



**Hoge
Gezondheidsraad**

**VOORSTEL VAN EUROPESE VERORDENING
BETREFFENDE PLANTEN VERKREGEN
MET BEPAALDE NIEUWE GENOMISCHE
TECHNIEKEN (NGTs)**

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ADVIES VAN DE HOGE GEZONDHEIDSRAAD nr. 9801

Voorstel van Europese verordening betreffende planten verkregen met bepaalde Nieuwe Genomische Technieken (NGT's)

In this scientific advisory report, which offers guidance to public health policy-makers, the Superior Health Council of Belgium provides its opinion on the introduction of plants edited by cisgenesis or targeted mutagenesis using NGTs.

This report aims at the same time to provide policy-makers with some specific recommendations on the proposal of the European Commission.

Versie gevalideerd op het College van
6/3/2024¹

I INLEIDING EN ONDERWERP

Op 5 juli 2023 heeft de Europese Commissie een voorstel² aangenomen voor een nieuwe verordening betreffende met bepaalde Nieuwe Genomische Technieken (NGT's) verkregen planten en de daarvan afgeleide levensmiddelen en diervoeders, en tot wijziging van Verordening (EU) 2017/625. NGT's zijn nieuwe biotechnologische technieken die gerichte genomische veranderingen in organismen mogelijk maken. Ze werden ontwikkeld na de invoering van de huidige Europese GGO-wetgeving in 2001 (Richtlijn 2001/18/EG). De bekendste NGT is CRISPR-Cas9. De EU-Commissie concludeerde dat de huidige GGO-wetgeving niet langer aangepast is aan deze nieuwe ontwikkelingen en niet bevorderlijk is voor de ontwikkeling van innovatieve en nuttige producten. Daarom werd het huidige voorstel geïntroduceerd om de EU-strategieën "van boer tot bord" en "biodiversiteit" te ondersteunen. NGT's hebben een intrinsiek potentieel om bij te dragen aan een duurzamere wereld door een snellere veredeling van gewassen die bestand zijn tegen klimaatverandering en plagen/ziekten.

Het voorstel van de Europese Commissie heeft alleen betrekking op planten die genetisch materiaal van dezelfde plant bevatten (*gerichte mutagenese*) of van kruisbare planten (*cisgenese*, inclusief *intragenese*). Transgene planten vallen hier niet onder en blijven onderworpen aan de huidige GGO-wetgeving. Er worden twee categorieën van NGT-planten beschouwd:

¹ De Raad behoudt zich het recht voor om in dit document op elk moment kleine typografische verbeteringen aan te brengen. Verbeteringen die de betekenis wijzigen, worden echter automatisch in een erratum opgenomen. In dergelijk geval wordt een nieuwe versie van het advies uitgebracht.

² <https://eur-lex.europa.eu/legal-content/NL/TXT/?uri=CELEX%3A52023PC0411> (geraadpleegd op 28/11/2023)

- Categorie 1 (NGT1): NGT1-planten die ook in de natuur kunnen voorkomen of door conventionele veredeling kunnen worden gemaakt, worden onderworpen aan een controleprocedure op basis van gedefinieerde criteria. NGT1-planten zouden worden behandeld als conventionele planten en worden vrijgesteld van de vereisten van de GGO-wetgeving. Informatie over NGT1-planten zou echter nog steeds beschikbaar zijn via de etikettering van zaden, een openbare databank en catalogi van plantenvariëteiten.
- Categorie 2 (NGT2): NGT2-planten voldoen niet aan de criteria van NGT1-planten en moeten nog steeds voldoen aan de vereisten van de huidige GGO-wetgeving. Ze zouden worden onderworpen aan een risicobeoordeling en goedkeuring voordat ze op de markt worden gebracht. Ze zouden worden geëtiketteerd als GGO's, met de mogelijkheid van een vrijwillig etiket om het doel van de genetische modificatie aan te geven. De risicobeoordeling, detectiemethode en monitoringvereisten zouden worden aangepast aan verschillende risicoprofielen. Er zouden regelgevende stimulansen beschikbaar komen voor NGT2-planten met eigenschappen die bijdragen aan duurzaamheidsdoelen.

Op 7 maart 2023 stemde de werkgroep Chemische Omgevingsfactoren van de Hoge Gezondheidsraad ermee in om op eigen initiatief een advies te schrijven over de recente evoluties in de plantenbiotechnologie. Een paar maanden later volgde de Europese Commissie met een wetgevingsprocedure: in juli 2023 werd een eerste ontwerp van een nieuwe verordening over NGT-planten gepubliceerd. Dit voorstel zal worden besproken en aangepast door de Europese Raad (lidstaten) en het Europees Parlement. Op 10 oktober 2023 is de Hoge Gezondheidsraad begonnen met het schrijven van een kort en bondig advies voor de Belgische beleidsmakers. In het kader van de onderhandelingen met de Europese Raad werd op 7 december 2023 een herzien voorstel over NGT-planten gepubliceerd³. Deze versie wordt verder in dit advies besproken.

Dit advies is geschreven met een aantal overwegingen in het achterhoofd:

- In verschillende adviezen (bv. HGR 9404, 9561, 9698) heeft de Raad de noodzaak benadrukt om de algehele blootstelling van het grote publiek aan pesticiden (gewasbeschermingsmiddelen) te verminderen. Hoewel dit een belangrijk doel is, zijn er slechts enkele efficiënte alternatieven voor duurzame gewasbescherming. Boeren (en de maatschappij) hebben immers economisch voldoende opbrengsten nodig. NGT's bieden potentiële nieuwe middelen voor de "toolbox" voor gewasbescherming en dragen bij aan de doelstelling van 50 % vermindering van pesticiden binnen de Europese Green Deal tegen 2030 en de "van boer tot bord"-strategie.
- Gezien de voorspellingen van het zesde evaluatierapport (AR6) van het IPCC, waarin een mogelijke opwarming van de aarde met 3 tot 4 °C tegen het einde van de eeuw wordt genoemd (als de emissies zeer hoog blijven), moeten gewassen aangepast worden aan de klimaatverandering en bestand zijn tegen toenemende droogte, extreme regenval en nieuwe, migrerende plagen en ziekten. Bovendien zijn hogere opbrengsten met minder middelen nodig gezien de voorspelde verdere groei van de

³ <https://data.consilium.europa.eu/doc/document/ST-16443-2023-INIT/nl/pdf> (geraadpleegd op 19/1/24)

wereldbevolking. Voldoende, duurzaam, kwalitatief en veilig voedsel en diervoeder zijn essentieel voor de voedselzekerheid en de menselijke gezondheid.

- Gezien de spectaculaire toename van het areaal dat in de VS wordt gebruikt voor GGO-gewassen sinds hun introductie (1996-2020, van 0 naar 55 %. Bron: US Department of Agriculture⁴), kan verwacht worden dat niet-transgene, met NGT veredelde planten de Europese landbouw binnen enkele jaren zullen domineren. Op dit moment worden in Japan de eerste NGT-tomaten op de markt gebracht. Als gevolg hiervan zullen NGT-gewassen in de toekomst door alle Europese en Belgische consumenten geconsumeerd worden. Daarom moeten ze inherent veilig zijn.
- Ons land is pionier op het gebied van veel biotechnologische innovaties (bv. het onderzoek van Prof. Van Montagu, Prof. Schell, Prof. Fiers en Prof. Content in de jaren 1970 en 1980). Het is belangrijk om deze ontwikkelingen nauw op te volgen.
- Het invoegen van genen in planten met behulp van biotechnologie is een controversieel onderwerp binnen het publieke debat, dat soms zeer emotionele reacties oproept. Een extreem maar bekend voorbeeld is de vernietiging van een testveld met genetisch gemodificeerde aardappelen aan de Universiteit Gent door activisten in 2011. De Hoge Gezondheidsraad ziet het als een kerntaak om in een steeds complexere wereld state-of-the-art wetenschappelijke informatie te verschaffen, niet alleen voor beleidsmakers maar ook voor het grote publiek.
- Biotechnologische hulpmiddelen ontwikkelen zich razendsnel op veel gebieden, niet alleen bij planten. Gezien de ongekende impact op de menselijke gezondheid van nieuw ontwikkelde vaccins tegen SARS-CoV-2 die plaatsvond als gevolg van de COVID-19-pandemie, moeten we in gedachten houden dat België een belangrijke rol kan spelen in de snelle ontwikkeling van innovatieve biotechnologische producten (bv. mRNA of genetisch gemodificeerde virale vaccins). Dit kan zelfs worden versneld door publiek-private partnerschappen met filantropische en middenveldorganisaties. We moeten na deze COVID-19-crisis ook niet vergeten dat de scepsis afneemt als er gelijke toegang en informatie worden verstrekt en de bioveiligheid wordt gegarandeerd.

In dit advies richt de Hoge Gezondheidsraad zich op dit onderwerp vanuit een multidisciplinair "Eén wereld, één gezondheid"-perspectief. Daarom staan milieugezondheid, menselijke gezondheid en duurzame voedselzekerheid centraal in dit advies. Voor zeer technische opmerkingen over het voorstel wordt verwezen naar andere instanties (bv. de Adviesraad voor Bioveiligheid).

⁴ <https://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=107037> (geraadpleegd op 28/11/2023)

II CONCLUSIE EN AANBEVELINGEN

De klimaatverandering en de groeiende wereldbevolking brengen grote uitdagingen met zich mee voor de landbouw, aangezien voedselzekerheid en duurzame voedselproductie de komende decennia gewaarborgd moeten worden. Daarnaast is de "van boer tot bord"-strategie van de Europese Unie erop gericht om het gebruik van pesticiden tegen 2030 met 50 % te verminderen. Gewassen moeten verder worden versterkt tegen stress van zowel biotische (ziekten en plagen) als abiotische aard (droogte, hitte, overstromingen, verzilting en een verminderde beschikbaarheid van nutriënten). Bovendien moet wegens de groeiende wereldbevolking de opbrengst per gewas worden verhoogd, terwijl de middelen, zoals land en water, zullen afnemen. Al deze aanpassingen in de plantenveredeling moeten veel sneller worden gerealiseerd dan haalbaar is met conventionele benaderingen.

De afgelopen twee decennia zijn gekenmerkt door belangrijke doorbraken in de biotechnologie, met de ontwikkeling van nieuwe, snelle en nauwkeurige Nieuwe Genomische Technieken (NGT's), waaronder het Site-Directed Nuclease "CRISPR-Cas"-systeem. Om het gebruik van deze NGT's als hulpmiddelen bij de plantenveredeling toe te laten, heeft de Europese Commissie in 2023 een voorstel ingediend voor een "*Verordening betreffende met bepaalde nieuwe genomische technieken verkregen planten en de daarvan afgeleide levensmiddelen en diervoeders, en tot wijziging van Verordening (EU) 2017/625*".

In dit advies evalueert en verschaft de Hoge Gezondheidsraad inzicht in de toepassingen van NGT's bij plantenveredeling. Daarna bespreekt het advies de mogelijke gevolgen van het voorstel van de Commissie (versie van december 2023) voor de menselijke gezondheid en het milieu en de publieke acceptatie van NGT-planten.

Op basis van de wetenschappelijke literatuur en de interactie binnen de werkgroep kunnen de volgende conclusies worden getrokken over NGT's en hun toepassing:

- **NGT's kunnen zeer precieze, gerichte wijzigingen in het genoom aanbrengen.** Over het algemeen is het aantal mutaties aanzienlijk lager dan de spontane mutaties bij klassieke veredeling en willekeurige mutagenese.
- NGT's worden gebruikt voor gen-/genoombewerking op basis van *gerichte mutagenese*. Ze omvatten ook toepassingen met *cisgenese* en *inragenese*. **De uiteindelijke plantaardige producten die zijn afgeleid van het gebruik van NGT's zijn niet *transgeen* en hoewel ze genetische veranderingen hebben ondergaan, verschillen ze sterk van de genetisch gemodificeerde organismen (GGO's) waarop de huidige regelgeving van toepassing is. Daarom kunnen NGT-planten niet als GGO's worden beschouwd.**
- Op dit moment blijft de detectie en monitoring van NGT-planten een uitdaging. **Het is momenteel meestal onmogelijk om NGT-planten van conventioneel veredelde planten te onderscheiden door hun genomen te analyseren.**
- De verschillende Nieuwe Genomische Technieken zelf leveren geen intrinsieke veiligheidsproblemen op met betrekking tot hun gebruik. De discussie over de veiligheid van NGT-planten heeft voornamelijk te maken met de eigenschap die wordt

gemodificeerd of geïntroduceerd. **De impact op de menselijke gezondheid en het milieu hangt af van het doel van de NGT-plant.**

- **NGT-planten hebben het potentieel om bij te dragen aan een klimaatrobuuste, duurzame landbouwproductie met hogere opbrengsten die minder hulpbronnen vereisen.** Binnen een geïntegreerd bestrijdingssysteem van ziekten en plagen kunnen ze bijdragen aan het verminderen van het gebruik van pesticiden.
- **NGT's zijn geen magische oplossing voor alle problemen in de landbouw. Ze zijn slechts een van de vele hulpmiddelen die kunnen worden gebruikt om een duurzame voedselproductie in de toekomst te garanderen.**

Vanuit het "One world, one health"-perspectief beoordeelt de HGR het voorstel van verordening van de Commissie als volgt:

- **De HGR verwelkomt een Europese verordening voor NGT-planten die afwijkt van de bestaande GGO-verordening.** NGT-planten hebben het potentieel om bij te dragen aan innovatieve oplossingen voor grote uitdagingen in de landbouw. Aangezien deze planten geen GGO's zijn, is er een apart wetgevend kader nodig.
- Om praktische redenen gaat de HGR akkoord met de onderverdeling van NGT-planten in twee categorieën. Er zal een controleprocedure worden gebruikt om onderscheid te maken tussen NGT1 en NGT2. **NGT1-planten worden op dezelfde manier behandeld als conventionele planten,** aangezien ze ook door conventionele veredeling kunnen worden verkregen en in de praktijk niet te onderscheiden zijn van conventioneel geteelde planten. **De risico's van NGT1-planten worden vergelijkbaar ingeschat met de risico's van planten verkregen door conventionele veredeling. Als een plant NGT2 is, vereist het huidige voorstel een gedetailleerde risicobeoordeling per geval,** vergelijkbaar met die voor GGO's, waarin agronomische, milieu-, fenotypische, compositorische, toxicologische, allergene en voedingsaspecten zijn opgenomen.

