt all "cisgenic" OR ctxt all "intragenes*" OR ctxt all "intragenic") AND (ctxt any "faba*" OR ctxt any "chickpea*" OR ctxt all "Cicer arietrinum" OR ctxt all "C arietrinum" OR ctxt any "legume*" OR ctxt any "lentil*")	4
(ctxt any "cisgenes" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt all "Lens culinaris" OR ctxt all "L culinaris" OR ctxt any "pea" OR nftxt = "Pisum sativum" OR nftxt = "P sativum" OR nftxt = "alfalfa")	32
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any peas OR ctxt all "Medicago sativa" OR cxt all "M sativa" OR ctxt any "apple?" OR ctxt all "malus domestica" OR ctxt all "m domestica")	4
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (OR ctxt any "pear?" OR ctxt all "Pyrus communis" OR ctxt all "P communis" OR ctxt any "quince?" OR ctxt all "Cydonia oblonga" OR ctxt all "C oblonga")	2
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "peach*" OR ctxt any "prunus" OR ctxt any "nectarine?" OR ctxt any "apricot?" OR ctxt any "cherry" OR ctxt any "cherries")	2
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "plum?" OR ctxt any "sloe?" OR ctxt any "medlar*" OR ctxt all "Mespilus germanica" OR ctxt all "M germanica" OR ctxt all "Ficus carica")	1
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt all "F carica" OR ctxt any "kiwi?" OR ctxt any "kiwifruit*" OR ctxt all "Actinidia chinensis" OR ctxt all "Actinidia deliciosa" OR ctxt = "A chinensis")	0
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt = "A deliciosa" OR ctxt any "avocado?" OR ctxt = "Persea americana" OR ctxt = "P americana" OR ctxt any "banana?" OR ctxt any "musa")	2
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "orange?" OR ctxt any "citrus" OR ctxt any "satsuma?" OR ctxt any "clementine?" OR ctxt any "mandarin?" OR ctxt any "lemon?")	4
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "lime" OR ctxt any "limes" OR ctxt any "grapefruit*" OR ctxt any "grape?" OR ctxt all "grapevine" OR ctxt all "Vitis vinifera")	3
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt all "v vinifera" OR ctxt any "strawberr*" OR ctxt = "Fragaria ananassa" OR ctxt = "F ananassa" OR ctxt any "curcubit*" OR ctxt any "melon?")	3
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "cantaloupe*" OR ctxt = "cucumis melo" OR ctxt = "C melo" OR ctxt any "watermelon?" OR ctxt = "Citrullus lanatus" OR ctxt = "C lanatus")	1
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "olive*" OR ctxt = "Olea europaea" OR ctxt = "O europaea" OR ctxt any "aubergine*" OR ctxt any "eggplant*" OR ctxt = "solanum melongena")	1
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt = "S melongena" OR ctxt any "celeriac*" OR ctxt any "apium" OR ctxt any "cucumber*" OR ctxt any "pumpkin*" OR ctxt any "squash*")	10
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "beetroot*" OR ctxt any "beet?" OR ctxt = "Red Beet*" OR ctxt any "carrot*" OR ctxt = "Daucus carota" OR ctxt = "D carota")	3
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "pepper*" OR ctxt = "Capsicum annuum" OR ctxt = "C annuum" OR ctxt any "potato*" OR ctxt = "Solanum tuberosum" OR ctxt = "S tuberosum")	10
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "tomato*" OR ctxt = "Solanum lycopersicum" OR ctxt = "S lycopersicum" OR ctxt any "brassica" OR ctxt = "Brussels sprout*" OR ctxt any "broccoli")	17

Search string	Results
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "cabbage*" OR ctxt any "cauliflower*" OR ctxt any "kale" OR ctxt any "lettuce*" OR ctxt = "Lactuca sativa" OR ctxt = "L sativa")	4
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "spinach*" OR ctxt = "Spinacia oleracea" OR ctxt = "S oleracea" OR ctxt any "turnip*" OR ctxt any "rape" OR ctxt any "canola")	3
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt any "colza" OR ctxt any "sunflower*" OR ctxt = "Helianthus annuus" OR ctxt = "H annuus" OR ctxt any "sugarbeet*" OR ctxt = "sugar beet*"	4
(ctxt any "cisgenes*" OR ctxt any "cisgenic" OR ctxt any "intragenes*" OR ctxt any "intragenic") AND (ctxt = "Saccharum officinarum" OR ctxt = "S officinarum" OR ctxt any "squash*" OR ctxt = "beta vulgaris" OR ctxt any "sugarcane*"))	5
After de-duplication	56 results

Date of the search 10 November 2021.

Table A.9: Search Strings. Google Patents

Search string	Results
((TI = "cisgenesis") OR (TI = "intragenesis") OR (TI = "cisgenic") OR (TI = "intragenic") OR (AB = "cisgenesis") OR (AB = "intragenesis") OR (AB = "cisgenic") OR (CL = "cisgenesis") OR (CL = "intragenesis") OR (CL = "cisgenic") OR (CL = "intragenic") OR (AB = "intragenic")) (((TI = crop) OR (TI = crops) OR (TI = plant) OR (TI = plants) OR (TI = vegetable) OR (TI = vegetables) OR (TI = fruit) OR (TI = fruits) OR (Ti = cereal) OR (TI = cereals) OR (TI = grain) OR (TI = grains) OR (TI = oilseed) OR (AB = crop) OR (AB = crops) OR (AB = plant) OR (AB = plants) OR (AB = vegetable) OR (AB = vegetables) OR (AB = fruit) OR (AB = fruits) OR (AB = cereal) OR (AB = cereals) OR (AB = grain) OR (AB = grains) OR (AB = oilseed) OR (CL = crop) OR (CL = crops) OR (CL = plant) OR (CL = plants) OR (CL = vegetable) OR (CL = vegetables) OR (CL = fruit) OR (CL = fruits) OR (CL = cereal) OR (CL = cereals) OR (CL = grain) OR (CL = grains) OR (CL = oilseed) OR (TI = maize) OR (TI = "zea mays") OR (TI = "z mays") OR (TI = oryza) OR (TI = "O sativa") OR (TI = rye) OR (TI = "Secale cereale") OR (TI = sorghum) OR (TI = triticale) OR (TI = wheat) OR (TI = "Triticum aestivum") OR (TI = buckwheat) OR (TI = soyabean) OR (TI = soybean) OR (TI = "Glycine max") OR (TI = "G max") OR (TI = soja) OR (TI = soya) OR (TI = spelt) OR (TI = spelta) OR (TI = bean) OR (TI = beans) OR (TI = faba) OR (TI = chickpea) OR (TI = "Cicer arietrinum") OR (TI = "C arietrinum") OR (TI = legume) OR (TI = lentil) OR (TI = "Lens culinaris") OR (TI = "L culinaris") OR (TI = pea) OR (TI = peas) OR (TI = "Pisum sativum") OR (TI = "P sativum") OR (TI = alfalfa) OR (TI = "Medicago sativa") OR (TI = "M sativa") OR (TI = apple) OR (TI = apples) OR (TI = "malus domestica") OR (TI = "m domestica") OR (TI = pear) OR (TI = pears) OR (TI = "Pyrus communis") OR (TI = "P communis") OR (TI = quince) OR (TI = quinces) OR (TI = "Cydonia oblonga") OR (TI = "C oblonga") OR (TI = peach) OR (TI = peaches) OR (TI = prunus) OR (TI = nectarine) OR (TI = nectarines) OR (TI = apricot) OR (TI = apricots) OR (TI = cherry) OR (TI = cherries) OR (TI = plum) OR (TI = plums) OR (TI = sloe) OR (TI = sloes) OR (TI = medlar) OR (TI = "Mespilus germanica") OR (TI = "M germanica") OR (TI = "Ficus carica") OR (TI = "F carica") OR (TI = kiwi) OR (TI = kiwis) OR (TI = kiwifruit) OR (TI = "Actinidia chinensis") OR (TI = "Actinidia deliciosa") OR (TI = "A chinensis") OR (TI = "A deliciosa") OR (TI = avocado) OR (TI = avocados) OR (TI = "Persea americana") OR (TI = "P americana") OR (TI = banana) OR (TI = bananas) OR (TI = musa) OR (TI = orange) OR (TI = oranges) OR (TI = citrus) OR (TI = satsuma) OR (TI = satsumas) OR (TI = clementine) OR (TI = clementines) OR (TI = mandarin) OR (TI = mandarins) OR (TI = lemon) OR (TI = lemons) OR (TI = lime) OR (TI = limes) OR (TI = grapefruit) OR (TI = grape) OR (TI = grapes) OR (TI = grapevine) OR (TI = "Vitis vinifera") OR (TI = "v vinifera") OR (TI = strawberr) OR (TI = "Fragaria ananassa") OR (TI = "F ananassa") OR (TI = curcubit) OR (TI = melon) OR (TI = melons) OR (TI = cantaloupe) OR (TI = "cucumis melo") OR (TI = "C melo") OR (TI = watermelon) OR (TI = watermelons) OR (TI = "Citrullus lanatus") OR (TI = "C lanatus") OR (TI = olive) OR (TI = "Olea europaea") OR (TI = "O europaea") OR (TI = aubergine) OR (TI = eggplant) OR (TI = "solanum melongena") OR (TI = "S melongena") OR (TI = celeriac) OR (TI = apium) OR (TI = cucurbit) OR (TI = cucumber) OR (TI = pumpkin) OR (TI = squash) OR (TI = beetroot) OR (TI = beet) OR (TI = beets) OR (TI = "Red Beet") OR (TI = carrot) OR (TI = "Daucus carota") OR (TI = "D carota") OR (TI = pepper) OR	157