De HGR wil de volgende aanbevelingen doen:

- De HGR vindt het belangrijk dat de hele samenleving voordeel heeft van NGT's en NGT-planten. Boeren en kwekers moeten toegang hebben tot zowel technieken als plantmateriaal tegen een aanvaardbare prijs. **De toekomstige uitwerking van de regels voor patentering, zoals aangekondigd door de Europese Commissie, moet daarom voorkomen dat er monopolies ontstaan die de betaalbare en algemene toegang tot deze nieuwe technieken en planten bemoeilijken.**
- De HGR onderstreept **het belang van een goede verificatieprocedure,** zoals momenteel wordt uiteengezet in het NGT-voorstel. Bovendien is alle bestaande Europese wetgeving met betrekking tot planten, nieuwe voedingsmiddelen (Verordening (EU) 2015/2283) en gezondheidsclaims (Verordening (EG) nr. 1924/2006) ook van toepassing op NGT-planten. **Op deze manier wordt een even hoog niveau van bescherming van de menselijke gezondheid en het milieu gewaarborgd.**

- De HGR geeft **enkele specifieke technische opmerkingen over het voorstel, met name over de criteria voor NGT1-planten, in bijlage I**. Deze opmerkingen staan in hoofdstuk 4 van het advies.
- De HGR vindt het belangrijk dat een Europese instelling in de toekomst beoordeelt of er **een "voordeel voor de maatschappij" is bij de introductie van nieuwe rassen/planten, ongeacht de technieken die gebruikt zijn om deze planten te produceren** (conventioneel, NGT, GGO ...). **Vanuit ethisch perspectief moeten de voordelen voor de maatschappij altijd opwegen tegen de mogelijke nadelen.**
- De HGR vindt **traceerbaarheid** belangrijk, evenals **transparantie** voor het publiek. Daarom verwelkomt de Raad de **etikettering van zaden** en het feit dat er een **voor het publiek toegankelijke databank** van NGT-planten zal worden opgezet.
- De HGR benadrukt hier dat het belangrijk is dat **ook de invoer van NGT-planten/zaden van buiten de Europese Unie gecontroleerd wordt**. Dezelfde veiligheidsnormen moeten worden toegepast.
- De HGR vindt het belangrijk dat de **NGT-regelgeving regelmatig wordt ge(her)evalueerd in het licht van de toekomstige wetenschappelijke inzichten**.
- De HGR pleit voor initiatieven die **de kennis van NGT's bij het grote publiek vergroten op basis van gedegen wetenschappelijke argumenten**.

Zoals uit deze conclusies en aanbevelingen volgt, is ook het ethische perspectief belangrijk voor de Hoge Gezondheidsraad. Maatschappelijke en agronomische voordelen (bv. resistentie tegen biotische of abiotische stressfactoren) moeten altijd worden afgewogen tegen de risico's voor de mens en zijn omgeving. Ook NGT-toepassingen moeten met beide rekening houden. Correcte en transparante informatie aan het grote publiek is essentieel. Burgers hebben niet alleen recht op accurate informatie, maar deze informatie moet ook hun vertrouwen in de voedselvoorzieningsketen garanderen.

III METHODOLOGIE

Het initiatief voor dit advies werd genomen in de permanente werkgroep "Chemische Omgevingsfactoren". In het verleden richtte deze groep zich vooral op milieukwesties met betrekking tot chemische verontreinigende stoffen. In de loop der tijd heeft de groep een bredere kijk op milieugezondheid ontwikkeld vanuit het perspectief "One world, one health".

Na analyse van het projectvoorstel hebben de Raad en de co-voorzitters van de werkgroep Chemische Omgevingsfactoren de noodzakelijke deskundigheidsgebieden vastgesteld. Vervolgens werd een *ad-hoc* werkgroep opgericht met deskundigen op het gebied van plantengenetica, functionele plantenbiologie, plantenveredeling, plantenfysiologie, gewasbescherming, chemie, bio-ingenieurswetenschappen, sociologie, agro-economie, milieugezondheid, toxicologie, farmacie en voeding. De deskundigen van deze werkgroep leverden een algemene en een *ad-hoc* belangenverklaring af en de Deontologische Commissie evalueerde het potentiële risico van belangenconflicten.

Dit advies is gebaseerd op een overzicht van de (*peer-reviewed*) wetenschappelijke literatuur die is verschenen in wetenschappelijke tijdschriften en rapporten van ter zake bevoegde nationale en internationale organisaties, evenals op de opinie van de deskundigen. De wetenschappelijke literatuur werd verzameld met behulp van zoekmachines zoals Google Scholar en databanken zoals PubMed, Web of Science en Scopus.

Nadat het advies door de werkgroep was goedgekeurd, werd het uiteindelijk door de Raad gevalideerd.

Sleutelwoorden en MeSH descriptor terms⁵

MeSH terms*	Keywords	Sleutelwoorden	Mots clés	Schlüsselwörter
<i>Climate Change</i>	<i>Climate Change</i>	Klimaat- verandering	<i>Changement climatique</i>	<i>Klimawandel</i>
<i>Clustered Regularly Interspaced Short Palindromic Repeats</i>	<i>Clustered Regularly Interspaced Short Palindromic Repeats</i>	<i>Clustered Regularly Interspaced Short Palindromic Repeats</i>	<i>Clustered Regularly Interspaced Short Palindromic Repeats</i>	<i>Clustered Regularly Interspaced Short Palindromic Repeats</i>
<i>Genetically Modified Plants</i>	<i>Genetically Modified Plants</i>	Genetisch Gewijzigde Planten	<i>Plantes génétiquement modifiées</i>	<i>Gentechnisch veränderte Pflanzen</i>
<i>Genetically Modified Food</i>	<i>Genetically Modified Food</i>	Genetisch gewijzigde voeding	<i>Aliments génétiquement modifiés</i>	<i>Gentechnisch veränderte Lebensmittel</i>
<i>Legislation</i>	<i>legislation</i>	wetgeving	<i>législation</i>	<i>Gesetzgebung</i>
<i>Sustainable Development</i>	<i>sustainable development</i>	duurzame ontwikkeling	<i>développement durable</i>	<i>Nachhaltige Entwicklung</i>
<i>Toxicity</i>	<i>toxicity</i>	toxiciteit	<i>toxicité</i>	<i>Toxizität</i>
-	<i>human exposure</i>	humane blootstelling	<i>exposition humaine</i>	<i>menschliche Exposi tion</i>

MeSH (Medical Subject Headings) is de thesaurus van de NLM (National Library of Medicine) met gecontroleerde trefwoorden die worden gebruikt voor het indexeren van artikelen voor PubMed <http://www.ncbi.nlm.nih.gov/mesh>.

⁵ De Raad wenst te verduidelijken dat de MeSH-termen en sleutelwoorden worden gebruikt voor referentiedoeleinden en een snelle definitie van de scope van het advies. Voor nadere inlichtingen kunt u het hoofdstuk "methodologie" raadplegen.

Lijst van gebruikte afkortingen

BAC	<i>Biosafety Advisory Council</i>
Bt	<i>Bacillus thuringiensis</i>
CRISPR	<i>Clustered Regularly Interspaced Short Palindromic Repeats</i>
CRISPRa	<i>CRISPR activation</i>
CRISPRi	<i>CRISPR interference</i>
Cas	<i>CRISPR-associated</i>
Cas9	<i>CRISPR-associated protein 9</i>
Cpf1	<i>CRISPR from Prevotella and Francisella 1</i>
CRA-W	<i>Centre wallon de Recherches agronomiques</i>
crRNA	<i>CRISPR ribonucleic acid</i>
DNA	<i>Deoxyribonucleic acid</i>
dPCR	<i>Digital polymerase chain reaction</i>
DSB	<i>Double-strand break</i>
EC	<i>Europese Commissie</i>
EFSA	<i>European Food Safety Authority</i>
EGT	<i>Established genomic techniques</i>
EU	<i>Europese Unie</i>
F1	<i>F1 hybrid</i>
GE	<i>Gene/Genome edited</i>
GM	<i>Genetically modified</i>
GMO	<i>Genetically modified organism</i>
gRNA	<i>Guide ribonucleic acid</i>
HDR	<i>Homology-directed repair</i>
HT	<i>Herbicide tolerant</i>
ILVO	<i>Instituut voor Landbouw-, Visserij- en Voedingsonderzoek</i>
LNA	<i>Locked nucleic acid</i>
MGB	<i>Minor groove binder</i>
NGT	<i>Nieuwe Genomische Technieken</i>
NHEJ	<i>Non-homologous end joining</i>
ODM	<i>Oligonucleotide-directed mutagenesis</i>
PAM	<i>Protospacer adjacent motif</i>
PEG	<i>Polyethylene glycol</i>
PNA	<i>Peptide nucleic acid</i>
PCR	<i>Polymerase chain reaction</i>
QTL	<i>Quantitative trait locus</i>
RNA	<i>Ribonucleic acid</i>
SDN	<i>Site-directed nuclease</i>
sgRNA	<i>Single guide ribonucleic acid</i>
SNV	<i>Single nucleotide variation</i>
SHC	<i>Superior Health Council</i>
SNP	<i>Single-nucleotide polymorphism</i>
TALEN	<i>Transcription activator-like effector nuclease</i>
T-DNA	<i>Transfer deoxyribonucleic acid</i>
Ti plasmid	<i>Tumor-inducing plasmid</i>
tracrRNA	<i>Trans-activating CRISPR ribonucleic acid</i>
VIB	<i>Vlaams Instituut voor Biotechnologie</i>

IV GLOSSARIUM

Om de lezer vertrouwd te maken met een aantal belangrijke begrippen in dit advies is een verklarende woordenlijst (Engelstalig) opgenomen met begrippen zoals gedefinieerd in de woordenlijst van de EFSA⁶ en EFSA (2022)⁷. Deze definities zijn slechts indicatief; elke definitie heeft onvermijdelijk haar specifieke beperkingen.

Word	Definition	Source
Allergenicity	The ability to trigger an abnormal immune response that leads to an allergic reaction in a person.	EFSA Glossary
Breeder's gene pool	The sources of genes available for conventional plant breeding.	EFSA (2022)
Breeding programme	A structured programme to improve a population of plants or animals by breeding for certain characteristics.	EFSA Glossary
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.	EFSA (2022)
Comparative assessment	Required in law, an assessment designed to compare the safety of a genetically modified (GM) organism against its non-GM bred counterpart.	EFSA Glossary
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.	EFSA (2022)
DNA	A complex chain-like molecule that carries the genetic material, present in living organisms and some viruses. DNA (deoxyribonucleic acid) is capable of copying itself and carries the instructions for all the proteins used to create and sustain life.	EFSA Glossary
DNA sequence	The exact order of units in a DNA chain.	EFSA Glossary
Double-strand break (DSB)	The mechanical, chemical or enzymatical cleavage of both strands of the DNA.	EFSA (2022)
Environmental Risk Assessment	The process of assessing potential harm to the environment caused by a substance, activity or natural occurrence. This may include the introduction of GM plants, the use of pesticides, or the spread of plant pests.	EFSA Glossary
Exogenous DNA	DNA originating outside the plant being modified which can be introduced naturally or by technological intervention.	EFSA (2022)
Genetic diversity	Genetic variation between and within species.	EFSA Glossary
Genetic Engineering (GE)	Process that alters the genetic material of an organism by modifying, removing or introducing new DNA to its genome.	EFSA Glossary

⁶ <https://www.efsa.europa.eu/nl/glossary-taxonomy-terms> (geraadpleegd op 5/2/24)

⁷ <https://doi.org/10.2903/j.efsa.2022.7621> (geraadpleegd op 5/2/24)

Genetically Modified Organism (GMO)	An organism which contains genetic material that has been deliberately altered and which does not occur naturally through breeding or selection.	EFSA Glossary
Genome	The haploid set of chromosomes of a given organism which contains all the genetic information necessary for its maintenance.	EFSA (2022)
Genetic mutation	Permanent change of the nucleotide sequence in the genome of a given organism.	EFSA (2022)
Genome editing techniques	Processes that change the genetic material of animals, plants and microorganisms with precision in subtle or more extensive ways.	EFSA Glossary
GMO non-target organism	Other organism that is not genetically modified but which may interact with, or be affected by, the presence of a GM organism.	EFSA Glossary
Health Claim	Any practice (e.g., a statement or visual) used in food marketing to suggest that health benefits can be gained from consuming a given food, nutrient or ingredient.	EFSA Glossary
Homology-directed repair (HDR)	A molecular mechanism which allows the repair of DNA double-strand breaks using a homologous sequence of DNA as template.	EFSA (2022)
Human biomonitoring	A direct measurement of the level of toxic chemical compounds present in the body. Often, these measurements are made using blood and urine.	EFSA Glossary
Insecticide	A substance that kills insects.	EFSA Glossary
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.	EFSA (2022)
New Genomic Techniques (NGTs)	Molecular breeding techniques that can alter the genetic material of an organism and that have been developed since the adoption of the EU's GMO legislation in 2001.	EFSA Glossary
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.	EFSA (2022)
Novel Food	Foodstuff or food ingredient that was not used for human consumption to a significant degree within the European Union before 15 May 1997.	EFSA Glossary
Nutrition claim	A statement that implies that a foodstuff has beneficial nutritional properties, such as being "low fat" or "high in fibre".	EFSA Glossary
Oligonucleotide	A stretch of nucleic acid consisting of a relatively low number of nucleotides.	EFSA (2022)
Omics	High-powered technologies used for holistic analysis of the molecules that make up the cells of living organisms; for example, Genomics is the study of the entire genome, while Proteomics analyses the complete complement of proteins within a biological sample.	EFSA Glossary

Post-market environmental monitoring of GM plants	Monitoring of the effects of a new product (e.g., a GM plant) following its release onto the market. This may reveal adverse effects which were not predicted in the risk assessment conducted prior to market release.	EFSA Glossary
Protein	A type of molecule composed of complex strings of amino acids (protein building blocks).	EFSA Glossary
Risk characterisation	The final stage of risk assessment, in which the likelihood that a particular substance will cause harm is calculated in the light of the nature of the hazard and the extent to which people, animals, plants and/or the environment are exposed to it.	EFSA Glossary
RNA	A type of nucleic acid found in the body, similar to DNA but single stranded. The best-known function of RNA (ribonucleic acid) is transmitting instructions from DNA to the cellular machinery responsible for making proteins.	EFSA Glossary
RNA interference	The blocking of normal gene activities by RNA molecules. This is a natural process but can also be harnessed by biologists as a way of researching how genes work in the body.	EFSA Glossary
Ribonucleoprotein (RNP)	A macromolecule complex composed of protein and RNA polymers.	EFSA (2022)
Sequence	Usually refers to the linear order of nucleotides in DNA and RNA or amino acids in proteins.	EFSA (2022)
Site-directed mutagenesis	A molecular biology method that is used to make specific and intentional changes (insertions, deletions and substitutions) to a genomic locus.	EFSA (2022)
Site-directed nuclease (SDN)	An enzyme which recognises a specific sequence and cleaves the DNA usually creating a double-strand break.	EFSA (2022)
Targeted mutagenesis	Technique that induces specific mutation(s) in targeted locations of the genome without inserting new genetic material.	EFSA Glossary
Toxicity	The potential of a substance to cause harm to a living organism.	EFSA Glossary
Traceability	The ability to track the journey of a foodstuff or ingredient through all stages of production, processing and distribution.	EFSA Glossary
Transformation	The process by which a prokaryotic or eukaryotic cell takes up exogenous DNA.	EFSA (2022)
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.	EFSA (2022)

V UITWERKING EN ARGUMENTATIE

1 Short introduction to plant breeding, GMOs and NGTs

1.1 History and context

Since the beginning of agriculture 10 000 years ago, humans have strived to develop and improve plants with various desired traits (Taiz, 2013; Ahmar et al., 2020), such as higher yield or nutritional value, resistance to biotic (pests and diseases) and abiotic stresses (drought, salinisation, little nutrients), and aesthetic properties for consumers. Due to the discovery of Mendelian laws in 1866, plant breeding rapidly developed during the 20th century. Time-consuming conventional (cross-)breeding techniques gradually improved as plant physiology became better understood (Taiz et al., 2015). Moreover, the use of random mutagenesis by chemicals or radiation facilitated the search for more interesting variations since the 1930s (Ahmar et al., 2020). Later, a revolution in plant breeding was started by the discovery of tumor-inducing plasmids in *Agrobacterium tumefaciens* by Zaenen et al. (1974) and Van Larebeke et al. (1975) at Ghent University, which allowed circumventing species boundaries by introducing foreign genes in the plant genome (transgenesis). In 2019, the worldwide acreage of Genetically Modified (GM) crops reached 190 million hectares (Statista, 2023). Furthermore, since the 1990s, diverse novel techniques have allowed faster breeding and the selection of superior hybrid lines, e.g., molecular marker-assisted selection (using amongst others Single Nucleotide Polymorphisms [SNPs]), the mapping of quantitative trait loci (QTLs), high-throughput phenotyping, whole-genome sequence-based approaches, speed breeding and developments in plant tissue culture (Ahmar et al., 2020). During the last decade, another genomic revolution took place, as programmable Site-Directed nucleases (SDNs), such as CRISPR-Cas, have been found to edit genes in a faster, more efficient, and more precise way than before (Chen et al., 2019; Ahmar et al., 2020). While it often takes 8–12 years to create a new variety via conventional cross- and mutation breeding, by using genome editing techniques, such as CRISPR-Cas, the breeding process can be shortened significantly (depending on the species/crop, often a third of the time) (Chen et al., 2019; Wang et al., 2022). It should be emphasised that gene editing can accelerate, but not replace, the breeding process. Gene editing is thus an additional tool that can be used within breeding programs. The need for innovative plant breeding is now more urgent than ever. As the world population will reach 9 billion individuals by 2050 and the middle class in developing countries will grow further, the current rate of food production needs to double in order to provide sufficient food for everyone (Taiz et al., 2013). Besides, the threatening scenario of a possible 3-4 °C rise in temperature by the end of the century due to global warming (IPCC, 2023: AR6) puts current farming systems at great risk, through the concentration of weather extremes, increase in drought, shifting diseases/pests, among others. Climate-adapted crops are therefore needed in agriculture, to ensure a sustainable supply of sufficient, healthy, and nutritious food and feed.