Search string	Results
(TI = "Capsicum annuum") OR (TI = "C annuum") OR (TI = potato) OR (TI = "Solanum tuberosum") OR (TI = "S tuberosum") OR (TI = tomato) OR (TI = "Solanum lycopersicum") OR (TI = "S lycopersicum") OR (TI = brassica) OR (TI = "Brussels sprout") OR (TI = broccoli) OR (TI = cabbage) OR (TI = cauliflower) OR (TI = kale) OR (TI = lettuce) OR (TI = "Lactuca sativa") OR (TI = "L sativa") OR (TI = spinach) OR (TI = "Spinacia oleracea") OR (TI = "S oleracea") OR (TI = turnip) OR (TI = rape) OR (TI = canola) OR (TI = colza) OR (TI = sunflower) OR (TI = "Helianthus annuus") OR (TI = "H annuus") OR (TI = sugarbeet) OR (TI = "sugar beet") OR (TI = "beta vulgaris") OR (TI = sugarcane) OR (TI = "Saccharum officinarum") OR (TI = "S officinarum") OR (AB = maize) OR (AB = "zea mays") OR (AB = "z mays") OR (AB = oryza) OR (AB = "O sativa") OR (AB = rye) OR (AB = "Secale cereale") OR (AB = sorghum) OR (AB = triticale) OR (AB = wheat) OR (AB = "Triticum aestivum") OR (AB = buckwheat) OR (AB = soyabean) OR (AB = soybean) OR (AB = "Glycine max") OR (AB = "G max") OR (AB = soja) OR (AB = soya) OR (AB = spelt) OR (AB = spelta) OR (AB = bean) OR (AB = beans) OR (AB = faba) OR (AB = chickpea) OR (AB = "Cicer arietrinum") OR (AB = "C arietrinum") OR (AB = legume) OR (AB = lentil) OR (AB = "Lens culinaris") OR (AB = "L culinaris") OR (AB = pea) OR (AB = peas) OR (AB = "Pisum sativum") OR (AB = "P sativum") OR (AB = alfalfa) OR (AB = "Medicago sativa") OR (AB = "M sativa") OR (AB = apple) OR (AB = apples) OR (AB = "malus domestica") OR (AB = "m domestica") OR (AB = pear) OR (AB = pears) OR (AB = "Pyrus communis") OR (AB = "P communis") OR (AB = quince) OR (AB = quinces) OR (AB = "Cydonia oblonga") OR (AB = "C oblonga") OR (AB = peach) OR (AB = peaches) OR (AB = prunus) OR (AB = nectarine) OR (AB = nectarines) OR (AB = apricot) OR (AB = apricots) OR (AB = cherry) OR (AB = cherries) OR (AB = plum) OR (AB = plums) OR (AB = sloe) OR (AB = sloes) OR (AB = medlar) OR (AB = "Mespilus germanica") OR (AB = "M germanica") OR (AB = "Ficus carica") OR (AB = "F carica") OR (AB = kiwi) OR (AB = kiwis) OR (AB = kiwifruit) OR (AB = "Actinidia chinensis") OR (AB = "Actinidia deliciosa") OR (AB = "A chinensis") OR (AB = "A deliciosa") OR (AB = avocado) OR (AB = avocados) OR (AB = "Persea americana") OR (AB = "P americana") OR (AB = banana) OR (AB = bananas) OR (AB = musa) OR (AB = orange) OR (AB = oranges) OR (AB = citrus) OR (AB = satsuma) OR (AB = satsumas) OR (AB = clementine) OR (AB = clementines) OR (AB = mandarin) OR (AB = mandarins) OR (AB = lemon) OR (AB = lemons) OR (AB = lime) OR (AB = limes) OR (AB = grapefruit) OR (AB = grape) OR (AB = grapes) OR (AB = grapevine) OR (AB = "Vitis vinifera") OR (AB = "v vinifera") OR (AB = strawberr) OR (AB = "Fragaria ananassa") OR (AB = "F ananassa") OR (AB = curcubit) OR (AB = melon) OR (AB = melons) OR (AB = cantaloupe) OR (AB = "cucumis melo") OR (AB = "C melo") OR (AB = watermelon) OR (AB = watermelons) OR (AB = "Citrullus lanatus") OR (AB = "C lanatus") OR (AB = olive) OR (AB = "Olea europaea") OR (AB = "O europaea") OR (AB = aubergine) OR (AB = eggplant) OR (AB = "solanum melongena") OR (AB = "S melongena") OR (AB = celeriac) OR (AB = apium) OR (AB = cucurbit) OR (AB = cucumber) OR (AB = pumpkin) OR (AB = squash) OR (AB = beetroot) OR (AB = beet) OR (AB = beets) OR (AB = "Red Beet") OR (AB = carrot) OR (AB = "Daucus carota") OR (AB = "D carota") OR (AB = pepper) OR (AB = "Capsicum annuum") OR (AB = "C annuum") OR (AB = potato) OR (AB = "Solanum tuberosum") OR (AB = "S tuberosum") OR (AB = tomato) OR (AB = "Solanum lycopersicum") OR (AB = "S lycopersicum") OR (AB = brassica) OR (AB = "Brussels sprout") OR (AB = broccoli) OR (AB = cabbage) OR (AB = cauliflower) OR (AB = kale) OR (AB = lettuce) OR (AB = "Lactuca sativa") OR (AB = "L sativa") OR (AB = spinach) OR (AB = "Spinacia oleracea") OR (AB = "S oleracea") OR (AB = turnip) OR (AB = rape) OR (AB = canola) OR (AB = colza) OR (AB = sunflower) OR (AB = "Helianthus annuus") OR (AB = "H annuus") OR (AB = sugarbeet) OR (AB = "sugar beet") OR (AB = "beta vulgaris") OR (AB = sugarcane) OR (AB = "Saccharum officinarum") OR (AB = "S officinarum") OR (CL = maize) OR (CL = "zea mays") OR (CL = "z mays") OR (CL = oryza) OR (CL = "O sativa") OR (CL = rye) OR (CL = "Secale cereale") OR (CL = sorghum) OR (CL = triticale) OR (CL = wheat) OR (CL = "Triticum aestivum") OR (CL = buckwheat) OR (CL = soyabean) OR (CL = soybean) OR (CL = "Glycine max") OR (CL = "G max") OR (CL = soja) OR (CL = soya) OR (CL = spelt) OR (CL = spelta) OR (CL = bean) OR (CL = beans) OR (CL = faba) OR (CL = chickpea) OR (CL = "Cicer arietrinum") OR (CL = "C arietrinum") OR (CL = legume) OR (CL = lentil) OR (CL = "Lens culinaris") OR (CL = "L culinaris") OR (CL = pea) OR (CL = peas) OR (CL = "Pisum sativum") OR (CL = "P sativum") OR (CL = alfalfa) OR (CL = "Medicago sativa") OR (CL = "M sativa") OR (CL = apple) OR (CL = apples) OR (CL = "malus domestica") OR (CL = "m domestica") OR (CL = pear) OR (CL = pears) OR (CL = "Pyrus communis") OR (CL = "P communis") OR (CL = quince) OR (CL = quinces) OR (CL = "Cydonia oblonga") OR (CL = "C oblonga") OR (CL = peach) OR (CL = peaches) OR (CL = prunus) OR (CL = nectarine) OR (CL = nectarines) OR (CL = apricot) OR (CL = apricots) OR (CL = cherry) OR (CL = cherries) OR (CL = plum) OR (CL = plums) OR (CL = sloe) OR (CL = sloes) OR (CL = medlar) OR (CL = "Mespilus germanica") OR (CL = "M germanica") OR (CL = "Ficus	