1.2 Genetic resources for new traits

To find new traits, several strategies can be followed (Figures 1-3), each of which generates in a different way, targeted or non-targeted, few to many mutations (Figures 4-5).

First, the existing **natural diversity** among cultivars, wild types and landraces is used for the introgression of specific traits by crossing. Besides, spontaneous mutations also generate new traits over time. However, conventional breeding via intraspecific crosses between similar types of parents lowers genetic diversity and commercial hybrids often substitute landraces or indigenous germplasm (Rauf et al., 2010). In addition to climate change and biodiversity loss, the narrowing genetic base of crop varieties and the widespread of dominant varieties are considered to be the important driving forces of decreasing genetic resources (Salgotra & Chauhan, 2023).

After the discovery of the mutagenic effects of X-rays on maize and barley by Stadler in 1930, **random mutagenesis** was increasingly used for plant breeding during the 20th century, especially after the establishment of the International Atomic Energy Agency (IAEA) (Kharkwal, 2023). By 2022, over 3 500 mutant varieties of > 240 plants (including cereals, pulses, oilseeds, vegetables, fruits, fibres and ornamentals) have been released (Kharkwal, 2023). **Mutation breeding** (mostly on seeds) is classified into three types of mutagenesis: radiation-induced mutagenesis (gamma rays, X-rays, ion beams), chemically-induced mutagenesis (e.g., ethyl methanesulfonate) and insertional mutagenesis (e.g., by the activation of transposable elements) (Ahmar et al., 2020). According to the IAEA (2023), exposure to radiation boosts DNA mutation rates by 1 000 to million-fold. Mutagenesis is “random” and can also cause “knock-out” mutations, while the majority of mutations are silent and remain undiscovered. The IAEA has a publicly accessible Mutant Variety Database on the internet⁸.

A third way to insert new traits in plants resorts to biotechnology (Figure 2). The following distinctions can be made (adapted from EFSA, 2022a):

- **Transgenesis:** Inserting gene(s) from any sexually incompatible species, or any synthetic gene (non existing in nature), into the genome of a given cell, and the inheritance of such gene(s) thereafter.
- **Intragenesis:** Inserting genetic material obtained from the breeders’ gene pool and transferred to the host using various delivery strategies; the incorporated sequences contain a re-arranged copy of sequences already present in the breeders’ gene pool.
- **Cisgenesis:** Inserting genetic material obtained from the breeders’ gene pool and transferred to the host using various delivery strategies; the incorporated sequences contain an exact copy of a sequence already present in the breeders’ gene pool
- **Targeted genome editing:** An umbrella term used to describe newer techniques allowing to integrate large fragments at a precise location or to induce small modifications, such as base substitutions, deletions or insertions, in the genome (targeted mutagenesis).

⁸ <https://nucleus.iaea.org/sites/mvd> (accessed on 17/1/2024)

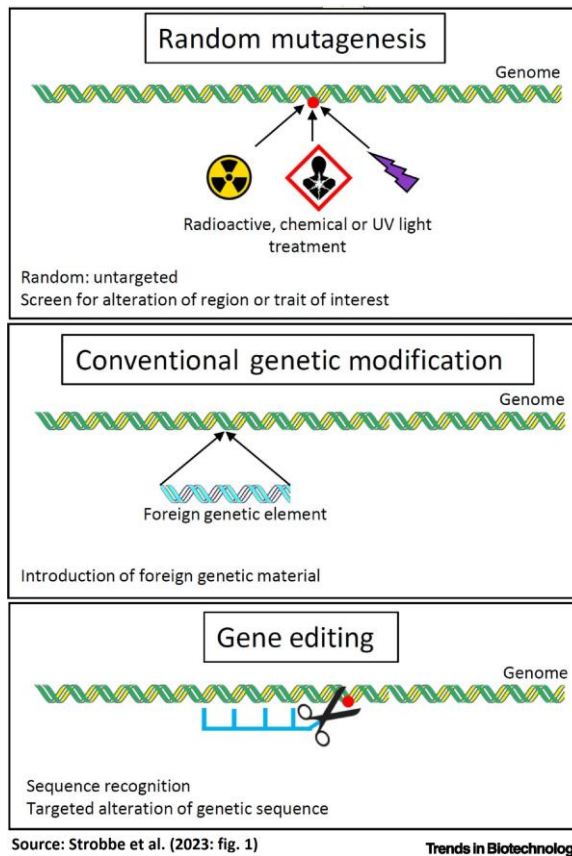


Figure 1. Three simple pathways to alter plant genes: random mutagenesis (conventional breeding), conventional genetic modification, gene editing. Source: Strobbe et al. (2023).

NEW GENOMIC TECHNIQUES (NGT)

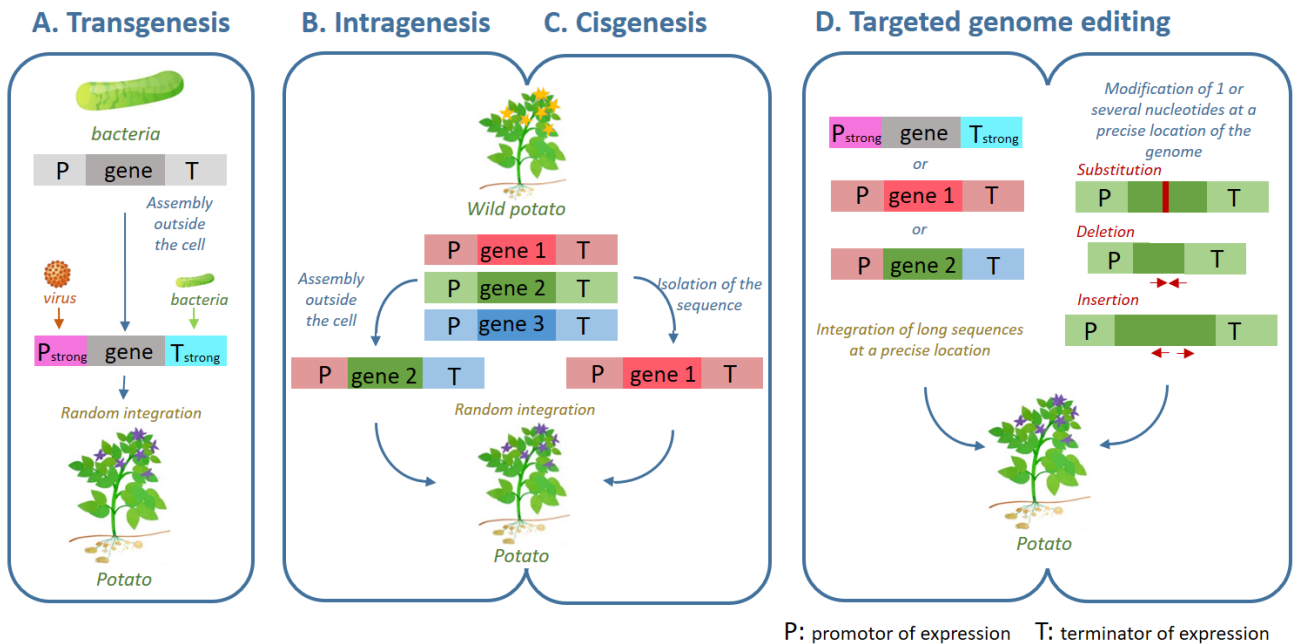


Figure 2. Main techniques of assisted plant modification. © CRA-W, F. Debode.

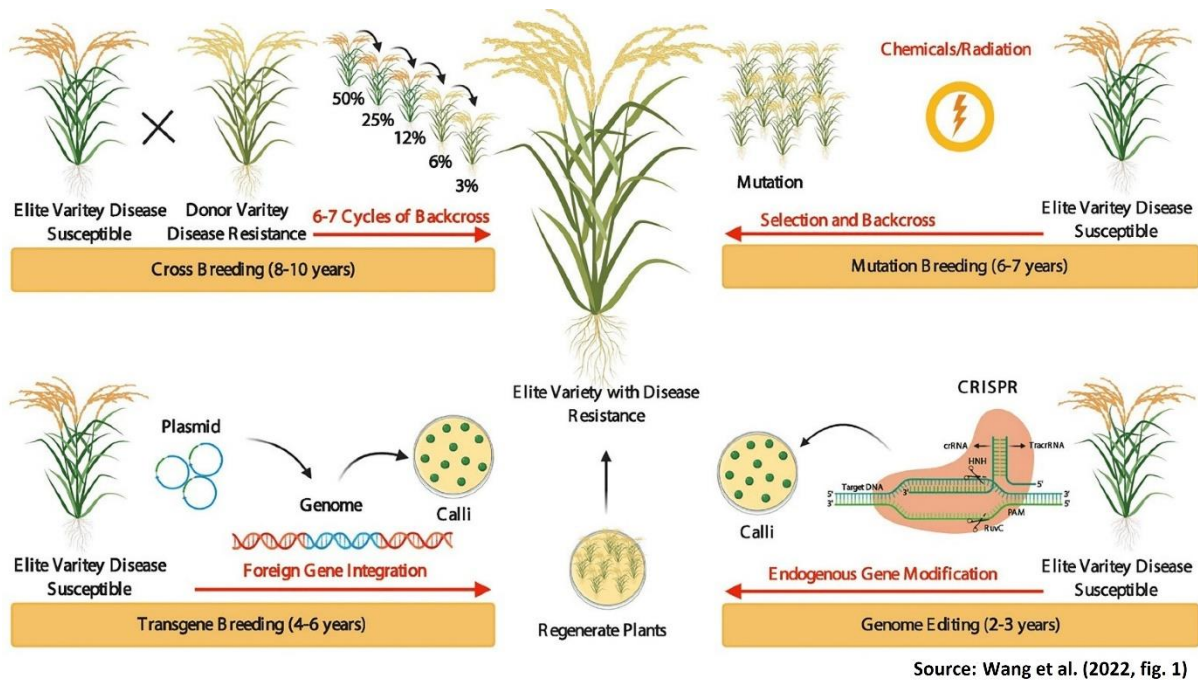


Figure 3. Crop-breeding techniques and the time needed for the development of a new cultivar. Times are only indicative, as these vary by species or crop type. Source: Wang et al. (2022).

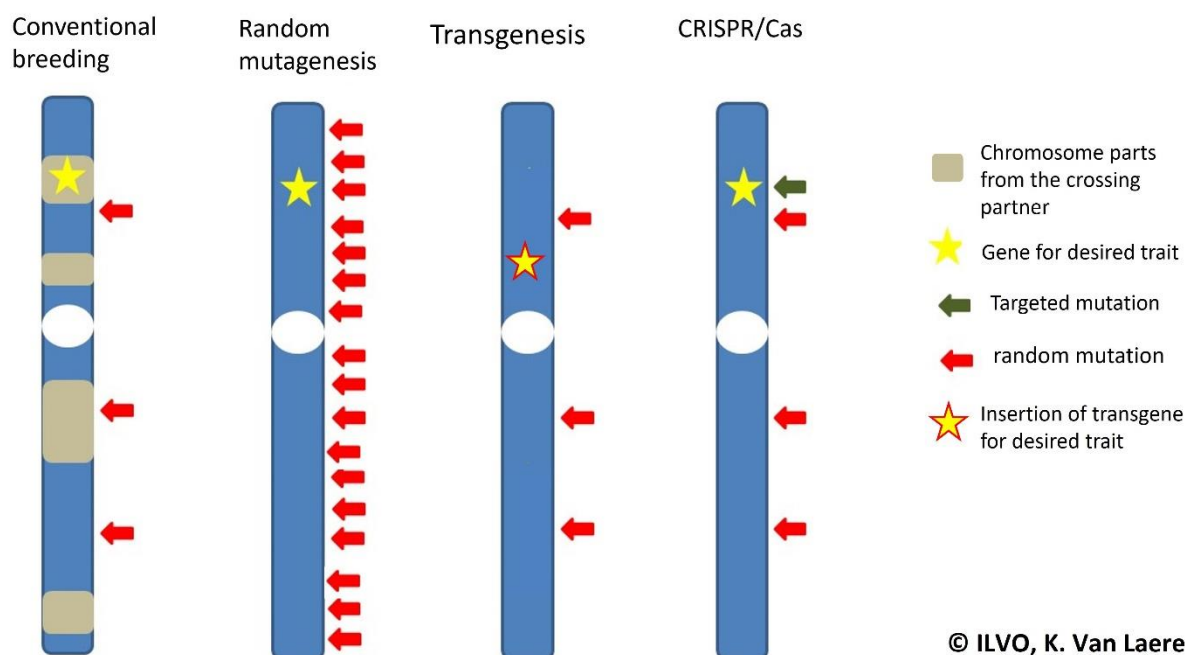


Figure 4. Schematic presentation of different techniques to create plants with a desired trait and the different mutations obtained, from left to right: conventional breeding by crossing (grey zones represent parts of the chromosome that originate from the other parent plant due to recombination), mutation breeding by random mutagenesis, classical genetic modification, and gene editing (CRISPR-Cas). © ILVO, K. Van Laere.

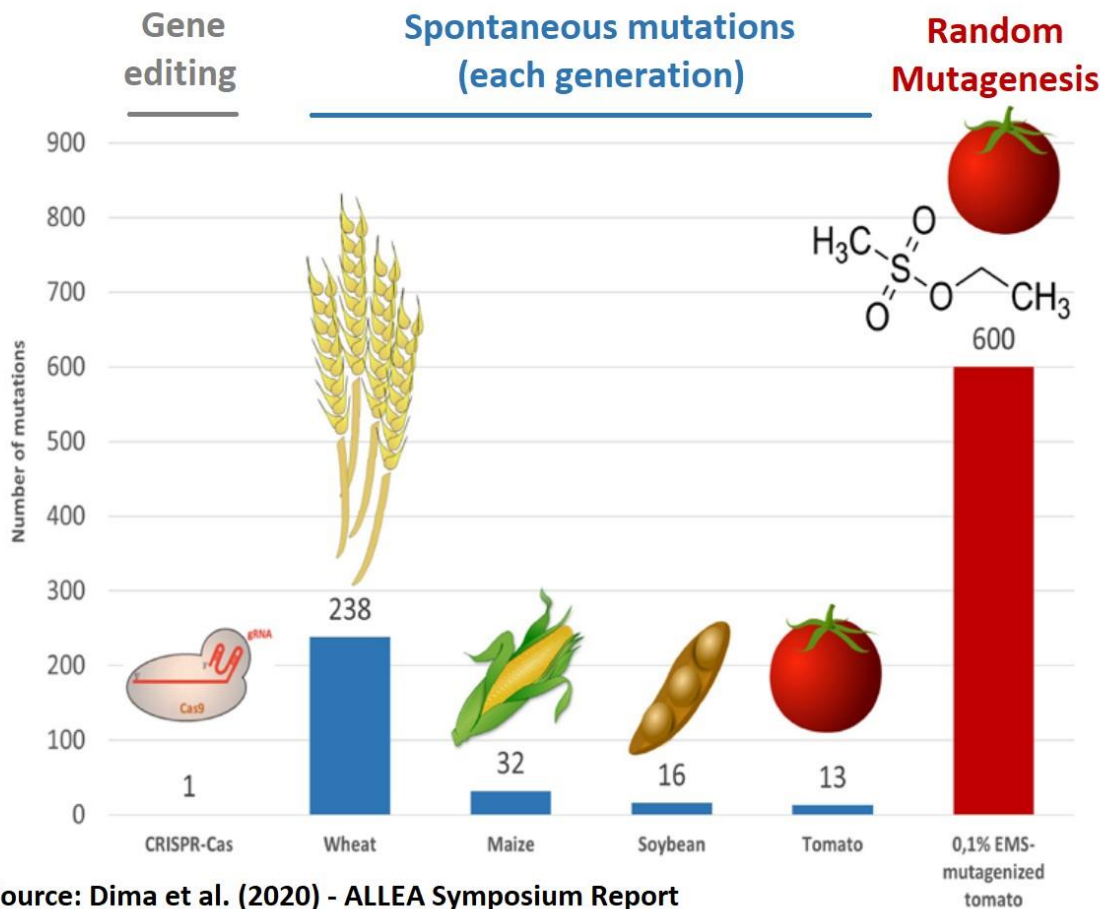


Figure 5. Estimated number of spontaneous mutations occurring in every individual plant compared to the hypothetical single change in the genome introduced using CRIPSR-Cas genome editing or random mutagenesis by ethyl methanesulfonate. The spontaneous mutations are extrapolated from the number of mutations in the model plant Arabidopsis, rescaled to the size of the genome of different crops. Source: Dima et al. (2020: fig. 5), ALLEA symposium report.

1.3 Conventional plant breeding

In conventional breeding, different methods are used to introduce new traits in plants. All these breeding programs are characterised by a long duration (often 10 years and more; see Figure 3). In self-pollinated crops, the bulk method, pedigree method and single-seed descent method are often used, while in cross-pollinated crops, recurrent selection (e.g., maize) and hybrid development are frequently applied (Suza & Lamkey, 2023). Although many variations on selection and crossing schemes exist, they are all based on the following principles (Goulet et al., 2017; Nirubana et al., 2021):

Hybridisation: Hybridisation is a cross between individuals from separate populations that differ in one or more heritable traits. Through many steps of selection and crossing, new varieties can be obtained. Two phenomena are often observed and used:

- ⇒ **Heterosis/hybrid vigour (among F1 hybrids):** The improved or increased function of any phenotypical trait in an F1 hybrid offspring.
- ⇒ **Transgressive segregation (population-level process):** The formation of extreme phenotypes or transgressive phenotypes observed in segregated hybrid populations compared to phenotypes observed in the parental lines. In contrast to heterosis, these extreme phenotypes are heritably stable.

Introgressive hybridisation (population-level process): When hybrids are fertile, they can be back-crossed multiple times with their parents. This can ultimately produce a plant that is almost identical to one parent (e.g., taste and fruit yield) but retains a specific trait from the other parent (e.g., disease resistance).

A disadvantage of hybridisation-based breeding is that the traits only come from the gene pool of crossable plants. Crossing within the primary gene pool (intraspecific) is relatively easy, leading to fertile hybrids. Within the secondary gene pool (interspecific), gene transfer between species is possible but difficult, often resulting in sterile hybrids. Within the tertiary gene pool (often intergeneric), gene transfer between distant species is only possible using embryo rescue, chromosome doubling or other *in vitro* techniques (Suza & Lamkey, 2023).

Some other difficulties can arise. Sometimes, it is known what DNA change is needed, but it is not found in nature. Moreover, it can be challenging in crops to break the link between positive and negative traits during crossing. Besides, there are really “difficult” plants, such as apple, pear (only flowering every few years, or needing a very long time between seed and new flowering plants) or banana (sterility). These bottlenecks in conventional plant breeding can partly be solved with CRISPR-Cas gene editing (see further).

As a conventional breeding tool, **random mutagenesis**, where random mutations are induced by radiation or chemicals (Figures 4-5), is also widely used. After the mutagenic treatment, selection is based on the modified phenotype, which is screened for new traits of interest. Due to the substantial application and the long history of safe use, it is exempted (Annex IB) from the current European GMO Directive 2001/18. As a result, these are treated as conventional plants without risk assessment.

1.4 Genetic Modification

1.4.1 GM techniques

Selected, often foreign genes (**transgenesis**) are inserted in plant genomes by genetic modification (GM). In the EU, these GM techniques are referred to as Established Genetic Techniques (EGTs) and were developed before 2001, when the European GMO Directive 2001/18/EC was adopted (EFSA, 2022). Article 2 (2) of this directive defines a “**Genetically Modified Organism (GMO)**” as “**an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination**”. To transform plant cells, different methods can be used to insert the foreign DNA into the cell (based on amongst others Chen et al., 2022):

- **Agrobacterium-mediated transformation** is the most common transformation method, as it is cost-effective and capable of transferring large DNA fragments into plant chromosomes (Chen et al., 2022). It uses the tumor-inducing (Ti) capacity of *Agrobacterium tumefaciens* which was discovered in the 1970s (Zaenen et al., 1974; Van Larebeke et al., 1975). Exogenous DNA is cloned into the (transfer) T-DNA region of the Ti-plasmid. The latter is a small circular extrachromosomal DNA molecule that contains a *vir* region with virulence genes that are responsible for the transfer of the T-DNA into the nucleus of the damaged host plant cells and the random integration and expression of the T-DNA into the DNA of the host plant cell. For a detailed review on the Ti plasmid, see Gordon & Christie (2015). Two ways exist for *Agrobacterium*-mediated transformation:
 - ⇒ **In vitro**: Plant explants (e.g., small leaf pieces) are co-cultivated with the *Agrobacterium* bacteria in the sterile conditions of *in vitro* plant tissue culture. As a selection marker (e.g., antibiotic resistance) is often included in the T-DNA, the transformed explants can be selected on a selection medium. Subsequently, the explants are put on a callus-inducing medium to produce callus and subsequently on a shooting and a rooting medium to regenerate calli into shoots (indirect organogenesis) and small *in vitro* plantlets that can then be transferred to soil. Other *in vitro* regeneration pathways are possible, such as somatic embryogenesis on the transformed plant tissues.
 - ⇒ **In planta**: *Agrobacterium* with modified Ti-plasmids can be injected into the plant (= agroinfiltration), after which *de novo* shoot regeneration on the place of infiltration can provide transformed plant material.
- **Particle bombardment (biolistics)** is another transformation method that can overcome competency barriers that can arise in *Agrobacterium*-mediated methods. In general, gold particles are coated with the DNA construct (promotor, terminator, reporter gene, gene of interest), and then fired into the cells of a plant explant (with callus). Using plant tissue culture (see above), transformed plants are regenerated from the transformed cells.

In addition to *Agrobacterium*-mediated transformation and biolistics, other strategies (using nucleic-acid-coated nanoparticles and virus-based RNA delivery systems) also exist, but are much less common.

1.5 Gene editing with New Genomic Techniques

1.5.1 NGT techniques

NGTs are new biotechnological techniques developed after the introduction of the current EU GMO legislation in 2001 (Directive in 2001 /18/EC). These techniques differ from the Established Genomic Techniques (EGTs) by the fact that NGTs work in a targeted way on short nucleic sequences (Figure 6). NGTs can very precisely edit DNA by targeted mutagenesis without inserting exogenous DNA, or they can allow the targeted integration of genetic sequences. **NGTs can be employed as a new tool in plant breeding; however, they do not replace plant breeding schemes.**

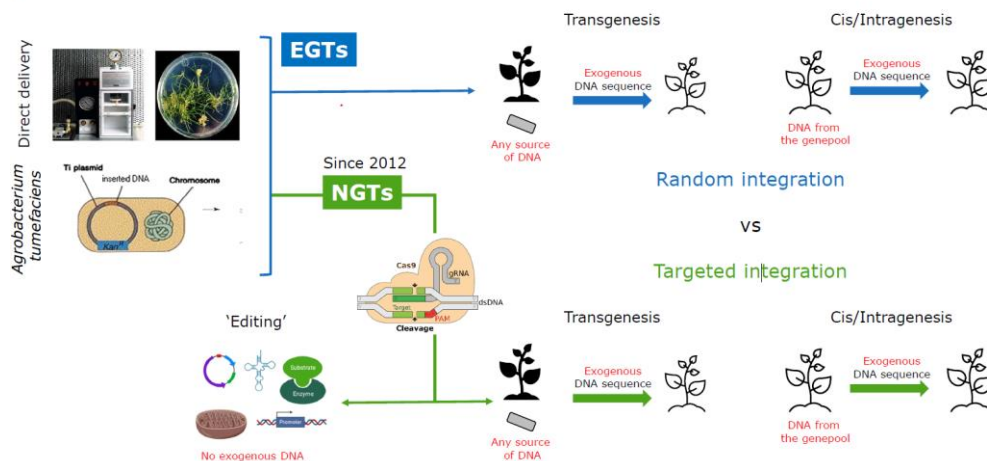


Figure 6. Schematic overview of the differences between EGTs and NGTs. NGTs “since 2012” refer to the discovery of the CRISPR-Cas technology. Source: Nogué & Papadopoulou (2022), EFSA GMO Panel and NIF unit.

An extensive overview of NGTs used for genome modification in plants, animals and microorganisms was given by the Joint Research Centre of the European Commission (Broothaerts et al., 2021). An important conclusion was: “NGTs allow to create genome alterations directly in elite germplasm or differentiated cells and thus shorten the development time for organisms with desired phenotypes and for cells to be used for gene therapy. As the changes are often small and often instructed by similar changes identified in other organisms, the resulting products containing the genome alterations display more predictable phenotypes and need less time for further testing.” Four main categories were distinguished by Broothaerts et al. (2021):

- **NGTs using Site-Directed Nucleases (SDNs) that create a double-strand break in DNA.** These include applications of site-directed nuclease-mediated genome editing (SDNs: endonucleases, zinc finger nucleases, TALEN and CRISPR-Cas), site-specific recombinase-mediated engineering, site-specific DNA transposition.
- **NGTs achieving genome editing without breaking the DNA double helix or generating only a single-strand DNA break.** These include applications of oligonucleotide-directed mutagenesis (ODM), base editing, prime editing.
- **NGTs inducing epigenomic changes or changes affecting DNA transcription into RNA.** These NGTs include applications of site-specific modulation of epigenetic state (DNA methylation modifiers, histone modifiers), site-specific activators and repressors (CRISPRa and CRISPRi).

- **NGTs acting specifically on RNA.**

These NGTs include RNA base editing, oligonucleotide-mediated RNA interference, CRISPR-Cas-mediated PAM-independent RNA interference, RNA splice isoform manipulation.

The most important tools use site-directed nucleases (SDNs), enzymes that recognise a specific sequence (protospacer adjacent motif or PAM) in the genome and cleave the DNA at that place, thus creating double-strand breaks (DSBs) in the genome. The genome editing is then the result of the repair of these DSBs by endogenous DNA repair mechanisms (Figure 7). They can be used with or without an added donor sequence that can function as a template during the repair process. A variety of genomic modifications is made after the DSB using two main repair pathways: non-homologous end joining (NHEJ, mostly in plants, without donor sequence template) and homology-directed repair (HDR, with a donor sequence as template for the repair process) (Chen et al., 2019). Definitions for three SDN approaches are given in EFSA (2022) (see also Nogué & Papadopoulou, 2022). We define them here as follows:

- **SDN-1:** Site-directed nuclease type 1 introduces random mutations (substitutions, insertions, and deletions) at the predefined target genomic locus.
- **SDN-2:** Site-directed nuclease type 2 makes use of template DNA to generate a predicted modification (i.e., intended sequence modification) at the predefined target genomic locus.
- **SDN-3:** Site-directed nuclease type 3 introduces a large stretch of donor DNA (up to several kilobases) into the predefined genomic locus.