Search string	Results
carica") OR (CL = "F carica") OR (CL = kiwi) OR (CL = kiwis) OR (CL = kiwifruit) OR (CL = "Actinidia chinensis") OR (CL = "Actinidia deliciosa") OR (CL = "A chinensis") OR (CL = "A deliciosa") OR (CL = avocado) OR (CL = avocados) OR (CL = "Persea americana") OR (CL = "P americana") OR (CL = banana) OR (CL = bananas) OR (CL = musa) OR (CL = orange) OR (CL = oranges) OR (CL = citrus) OR (CL = satsuma) OR (CL = satsumas) OR (CL = clementine) OR (CL = clementines) OR (CL = mandarin) OR (CL = mandarins) OR (CL = lemon) OR (CL = lemons) OR (CL = lime) OR (CL = limes) OR (CL = grapefruit) OR (CL = grape) OR (CL = grapes) OR (CL = grapevine) OR (CL = "Vitis vinifera") OR (CL = "v vinifera") OR (CL = strawberr) OR (CL = "Fragaria ananassa") OR (CL = "F ananassa") OR (CL = curcubit) OR (CL = melon) OR (CL = melons) OR (CL = cantaloupe) OR (CL = "cucumis melo") OR (CL = "C melo") OR (CL = watermelon) OR (CL = watermelons) OR (CL = "Citrullus lanatus") OR (CL = "C lanatus") OR (CL = olive) OR (CL = "Olea europaea") OR (CL = "O europaea") OR (CL = aubergine) OR (CL = eggplant) OR (CL = "solanum melongena") OR (CL = "S melongena") OR (CL = celeriac) OR (CL = apium) OR (CL = cucurbit) OR (CL = cucumber) OR (CL = pumpkin) OR (CL = squash) OR (CL = beetroot) OR (CL = beet) OR (CL = beets) OR (CL = "Red Beet") OR (CL = carrot) OR (CL = "Daucus carota") OR (CL = "D carota") OR (CL = pepper) OR (CL = "Capsicum annuum") OR (CL = "C annuum") OR (CL = potato) OR (CL = "Solanum tuberosum") OR (CL = "S tuberosum") OR (CL = tomato) OR (CL = "Solanum lycopersicum") OR (CL = "S lycopersicum") OR (CL = brassica) OR (CL = "Brussels sprout") OR (CL = broccoli) OR (CL = cabbage) OR (CL = cauliflower) OR (CL = kale) OR (CL = lettuce) OR (CL = "Lactuca sativa") OR (CL = "L sativa") OR (CL = spinach) OR (CL = "Spinacia oleracea") OR (CL = "S oleracea") OR (CL = turnip) OR (CL = rape) OR (CL = canola) OR (CL = colza) OR (CL = sunflower) OR (CL = "Helianthus annuus") OR (CL = "H annuus") OR (CL = sugarbeet) OR (CL = "sugar beet") OR (CL = "beta vulgaris") OR (CL = sugarcane) OR (CL = "Saccharum officinarum") OR (CL = "S officinarum"))) after:publication:20100101	
After de-duplication and time limit:	120