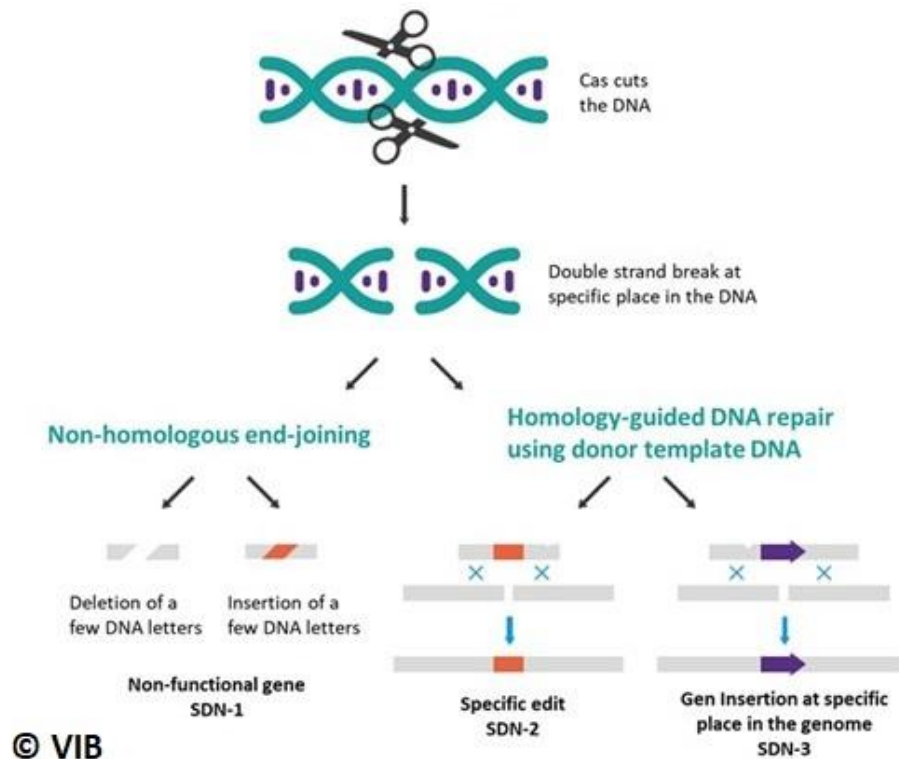


Figure 7. Repair of the double-strand breaks by DNA repair mechanisms: NHEJ and HDR using donor template DNA. © VIB

The most important SDN-based NGT is the **Clustered Regularly Interspaced Short Palindromic Repeats-associated protein (CRISPR-Cas) technique**, first applied for genome editing in 2012-2013. The CRISPR-Cas technique has its origin in type II prokaryotic adaptive immunity systems that protect bacteria/archaea against invading phages/plasmids (Broothaerts et al., 2021). The most common nuclease is Cas9, derived from *Streptococcus pyogenes*, but other CRISPR-associated nucleases are also used, such as CRISPR-Cpf1, which has a higher potency, specificity, and possibilities of wider application (Ahmar et al., 2020; Alok et al., 2020). The CRISPR loci consist of a cluster of repeated nucleotides, comprising a Cas9-encoding operon, transcription machinery, and consecutive repeats that originate from the DNA of the invading phages/plasmids, separated by spacer sequences (Ahmar et al., 2020). The recognition of the sequences of previously invading viral genomes due to the incorporation of their CRISPR loci induces the cleaving of these sequences in case of a subsequent invasion.

As part of the GenEdit project (funded by the Federal Public Service for Public Health, Food Chain Safety, and Environment), CRA-W created a database documenting more than 1 000 entries detailing modifications by NGT from research efforts. It is interesting to note that 90 % of the organisms were modified using a CRISPR-Cas technology, whereas only 5 % used the TALEN Technology. The success of CRISPR-Cas over other technologies can be explained by the following:

- **Simplicity of use.** The CRISPR-Cas technique is relatively easier to implement compared to TALEN. CRISPR-Cas utilises a guide RNA to target a given DNA sequence specifically, while TALEN requires the design and production of custom proteins for each target.
- **Flexibility.** CRISPR-Cas easily modifies the target sequence, simply by changing the guide RNA.
- **Efficiency.** CRISPR-Cas generally exhibits higher efficiency in genome editing compared to TALEN. CRISPR-Cas has been widely and successfully used in various species, including plants and animals.
- **Cost.** The CRISPR-Cas technique is more cost effective than TALEN in terms of designing and producing the tools required for genome editing.

The CRISPR-Cas9 system is designed for the editing of a specific nucleotide sequence (reviewed by, amongst others: Chen et al., 2019; Ahmar et al., 2020; Sandhya et al., 2020; Asmamaw Mengstie, 2022). CRISPR-Cas9 consists of guide RNA (gRNA) and the Cas9 nuclease (Figure 8). The gRNA comprises crRNA (crispr RNA, 17-20 nucleotides complementary to the target DNA) and tracrRNA (trans-activating crRNA, a conservative RNA strand with loops) that serves as a nuclease binding scaffold. While crRNA and tracrRNA are separate in natural form, they are synthetically combined for gene editing applications into sgRNA (single guide RNA). When the crRNA part of the synthetic sgRNA binds to the target DNA sequence, the latter will be cleaved by the Cas9 endonuclease 3-4 nucleotides after the PAM sequence, which is the Cas9 recognition site located next to the target DNA sequence. For SpCas9, most commonly used, this PAM site is 5'-NGG-3' with N being any nucleobase. The CRISPR-Cas9 elements (sgRNA and Cas9) can be delivered to the plant cells by different transformation and transfection methods (Figure 9). These include *Agrobacterium*-mediated transformation, particle bombardment of plant explants with plasmid DNA or Cas9/gRNA

ribonucleoproteins, polyethylene-glycol (PEG)-mediated transfection of protoplasts, protoplast electroporation, nanoparticle-mediated delivery, pollen magnetofection, viral vectors. After transformation/transfection, genome-edited plants are regenerated via plant tissue culture.

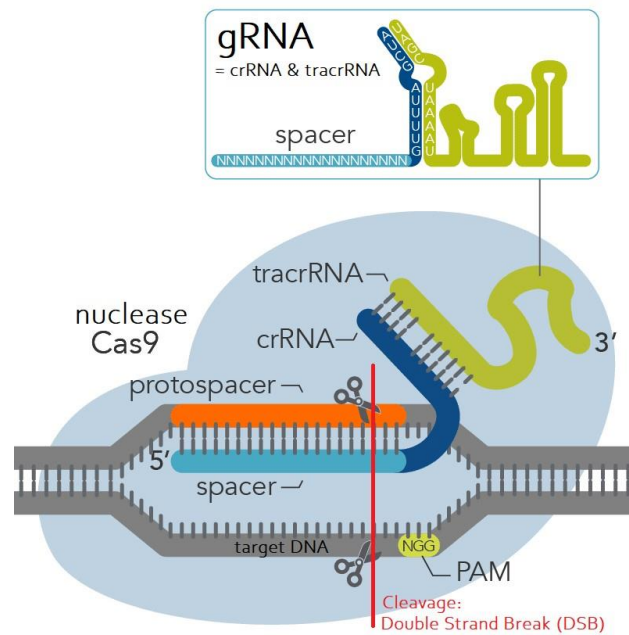


Figure 8. An overview of the CRISPR-Cas9 system, cleaving target DNA. Source: Image modified after Integrated DNA Technologies (IDT, 2023).

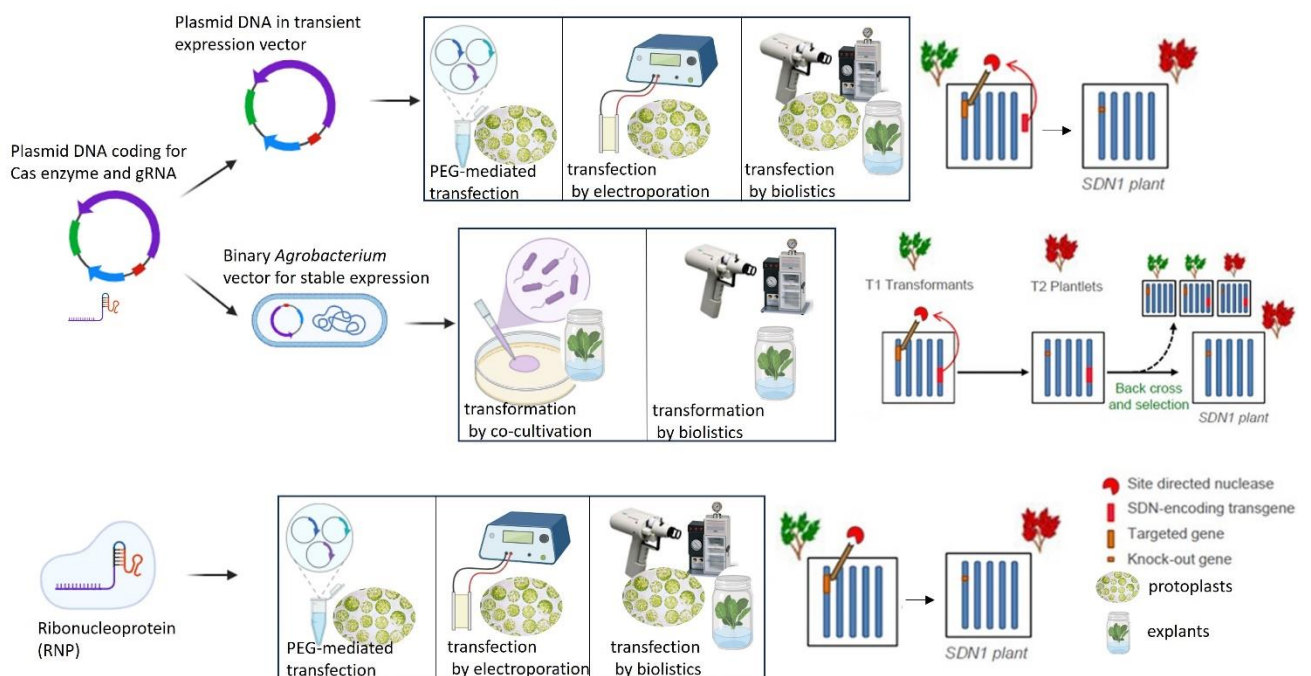


Figure 9. Transformation and transfection methods for the delivery of the CRISPR-Cas9 system and their different outcomes and strategies to obtain transgene-free NGT plants. © ILVO, K. Van Laere.

When applying *Agrobacterium*-mediated transformation, e.g., via floral dip or co-cultivation, the CRISPR-Cas9 elements are built into the plant genome as a transgene (stable expression of CRISPR-Cas elements). To obtain transgene-free NGT plants, back-crosses or selfings are performed during several generations so NGT plants without the transgene can be selected (Figure 9). When applying transfection of protoplasts with plasmid DNA or RNP using PEG, electroporation or bombardment, the CRISPR-Cas elements will not be incorporated into the plant genome (transient expression), the CRISPR-Cas elements will disintegrate from the plant cell soon after inducing the double-strand break at the target DNA site (see also Chen et al., 2019).

1.5.2 NGT crop use

CRISPR-Cas-mediated gene knock-out, insertion, and replacement can be applied to enhance yield, quality, disease resistance, and stress tolerance traits in crops and to improve hybrid breeding and crop domestication (Chen et al., 2019). The list with NGT applications grows every day, some examples are given by Ahmar et al. (2020: table 2), including resistance to powdery mildew in tomato, low-gluten wheat, salt-stress adaption and stomatal density regulation in rice, herbicide resistance in maize, condensed tannin content in poplar, early flowering in soybean, scab resistance in apple, drought tolerance in perennial ryegrass, etc.

Recently, in Belgium, the Flemish Institute for Biotechnology (VIB), in collaboration with the Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), was granted permission for the conduct of three field trials of genome-edited maize after greenhouse observations showed that the modified plants are more resistant to climate stress or easier to digest. CRISPR-modified maize plants show improved growth in the greenhouse when they experience drought. This will now be further tested in field conditions. If confirmed, these maize plants will be better adapted to tackle climate change with longer dry periods. In the Netherlands, at the Wageningen University, another breakthrough was announced in genome editing applications, i.e., in fighting potato late blight (*Phytophthora infestans*) in potatoes. At the moment, farmers are applying fungicides 15-20 times per growth season to protect their crops against late blight. Using CRISPR-Cas, non-transgene, resistant potatoes were created by modifying non-functional resistance genes from susceptible potato varieties to resistant gene variants found in wild potato species (WU, 2023; Moñino-López, 2023). **These results have the potential to drastically reduce fungicide use for this crop.**

In 2021, the Joint Research Centre of the European Commission also published a review of current and future worldwide market applications of NGTs (Parisi & Rodríguez-Cerezo, 2021). The study covers NGT applications in multiple stages: (1) commercial stage (currently marketed in at least one country), (2) pre-commercial stage (ready to be commercialised in at least one country, 5-year horizon), (3) advanced R&D stage (field trials, likely to reach the market in medium term), (4) early R&D stage (proof of concept). A total of 427 applications in plants were identified. One application was already marketed (soybean modified with TALEN, Group 1 NGT). 16 plants were in the pre-commercial stages (maize, soybean, rice, potato with herbicide tolerance, fungal resistance, modified oil or starch composition and non-browning properties, tomato fortified with dietary supplement GABA, herbicide tolerant pigeon pea and flax, pennycress and camelina with modified oil content). A total of 117 and 292 plant applications were identified within the advanced and early R&D stage respectively. The

genome-edited traits include disease resistance, abiotic stress tolerance (drought, salinity, heat), modified composition (starch and oil, fibre or vitamin content), reduced harmful properties (toxins, allergens, acrylamide) or gluten. **Most NGT plant applications are intended for a modified composition, biotic stress tolerance, plant yield and architecture** (Figure 10). **Cereals dominate NGT plant applications, followed at a distance by oil and fibre crops, vegetable crops, tuber/root vegetables and fruits** (Figure 11). **A total of 184 NGT-plant applications were developed by a private company, while 260 applications came from public and academic institutions.** The great majority of NGT plants were modified by CRISPR (70.8 %), followed by oligonucleotide-directed mutagenesis (ODM) (8.4 %) and TALEN (7.4 %).

In the GenEdit database (still in development by CRA-W), there are currently 1,019 applications listed. These include 833 plants, 185 animals, and 1 mushroom. However, only 10 of them are currently (2023) commercialised worldwide, including tomatoes with high levels of gamma-aminobutyric acid (GABA) from Japan, herbicide-tolerant canola in the US and Canada, maize with an increased starch amylopectin proportion, soybean with high-oleic oil content and non-browning mushroom in the USA. In this GenEdit database, among the 66 plant species listed, the major representatives are rice (286 applications), tomato (97), maize (75), wheat (41), soybean (38), potato (28), tobacco (24), and barley (21). China and the US are, by far, the main contributors with 551 and 240 entries, respectively (these numbers also include GE animals). Regarding trait categories, biotic stress tolerance represents 12 %, abiotic stress tolerance 7 %, herbicide tolerance 6 %, modified composition (fatty acids, protein, amylose, sugar, lignin, milk composition) 10 %, meat production by increasing the size and/or the musculature of the animals (GE animals, not treated in the proposal of regulation) 6%, yield and agronomic traits (grain size and weight, plant height, colour, ripening, flowering, male sterility) 36 %, medical purposes (represented by GE animals) 11 %, and research 9 % (optimisation of gene editing, understanding the role of genes).

These databases prove that the first CRISPR-Cas cultivars are already grown outside Europe and many cultivars and applications are in the pipeline. In Europe however, no product development has occurred so far, even though Europe is a technological stronghold in this domain and Europe's ambition on Farm to Fork and Green Deal strategies is very high. This requires the sustainable intensification and efficient genetic improvement of crops. A more adapted legislation for NGTs would fill this productivity gap in Europe.

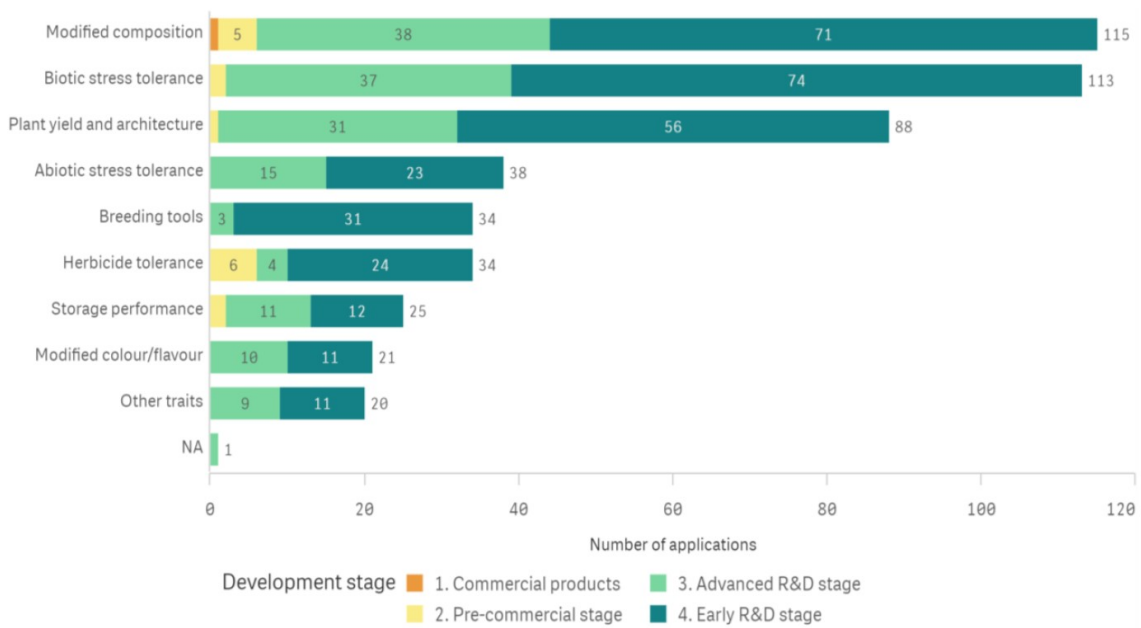


Figure 10. Entries in the database for NGT-derived plants by trait category and development stage. Source: Parisi & Rodríguez-Cerezo (2021: fig. 7a).

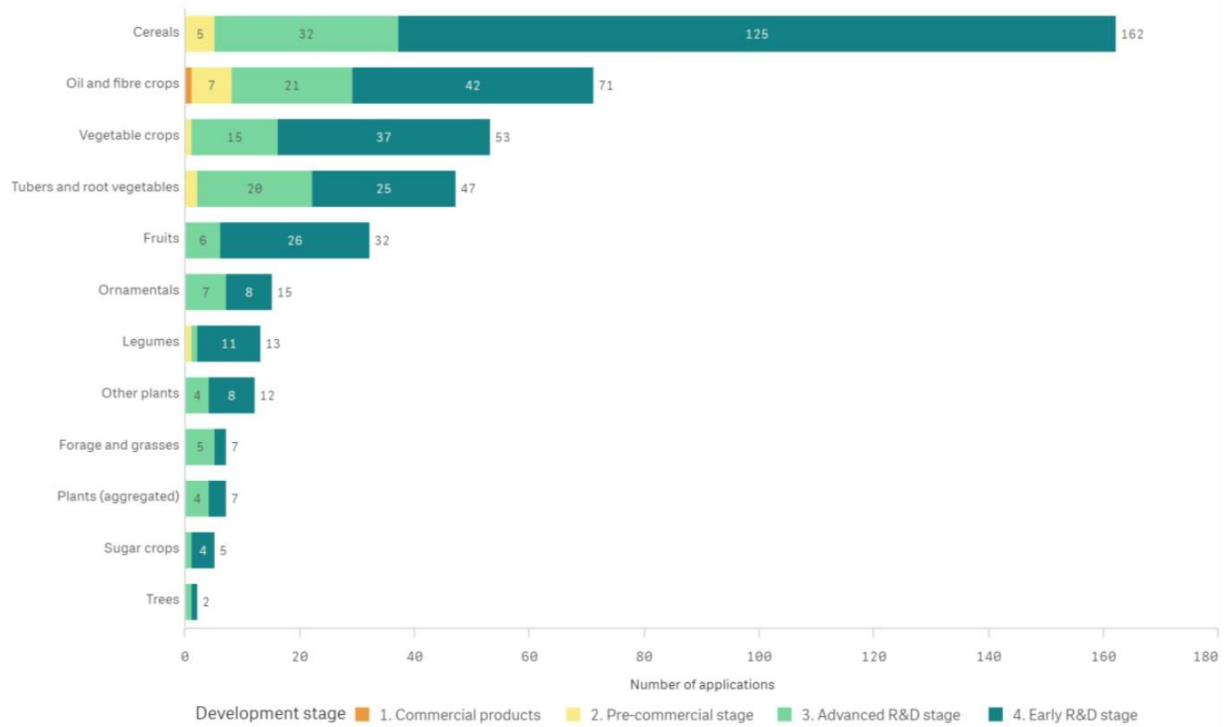


Figure 11. Entries in the database for NGT-derived plants identified in the four development stages by plant group. Source: Parisi & Rodríguez-Cerezo (2021: fig. 6).

1.5.3. *Detection of NGTs: state of the art*

As part of the necessary authorisation procedure for marketing GMOs in the EU, event-specific methods for the detection, identification, and quantification of GMOs and in their associated food and feed products are required. Polymerase chain reaction (PCR) methods can detect, identify, and quantify EU-authorized "traditional GMOs" by targeting the stable integration site of foreign DNA elements in the genome. However, plants produced using NGTs may not have any integration of foreign DNA or corresponding genetic elements commonly found in "traditional GMOs". Therefore, the detection of organisms produced by NGTs presents a significant challenge as the induced mutations (often small substitutions, insertions, or deletions) in NGTs cannot be distinguished from mutations that occurred naturally or via conventional breeding and random mutagenesis (Cao et al., 2011). The genome sequence of a genome-edited plant may only differ minimally from its parental sequence. As a result, new detection approaches must be developed and evaluated for the detection of these small modifications in pure and mixed products, and to distinguish edited from non-edited organisms. Some theoretical papers discuss possible methodologies for the detection of NGT-GE organisms (Bertheau et al., 2019; Grohmann et al., 2019).

Single nucleotide polymorphism (SNP) detection linked to NGT is possible, as demonstrated by studies such as Miyaoka et al. (2016) and Cheng et al. (2018). However, differentiating genome edits from natural mutations remains quite challenging (Cao et al., 2011) and will not be feasible in some cases. A case-by-case analysis focusing on the different potential mutation origins needs to be performed (Guertler et al., 2023).

The detection of organisms modified by NGTs in food and feed products is even more difficult because there is not just one individual from which we can extract large quantities of DNA. Instead, there is a large population of individuals. Additionally, mixed products contain several ingredients and, in processed products, DNA can be degraded.

Because NGTs do not rely on the introduction of foreign DNA, current methods might fail to meet the minimum performance criteria (Zanatta et al., 2023). In 2019, the European Network of GMO Laboratories (ENGL) published a guidance document titled "Detection of Food and Feed Plant Products Obtained by New Mutagenesis Techniques," which highlights that some issues cannot be resolved presently, due to a lack of experimental verification (ENGL, 2019). The document suggests that alternative screening strategies, targeting all known genome-edited events simultaneously, need to be developed to facilitate routine enforcement. A revision of this document renamed "Detection of Food and Feed by Targeted Mutagenesis and Cisgenesis" was published in 2023 (ENGL, 2023).

Real-time PCR is the reference method for the detection of traditional GMOs. To tackle the risk of non-specific amplification when using classical double-dye probes to detect single nucleotide variations (SNVs), several strategies have been reported, e.g., shorten the probes, maximise the importance of a mismatch. These strategies include coupling a minor groove binder (MGB) moiety at the 3' end of the probe, introducing locked nucleic acids (LNAs) or peptide nucleic acids (PNAs) in the probe sequence, etc. (Fouz et al., 2020; Zhang et al., 2021). Targeting SNVs and using other chemistries can have an impact on the performance criteria of the detection methods. For example, the real-time PCR method proposed by Chhalliyil et al. (2020) for the detection of Cibus rapeseed, a genome-edited rapeseed tolerant

to imidazolinone and sulfonylurea herbicides, uses LNA-modified primers to increase its specificity for the SNV. However, experimental evidence has shown that the method lacks robustness and specificity to detect the SNV (Weidner et al., 2022). As a result, the method was deemed unfit for the official control of oilseed rape products in the EU.

Digital PCR (dPCR) can also be used to detect and quantify genome-edited organisms using chemistries similar to those used in real-time PCR. Digital PCR has advantages, such as absolute quantification without dependence on calibration curves and a lower susceptibility to PCR inhibitors. Digital PCR, combined with an LNA-modified probe, has been used to detect and quantify genome-edited rice that contains a deletion or insertion of a few nucleotides (Zhang et al., 2021). Some approaches for the detection of SNVs use two probes for the same PCR amplicon, one for the mutated and one for the wild-type sequence (Mock et al., 2016). A general workflow using duplex digital PCR was also recently proposed for the detection of a genome-edited rice carrying a single nucleotide insertion (Fraiture et al., 2022). The dPCR method showed satisfactory sensitivity and specificity and met the minimum performance requirements for GMO testing as outlined by the ENGL. This study provided a good proof-of-concept on a sample of low complexity.

Where both qPCR and dPCR can only screen for mutated plants without providing details on the nucleotide level, high-throughput sequencing has the ability to detect mutations and give information of the exact DNA change in both plants (Délye et al., 2020; Vereecke et al. 2023) and animals (Maniego et al., 2022). However, the discriminatory power of this method when applied to mixtures or compound products is currently unknown. A targeted test was recently conducted on genome-edited rice, demonstrating good sensitivity and adequacy between the considered GE rice line content and allele frequency (Fraiture et al., 2023). Another advantage of amplicon sequencing is its potential for high multiplexing, though its application in the context of NGTs needs to be evaluated.

Two projects were recently funded by the European Commission for the detection of organisms modified by NGTs: the DETECTIVE project (detection of NGT products to promote innovation in the European Union; Grant agreement ID: 101137025) and the DARWIN project (Grant agreement ID: 101136462). These projects started in January 2024 and will assess both targeted and untargeted approaches.

In summary, while predicted off targets can be detected, distinguishing plants obtained through conventional breeding and NGT plants is often challenging, if not impossible, with the current techniques.

2 Current GMO legislation

An excellent overview of the history and application of the European GMO legislation is outlined in the NGT advisory report of the *Vlaamse Adviesraad voor Innoveren en Ondernemen* (VARIO, 2023). At the moment (2023), the key legal instruments are the “**horizontal GMO Directives**” **2009/41/EC** (contained use of Genetically Modified Micro-Organisms) and **2001/18/EC** (deliberate release of Genetically Modified Organisms), and other legislations dealing with specific products, in particular **Regulation (EC) 1829/2003** on GMOs destined for food or feed, and **Regulation (EC) 726/2004** on medicinal GMOs for human or veterinary use (Belgian Biosafety Server, 2023a, 2023b, 2023c). Regarding the introduction of GM plants, **Directive 2001/18/EC**⁹ is the most important, as it defines a common methodology to assess the risks for the environment associated with the release of GMOs and common objectives for monitoring after their deliberate release or placing on the market. The directive includes:

- An authorisation system for the deliberate release of GMOs, including a “case-by-case” assessment of the risks to human health and to the environment, excluding organisms obtained through techniques of genetic modification listed in Annex I B of Directive 2001/18/EC (including random mutagenesis);
- Differentiated rules for the experimental release of GMOs (field trials) and the placing on the market of GMOs (step-by-step approach, Figure 12);
- Requirements concerning the labelling and monitoring of released GMOs;
- An opt-out system for EU countries refusing the cultivation of GMO plants.

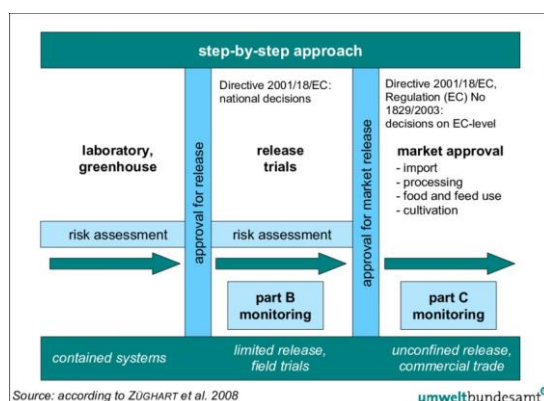


Figure 12. Step-by-step approach of the European legislation for the introduction of GMO plants.
Source: Umweltbundesamt (2011).

The complex authorisation procedure makes the current GMO legislation in the European Union one of the strictest in the world. The first step is risk assessment by EFSA and Member States' Biosafety Advisory Councils (BAC), followed by risk management by the European Commission and Member States. The procedure for field trials (Figure 13) and placing on the market differs (Figure 14). For field trials, the application is submitted to the member state, there is a notification requirement to other member states (see Biosafety Advisory Council, 2023). For the placement on the market, applications for commercialisation authorisations are submitted for the entire European market through a reporting Member State and involve all Member States in the authorisation process. A detailed explanation is given on the website of the Belgian Biosafety Server (2023c).

⁹ <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02001L0018-20210327> (accessed on 17/1/2024)

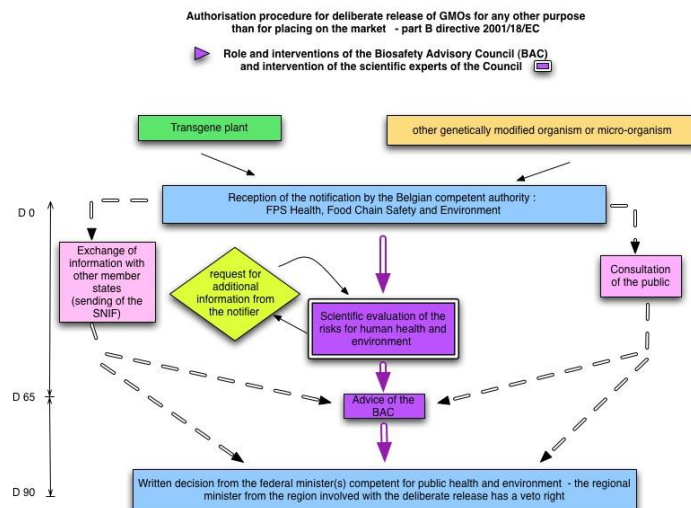


Figure 13. Regulatory scheme for notifications submitted for experimental trials with GM plants in Belgium. Source: Biosafety Advisory Council (2023).

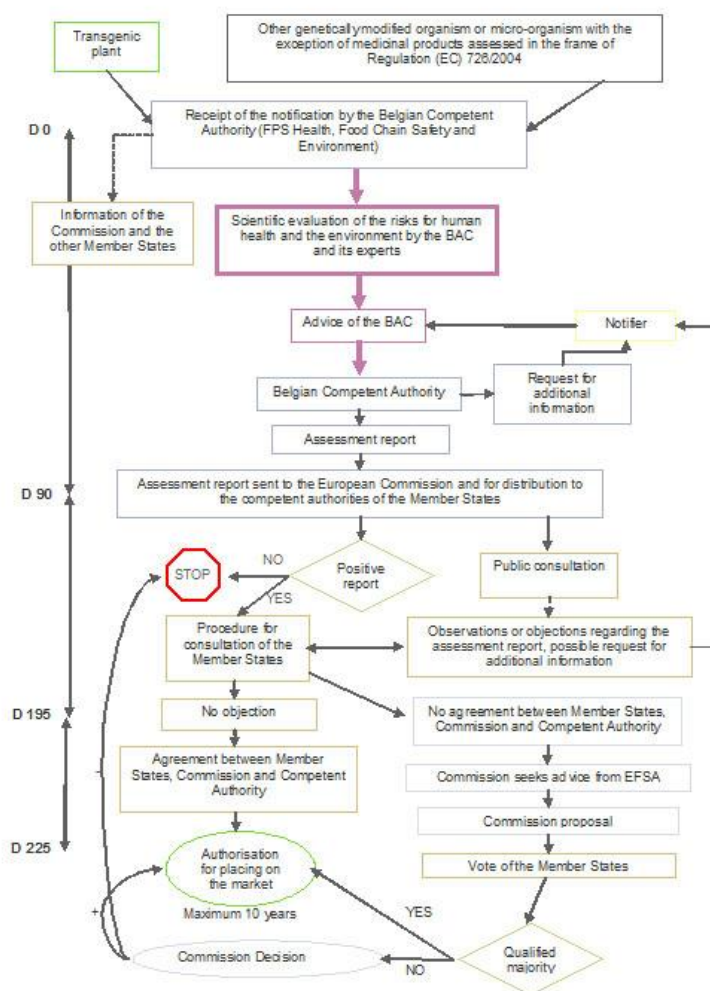


Figure 14. Regulatory scheme for notifications submitted for the placement on the market of GM plants in Belgium. Source: Belgian Biosafety Server (2023).