Date of the search: 10 November 2021.

A.3.1.4. Study selection process

The evidence retrieved through the literature searches will be screened for its relevance – against the eligibility criteria illustrated above (Tables A.2–A.3). Relevant evidence will need to comply with all the eligibility criteria of the review.

The study selection process will be undertaken in two steps:

1) Step 1: A rapid assessment based on title and abstract to exclude records that obviously are irrelevant;
2) Step 2: A detailed assessment of full-text documents.

Records that appear to be relevant and that appear of unclear relevance in Step 1 will be analysed further in Step 2, using the full-text document.

Each record will be screened independently by two reviewers (i.e. EFSA scientists) to minimise the risk of error. Results of the independent screenings will be compared. Any ambiguities between reviewers will be discussed to reach a consensus. If no consensus is reached, an additional independent opinion will be sought, from an expert member of the WG, in order to resolve differences of opinion.

The screening process will be undertaken in the review management software DistillerSR.

A.3.1.5. Appraisal of individual studies

In this step of the process, the risk of internal and external bias (RoB) and imprecision of each included study will be assessed. Each study will be appraised by two independent EFSA staff reviewers. Possible discrepancies that are not solvable via discussion between the two reviewers will be discussed by the whole working group.

A.3.1.6. Evidence synthesis/integration accounting for uncertainties

Evidence synthesis and integration will be based on a qualitative approach. The results of the studies will be discussed in a narrative way. Depending on the amount, the quality and the diversity of

the information that will be retrieved, there is a possibility that no meaningful synthesis will be possible. In this case, the data will be presented as such, without any further elaboration.

A.4. References

EFSA GMO Panel (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 (European Food Safety Authority), 2020. Draft framework for protocol development for EFSA's scientific assessments. EFSA supporting publication 2020:EN-1843. 46 pp. https://doi.org/10.2903/sp.efsa.2020.EN-1843