3 Proposed NGT legislation (Version 7/12/2023)

On 5 July 2023, the European Commission submitted a proposal for a regulation on plants obtained by certain NGTs and their food and feed products, aiming to enable the EU agri-food sector to contribute to the innovation and sustainability objectives of the European Green Deal and Farm to Fork and Biodiversity strategies, and to enhance the sector's competitiveness, while maintaining a high level of protection of health and the environment. An amended compromise text was sent to the European Council and was published on 7 December 2023. This draft¹⁰ is discussed here.

The proposed NGT legislation deals only with plants (Archaeplastida or Phaeophyceae; micro-algae are not included) modified via targeted mutagenesis and cisgenesis (including intragenesis) using genes from the breeders' gene pool (the total genetic information available for conventional breeding including from distantly related plant species that can be crossed by advanced conventional breeding techniques). Plants with inserted genetic material from non-crossable species (transgenesis) will always be covered by the classic GMO Directive 2001/18/EC, whatever the technique used. The regulation is a *lex specialis*: it only introduces specific provisions for NGT plants and their products. When there are no specific provisions for a certain (NGT) plant, Directive 2001/18/EC is applied.

The proposed legal framework introduces two categories of NGTs:

- **Category 1 NGT plants (NGT1) could also occur naturally or could be produced by conventional breeding techniques (criteria specified in Annex I). Hence, they are considered equal to conventional plants and treated in the same way.** Only the seeds of NGT1 plants will be labelled as NGT to provide transparency. The verification procedure of NGT1 plants prior to field trials should be conducted by the competent authorities of the member states. The progeny of NGT1 plants obtained by conventional breeding techniques should also be treated as NGT1 plants, without the need to go through the verification procedure prior to their release on the market. Conversely, the progeny deriving from the application of targeted mutagenesis or cisgenesis on an NGT1 plant shall be subject to the procedure to verify the fulfilment of the criteria of equivalence (Annex I), prior to its release or placing on the market as NGT1 plant. The criteria for an NGT1 plant can be changed in the future based on up-to-date scientific knowledge. **Intragenic plants are excluded from Category 1**, as novel hazards can be associated with intragenic plants compared to cisgenic and conventionally bred plants. **NGT plants that include tolerance to herbicides among the intended traits are also excluded from Category 1.**
- **Category 2 NGT plants (NGT2) includes all plants that are not NGT1 plants. NGT2 plants and their products should remain subject to the requirements of the Union GMO legislation given that, on the basis of current scientific and technical knowledge, their risks need to be assessed.** The principles of the **Risk Assessment** (Environmental risk assessment for NGT2 plants and also toxicology, allergenicity and nutritional assessment for NGT2 food and feed) for NGT2 plants are outlined in Annex II of the proposal. Given the wide variety of NGT2 plants, the amount

¹⁰ <https://data.consilium.europa.eu/doc/document/ST-16443-2023-INIT/en/pdf> (accessed on 19/1/24)

of information necessary for the risk assessment will vary on a case-by-case basis (based on considerations on the history of safe use, familiarity with the environment, and the structure of the modified/inserted sequences). As detection sometimes proves to be difficult, an analytical method should be provided by the notifier/applicant, but, if duly justified, the modalities to comply with analytical method performance requirements should be adapted. After deliberate release, post-market monitoring for environmental effects is needed according to the current GMO legislation. However, the competent authority can decide not to require this when justified based on certain objective criteria. **Regulatory incentives (accelerated procedure for risk assessment and enhanced pre-submission advice) are offered to potential notifiers/applicants when the plant/product contains traits with the potential to contribute to a sustainable agri-food system** (criteria in Annex III), to steer the development of NGT2 plants towards such traits. Additional incentives are afforded when the notifier/applicant is a medium-sized enterprise (SME), by granting fee waivers for the validation of detection methods to SMEs and pre-submission advice covering the design of studies to be carried out for risk assessment. **NGT2 plants featuring herbicide-tolerant traits should not be eligible for incentives.** Besides, an opt-out will be included for member states to restrict or prohibit the cultivation of NGT2 plants, analogous to classic GMOs.

An overview of how the proposed NGT regulation fits into the existing regulations is given in Table 1. The 'Annex 1' criteria for NGT1 plants are given in Table 2. Table 3 gives the Annex III criteria with traits of NGT2 plants justifying incentives.

The European Commission's proposal did not include an impact assessment on the impact of **patenting of plants** and related licensing and transparency practices may have on innovation in plant breeding, on breeders' access to plant genetic material and techniques and on the availability of plant reproductive material to farmers, as well as the overall competitiveness of the EU plant breeding industry. At the moment, Directive 98/44/EC¹¹ on the legal protection of biotechnological inventions sets out principles regarding the patentability of biological material including plants. It is noted in Recital 46a that "***It is important to ensure that farmers and breeders have access to techniques and material to promote the diversity of plant reproductive material, such as seeds, at affordable prices, while also strongly supporting innovation in both conventional and organic plant breeding by preserving investment incentives***". Therefore, Article 30bis stresses that the Commission shall conduct an impact study, the findings of which shall be reported not later than 31 December 2025. Based on the outcome of the study, the Commission shall inform on measures to follow-up or, if appropriate, submit a proposal.

¹¹ <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31998L0044> (accessed on 19/1/24)

Table 1. Regulatory categorisation and requirements of plants in the EU including the NGT proposal.
Adapted from Dima et al. (2023: table 1)

	Conventional plants	NGT1 plants: 'conventional-like'	NGT2 plants: 'GMO-like'	GMOs 'transgenic' Dir. 2001/18/EC	Annex I B GMOs 'exempted'
		Targeted editing		Non-targeted modification	
Process/product	Classical breeding techniques	Equivalent to conventional plants (Annex 1 criteria)	Not equivalent to conventional plants	Transgenesis	Non-targeted Mutagenesis induced by radiation/chemicals (and other GMO Annex IB techniques)
Risk assessment	No	No	Adapted GMO risk assessment	Full GMO risk assessment	No
Labelling	No	Seed bag labelling	GMO labelling and traceability	GMO labelling and traceability	No
Detection method	No	No	Yes (if a unique method is available)	Yes	No
Variety testing and registration	Yes	Yes	Yes	Yes	Yes
Organic farming	Yes	No	No	No	Yes
Cultivation opt-out	No	No	Yes (7/12/23)	Yes	No
Coexistence measures	No	No	Yes	Maybe allowed	No

Table 2. Proposal of criteria used to identify NGT1 plants (Annex I of the NGT proposal, 7/12/2023).

ANNEX I	
Criteria of equivalence of NGT plants to conventional plants	
<p>A NGT plant is considered equivalent to conventional plants when it differs from the recipient/parental plant by no more than 20 genetic modifications per monoploid genome of the types referred to in points 1 to 4, in any DNA sequence sharing sequence similarity with the targeted site that can be predicted by bioinformatic tools.</p>	
<p><u>Criteria specific to the use of targeted mutagenesis:</u></p> <p>(1) substitution or insertion of no more than 20 nucleotides; (2) deletion of any number of nucleotides;</p>	
<p><u>Criteria specific to the use of cisgenesis:</u></p> <p>(3) on the condition that the genetic modification does not interrupt an endogenous gene or that the resulting combination of DNA sequences in the recipient plant already occurs in a species from the breeders' gene pool:</p> <p style="margin-left: 40px;">(a) insertion of a continuous DNA sequence existing in the breeders' gene pool; (b) substitution of an endogenous DNA sequence with a continuous DNA sequence existing in the breeders' gene pool;</p> <p>(4) targeted inversion of a sequence of any number of nucleotides.</p>	

Table 3. Proposal of traits justifying incentives for NGT2 plants
(Annex III of the NGT proposal, 7/12/2023).

ANNEX III

Traits referred to in Article 22

Part 1

Traits justifying the incentives referred to in Article 22:

- (1) improved yield, including yield stability and yield under low-input conditions;
- (2) tolerance/resistance to biotic stresses, including plant diseases caused by nematodes, fungi, bacteria, viruses, insects and other pests;
- (3) tolerance/resistance to abiotic stresses, including adaptation to climate change conditions
- (4) more efficient use of natural resources, such as water and nutrients;
- (4 bis) reduced need for external inputs, such as plant protection products and fertilisers;
- (5) characteristics that enhance the sustainability of storage, processing and distribution;
- (6) improved quality or nutritional characteristics;
- (7) bioremediation.

Part 2

Traits excluding the application of the incentives referred to in Article 22: tolerance to herbicides.

4 Technical remarks on the proposal

4.1 Limitations of the present proposal

The Commission proposal concerns a *lex specialis* which only applies to certain NGTs, as opposed to a more general approach covering all types of genetic modifications. Such a *lex specialis* approach inherently entails limitations and risks. Indeed the proposal concerns a limited number of techniques, excluding potentially useful other techniques, currently existing or possibly developed in the future. In addition, problems with the current GMO legislation are not addressed and the proposed regulation risks being in conflict with other legislation, in particular Directive 2001/18/EC and Regulation (EC) 1829/2003.

4.2 The criteria for NGT1 plants in Annex I

Annex I of the Commission proposal gives criteria to define NGT1 plants and distinguish them from NGT2 plants (Table 2). NGT1 plants are considered to be equivalent to conventionally bred plants when these criteria are met.

First, Annex I limits the number of genetic modifications that an NGT1 plant may contain to a maximum of 20 per monoploid genome. The maximum number of genetic changes allowed will always be somewhat arbitrary, however, in light of the variability in conventionally bred plants (as revealed by large genome resequencing efforts, e.g. Niu et al., 2023; Wang et al., 2018; Yuan et al., 2021), a maximum of 20 genetic modifications as a limit for plants to be considered as NGT1, is quite conservative. **The equivalence criteria as rephrased in the proposal recently approved by the European Parliament (“The number of the following genetic modifications, which can be combined with each other, does not exceed 3 per any protein-coding sequence taking into account that mutations in introns and regulatory sequences are excluded from this limit”) may better reflect the large variability found in crop varieties resulting from and used in conventional breeding.**

Annex I further specifies that NGT1 plants obtained by targeted mutagenesis should not contain insertions or substitutions of more than 20 nucleotides. Therefore, if a plant contains, for example, an insertion of 25 nucleotides, it cannot be considered an NGT1, even if this is the only modification of the genome. **It will be important to provide transparency on the scientific basis used to choose this number and give information on the theoretical calculations to determine from which length onwards a sequence is considered as a “foreign DNA sequence” not occurring in the gene pool of a plant species.**

Thirdly, deletions of any number of nucleotides are allowed in NGT1 plants obtained by targeted mutagenesis according to Annex I. **To avoid unwanted effects (e.g. losing genes related to nutritional quality), it would be useful to specify that the deletions should not extend beyond a single targeted (protein-coding) sequence.**

The criteria specific to the use of cisgenesis mention that the genetic modification can not interrupt an endogenous gene. This is not in line with the criteria for targeted mutagenesis. Hence, it should be reconsidered.

Finally, it will be important that the European Commission follows up scientific knowledge on the types and extent of modifications that can occur naturally, through conventional breeding or by random mutagenesis in plant genomes and adapts the criteria in Annex I whenever justified by new scientific insights.

5 Impact on human health and the environment

The various New Genomic Techniques do not pose intrinsic safety and health concerns. The discussion on the safety of NGT plants mainly depends on the trait being modified or introduced. The impact on human health and the environment depends on the objective of the NGT plant.

5.1 Potential impact on human health

5.1.1 General remarks

When thinking about possible risks to human health, a potential health concern may be that toxins could be produced by the plant for resistance, which are subsequently consumed by humans. The best-known example of this resistance strategy are Bt crops (widely consumed worldwide), which produce the Cry protein that is safe for humans (EPA: Mendelsohn et al., 2003). However, this example is a classic transgenic GMO, so the comparison with NGTs is incorrect. NGTs involve searching for traits in related plants that can be introduced via cisgenesis and targeted mutagenesis (NGT1, NGT2) or intragenesis (NGT2 only). Moreover, **the majority of disease/plague-resistant or tolerant NGT plants do not use toxins and follow other strategies** (Borrelli et al., 2018; Schenke & Cai, 2020). Indeed, resistance/tolerance usually relies on switching off/modifying susceptibility genes. After recognition, a hypersensitive response follows. This is an entirely natural defence mechanism of the plant that is enhanced. In addition, NGT plants are often not introduced "directly" on the market, but form the "elite material" that is then further used in breeding programs for subsequent crossing and selection steps. Many NGT applications are identical to what happens in conventional breeding, only the development process is much faster and more targeted (see Fig. 2). As explained before, genome/gene editing resulting from cisgenesis and targeted mutagenesis through NGTs is in most cases impossible to detect and distinguish from plants obtained by conventional breeding techniques (ENGL, 2023; see 1.5.3). **Besides, the European Food Safety Authority concluded that the hazards arising from the use of a related plant-derived gene by cisgenesis are similar to those from conventional plant breeding** (EFSA, 2022b). It can therefore be expected that NGT1 plants will not pose additional hazards. For NGT2 plants, the full risk assessment procedure of GMOs remains the rule, ensuring optimal protection. Concerning the hazard identification and hazard characterisation of NGT2 food and feed, an analysis of agronomic, phenotypic and compositional characteristics is included, in addition to toxicology, allergenicity and a nutritional assessment. The proposed Regulation clearly describes under which objective conditions this risk assessment can be shortened (e.g., when sufficient data already exist on the history of safe-use of the NGT plant outside the EU, see Annex II of the proposal).

It should be noted that NGT1 plants will need to be tested similarly to varieties obtained through conventional breeding techniques. When a new variety is notified, the applicant has to fill in a questionnaire that includes all the (mainly phenotypic) characteristics of the crop, including, e.g., special new nutritional aspects. Afterward, Distinctness, Uniformity and Stability (DUS) testing will determine the characteristics of a new variety compared to the existing varieties. For vegetables and ornamental crops, DUS testing is sufficient; for agricultural crops, Value for Culture and Use (VCU) testing is also required. In VCU tests, new

varieties are sown several years apart in different institutes to test their value in different conditions (e.g., variation of soil or variation of weather conditions).

It is important for the Superior Health Council that plants with altered nutritional composition or with a health claim cannot simply be marketed without a health impact assessment (e.g., GABA-enriched tomatoes in Japan, advertised for blood pressure-lowering function). While adequate risk assessment is provided for NGT2 plants in the NGT proposal, NGT1 plants are treated like conventional plants. However, the NGT proposal addresses this concern in Recital 22: ***“In this regard, category 1 NGT food featuring a significantly changed composition or structure that affects the nutritional value, metabolism or level of undesirable substances of the food will be considered as novel food and thus fall into the scope of Regulation (EU) 2015/2283¹² of the European Parliament and of the Council and will be risk assessed in that context”***. Within the Novel Foods regulation, the safety assessment is carried out by the European Food Safety Authority based on dossiers provided by the applicants, containing data on the compositional, nutritional, toxicological and allergenic properties of the novel food as well as information on respective production processes, and the proposed uses and use levels¹³. **In this way, NGT1 plants with modified nutritional composition are also adequately screened.** Besides, Union rules on nutrition and health claims are established in **Regulation (EC) No 1924/2006¹⁴**, which will also be applicable to food/feed including NGT plants. In this way, it is ensured that **claims on labels and advertising are clear, accurate and based on scientific evidence.**

To keep an overview of the different NGTs, a European register is kept up to date. The Superior Health Council stresses here that it is important that there should also be control over the import of plants/seeds from outside the European Union, as the same level of safety is needed.

5.1.2 EFSA assessment

The proposed Regulation is scientifically based on several studies by the European Commission's Joint Research Centre (Broothaerts et al., 2021; ENGL, 2023) and the European Food Safety Authority (EFSA, 2021, 2022a, 2022b). The latter addressed the risk assessment of NGT plants for both humans and the environment. Earlier, EFSA (2012a) issued an opinion on plants developed through cisgenesis and intragenesis. Given the significant scientific progress in the subsequent decade, the conclusions were reviewed and updated by EFSA (2022b), while criteria for the risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis were outlined in a separate document (EFSA, 2022a). EFSA (2022b) concluded that:

- The conclusions of EFSA (2012a) remain valid. 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.
- 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

¹² <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32015R2283> (accessed on 19/1/2024)

¹³ <https://www.efsa.europa.eu/en/topics/topic/novel-food#efsas-role> (accessed on 19/1/2024)

¹⁴ <https://eur-lex.europa.eu/eli/reg/2006/1924/2014-12-13> (accessed on 19/1/2024)

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.
- The EFSA 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.

The proposed Regulation is thus in line with EFSA's recommendations. During the verification procedure, it is determined whether a plant is NGT1 or NGT2. If NGT1 (cisgenic and not intragenic), the hazards from the use are estimated to be similar to plants from conventional breeding. If the Novel Food Regulation applies, a risk assessment is carried out by EFSA. If a plant is NGT2, a detailed case-by-case risk assessment follows as exists for GMOs today.

5.1.3 Possible applications impacting human health

As concluded above, the impact on health and the environment depends on the objective of the NGT plant. Some examples studying human health can be given here:

- Indirect effect on human health: Disease/plague resistant plants can significantly reduce the amount of pesticides used, leading to a lower exposure of the general population to pesticides. Since their introduction, the use of pesticides has greatly increased yields, improved food safety and made the yield of crops more secure, which also benefited people's health (Cooper & Dobson, 2007). However, at the same time, the complex cocktail of substances has also been linked to the development of diseases (e.g., cancer), neurotoxicity and endocrine disruption observed in several epidemiological studies (e.g., Alavanja et al., 2004; SHC, 2019). Although many harmful active substances were banned and strict risk assessment and continuous monitoring (residue analysis and human biomonitoring) made the use of crop protection products in Europe safer, a key goal of the European Green Deal remains to halve the pesticide use by 2030. NGT plants help to reach that goal. Recent breakthroughs include non-transgenic potatoes being made resistant to late blight via CRISPR-Cas (WU, 2023; Moñino-López, 2023). These NGT potatoes have the potential to reduce fungicide use drastically and increase yields. For comparison in GMO plants, the introduction of insect-tolerant transgenic crops in the US also led to a reduction in insecticide use (Perry et al., 2013; Klümper & Qaim, 2014). All these initiatives indirectly contribute to improved human health.

- Direct effect on human health: NGTs are increasingly utilised in the biofortification of cereal crops (rice, wheat, barley, maize) and vegetable crops such as potato and tomato. The CRISPR-Cas-based crop genome editing has been utilised in imparting/producing qualitative enhancement in aroma, shelf life, sweetness, and quantitative improvement in starch, protein, gamma-aminobutyric acid (GABA), oleic acid, anthocyanin, phytic acid, gluten, and steroidal glycoalkaloid contents (Kumar et al., 2022). In December 2021, GABA-enriched tomatoes (Saantech Seed) were introduced in Japan, being the first plant-based CRISPR-edited food that entered the market¹⁵. It is claimed that these levels enhance the blood pressure-lowering function of tomatoes (Nonaka et al., 2017; Gramazio et al., 2020). In the EU, such claims will only be allowed to be made if they comply with Regulation (EC) No 1924/2006.
- Within the proposed regulation, NGT plants will not lead to more allergies: Within the polarised public debate, several worrying claims have been intentionally or unintentionally made, contributing to misinformation in the public. NGT plants are not GMOs (GM plants), given that all NGTs in the proposal of European Regulation are not transgenic. However, many of the arguments used against GMOs also recur with NGTs. A well-known example is the idea that GMO foods would lead to more allergies, despite vigorous testing and risk assessments. However, the opposite is true for several applications (Lee et al., 2017; FDA, 2023): biotechnology was used to remove a major allergen in soybean, thus demonstrating that genetic modifications can be used to reduce the allergenicity of food and feed (Herman, 2003). The unsubstantiated dangers associated with genetic engineering lead to the false perception that non-GM products are safer (d'Agnolo, 2005; Lee et al., 2017). In animals, no evidence of allergic reactions or immunotoxic effects was found by de Santis et al. (2019) in GM-fed animals versus non-GM-fed animals. In the NGT proposal, allergenicity tests are included in the risk assessment of NGT2 plants, as is the case for GMO plants.

In short, a proper verification procedure is essential. Furthermore, all NGT plants should comply with the existing EU legislation concerning plants, novel foods, and health claims, etc. In this way, an equally high level of protection of human health and the environment is ensured.

¹⁵ <https://www.nature.com/articles/d41587-021-00026-2> (accessed on 19/12/2023)

5.2 Potential impact on the environment

Environmental risks are also considered in the EFSA opinions and are taken into account in the risk assessment and monitoring of NGT2 plants. The following additional remarks can be made.

5.2.1 *Agro-ecological diversity*

A very one-sided use of herbicides (especially glyphosate) on herbicide-tolerant transgenic GMO crops in the United States led to the development and spread of herbicide-resistant weeds in recent decades (e.g., Owen & Zelaya, 2005). This increased the use of herbicides in agricultural regions where the development of resistant weeds was observed. This risk also exists with herbicide-tolerant NGT plants. **However, the European Commission learned from the US example and has excluded herbicide tolerance from NGT1 plants.** As a result, they will be treated as NGT2 plants, requiring the classic GMO risk assessment procedure. Moreover, herbicide-tolerant NGT plants can never qualify for incentives for a faster risk assessment. Hence, this aspect of the proposed Regulation contributes to the protection of the environment, biodiversity and agro-ecology.

5.2.2 *Crop diversity*

Conventional breeding and selection have narrowed the genetic base of crop varieties over time (Rauf et al., 2010; Salgotra & Chauhan, 2023). However, natural variety within species is an important source of beneficial traits, such as disease resistance or drought tolerance. NGTs can be used to increase the genetic diversity in agricultural systems, by further exploiting the genetic diversity present in wild plants. Recently, *de novo* domestication of *Solanum pimpinellifolium* could be achieved by the CRISPR-Cas9-mediated editing of six loci for yield and productivity in present-day tomatoes (*Solanum lycopersicum*). This increased the fruit size and number of *S. pimpinellifolium* by three and ten, while the lycopene accumulation increased by 500% compared to classic *S. lycopersicum* (Zsögön et al., 2018). These authors concluded that similar efforts may also be undertaken in other crops such as maize, wheat and sorghum.

5.2.3 *Crop yield*

While domestication of most crops took thousands of years, NGTs can help us achieve *de novo* domestication of other plants in a much shorter time frame. These new domestic plants may result in higher yields and theoretically contribute to less agricultural land needed to grow crops and more land reserved for nature.

5.2.4 *Restoring degraded ecosystems*

When used sustainably, genome editing with NGTs is a potential tool for restoring ecosystems (Breed et al., 2019). Given that the majority of earth's terrestrial environment are degraded and that climate change might create conditions that exceed the physiological tolerance of certain varieties, Breed et al. (2019) identified potential future applications in the recovery of degraded landscapes:

- “Using CRISPR–Cas9 to develop novel genotypes suited to challenging new conditions, while retaining existing desirable traits and a local genetic background”;
- “Developing gene drives to target essential fitness genes of foundation or keystone restoration species”;
- “Developing suppression gene drives to target essential survival or reproduction genes of unwanted pest species (such as exotic weeds and herbivores)”.

5.2.5 Climate-robust plants

At the moment, climate change already affects agricultural production. For example, rice (*Oryza sativa*) is vulnerable to both abiotic (drought, heat, salinity, heavy metals) and biotic (rice blast, bacterial blight) stresses. With the use of CRISPR-Cas-systems, several climate-resilient rice lines have been developed since 2013. These were extensively reviewed by Shaheen et al. (2023). This review shows how important these techniques will be to adapt further to rapidly changing conditions, given the shortcomings of classical breeding and EGTs. It was hypothesised that “development and characterization of CRISPR-edited plants against various stresses at the same time has huge potential that can transform and speed up the future breeding programs” (Shaheen et al., 2023). Other reviews also show the large potential for NGT plants as a strategy to make agriculture climate-robust (e.g., in cereals, Massel et al., 2021). Potentially, gene editing to increase drought resistance might also be an interesting additional tool to save endangered, non-agricultural plant species in specific contexts.

5.2.6 Bio-based economy and environment-friendly production

Several innovative applications for industry could contribute to a more environment-friendly production process. Two examples from the research field:

- The Horizon Europe-funded GeneBEcon¹⁶ project researches and innovates the use of NGTs to provide farmers and bio-based industries with climate-friendly and less polluting agricultural solutions. A case study is the development of a virus-resistant potato with an industrial tuber starch quality. This way of producing starch will put less pressure on the environment.
- ILVO¹⁷ is conducting research in industrial chicory (root chicory, *Cichorium intybus* var. *sativum*) to reduce bitterness in the root via CRISPR-Cas. Chicory is used in food because of its high content in inulin, which is a very healthy prebiotic fibre. Today, to fully utilise the inulin, a lot of environmentally polluting and energy-consuming processing steps are performed to remove the bitterness from the root and then extract the inulin. With ordinary breeding, bitterness can be partially reduced, but the result remains insufficient. With CRISPR-Cas, the bitterness can be reduced completely. That way, environmentally damaging steps are no longer necessary in chicory processing.

5.2.7 Integrated Pest Management

Long-term dynamics of host-pathogen interactions need to be taken into account. To counter selection pressure in pests and diseases and to avoid the development of higher virulence or

¹⁶ <https://genebecon.eu/about/> (accessed on 25/1/2024)

¹⁷ <https://ilvo.vlaanderen.be/nl/nieuws/minder-bitter-witloof-en-cichorei-onderzoekers-slagen-erin-bittergenen-uit-te-schakelen-met-crispr-cas9-technologie> (accessed on 25/1/2024)

new variants, it is important to employ NGT plants within an Integrated Pest Management system. **NGTs are not a magic solution for all problems in agriculture. They are just one of many tools that can be used to ensure sustainable food production in the future.**

6 Public acceptance

Consumer and stakeholder research on the acceptance of gene editing has been reviewed in recent studies (Woźniak-Gientka et al., 2022; Spök et al., 2022; Strobbe et al., 2023). Despite the heterogeneity in the scope of research (e.g., targeted product, improved trait, study location), the limited but growing number of consumer acceptance studies indicates that gene-edited foods are generally more accepted than GM foods (Strobbe et al., 2023). Nevertheless, the share of consumers without or with low awareness (50-62 %) or knowledge of genome editing (50-96 %) was high. While positive information about gene editing or its benefits could increase consumer acceptance (e.g., Son et al., 2021), negative information could substantially lower acceptance of such technologies, as shown for GM foods (e.g., De Steur et al., 2017; Valente & Chaves, 2018). In this context, the current lack of adequate knowledge and tailored information might increase misconceptions and, hence, alter the initial positive consumer attitudes towards gene editing in foods.

In the European Union, the 2019 food safety Eurobarometer survey demonstrated that a much smaller share of respondents had safety concerns about genome editing in foods (4 %) as compared to GM ingredients in foods (27 %) (EFSA, 2019). This might be linked to the relatively lower awareness of gene editing (60 % of people were aware of GM ingredients in foods, versus 21 % of gene editing in foods). However, as (mis)information on gene editing will increase, as it did for genetic modification earlier, it remains to be seen how this will affect public acceptance of NGTs.

The Superior Health Council therefore advocates for initiatives that can increase the general public's knowledge of NGTs based on sound scientific arguments.

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VII SAMENSTELLING VAN DE WERKGROEP

De samenstelling van het Bureau en het College alsook de lijst met de bij KB benoemde experts is beschikbaar op de website van de HGR: [wie zijn we?](#).

Al de experts hebben **op persoonlijke titel** aan de werkgroep deelgenomen. Hun algemene belangenverklaringen alsook die van de leden van het Bureau en het College kunnen worden geraadpleegd op de website van de HGR ([belangenconflicten](#)).

De volgende experts hebben hun medewerking en goedkeuring verleend bij het opstellen van het advies. Het voorzitterschap werd waargenomen door **Pieter SPANOGHE** en het wetenschappelijk secretariaat door Stijn EVERAERT.

ANGENON Geert	Plantengenetica	VUB, ARB*
DE STEUR Hans	Sociologie, agro-economie	UGent
DEBODE Frédéric	Bio-ingenieurswetenschappen	CRA-W, ARB*
FONTAINE Véronique	Microbiologie, virologie, immunologie	ULB, ARB*
FRAEYMAN Norbert	Farmacologie	UGent
LIBBRECHT Julien	Ethiek	BRCB**
SCHOETERS Greet	Milieugezondheid, toxicologie	UAntwerpen
SPANOGHE Pieter	Gewasbeschermingschemie	UGent
VANDERSCHUREN Herve	Plantengenetica	ULiège, KU Leuven
VAN DER STRAETEN Dominique	Functionele plantenbiologie	UGent
VAN LAERE Katrijn	Plantenveredeling	ILVO
VAN LAREBEKE Nicolas	Milieugezondheid, cancerologie	UGent, VUB
VERBRUGGEN Nathalie	Plantengenetica, plantenfysiologie	ULB
WILLEKENS Julie	Farmacie, voeding	Antigifcentrum

*ARB = Adviesraad voor Bioveiligheid

**BRCB = Belgisch Raadgevend Comité voor Bio-ethiek

De volgende administraties/ministeriële kabinetten werden gehoord maar werden niet betrokken bij de goedkeuring van het advies:

DE MESMAEKER Mathias	Expert GGO's	FOD Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu
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