

10 questions and answers: What do we really know about NGT plants?

And what should we know before making decisions on future regulation?

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Introduction

The EU is currently discussing the future regulation of NGT plants. Industry, affiliated experts and the EU Commission are all advocating deregulation. However, many experts disagree with this, as there are still a number of controversial issues, such as risks associated with NGT plants and questions linked to the socioeconomic role of patents. This short overview provides information which is essential for final decision making, but so far is not fully available.

The first four questions relate to whether or not NGT plants currently being developed can be equated to conventional breeding. The three subsequent questions deal with unintended effects, cumulative effects and sustainability effects that could result from the introduction of NGTs. The final three questions concern the NGT patent landscape and are related to the legal framework governing patents on NGTs. This backgrounder concludes that the proposal made by the EU Commission in regard to the future regulation of NGTs cannot ensure health or environmental safety, and the European Patent Office will continue to grant patents on NGTs. Finally, we include recommendations on how to ensure a science-based and fit for purpose regulation of NGT plants.

(1) What can we learn from data on pending applications of NGT plants?

If databases, such as those from EU-SAGE¹ and JRC², are to be believed, a great many NGT applications in plants are currently being developed across a broad range of species and traits. However, none of these databases provide any information relevant to the future regulation of NGT plants. These issues are, however, dealt with in several opinions of the European Food Safety Authority (e. g. EFSA, 2020 a and b; 2021; 2022a and b). But these opinions are noticeably abstract or theoretical and very rarely based on the assessment of actual case studies. Apart from EFSA, there have only been few attempts to assess the data of specific cases (Kawall, 2021a and b; Koller & Cieslak 2023a; Eckerstorfer & Heissenberger, 2023; ANSES, 2023). These studies demonstrate that NGT plants can overcome the boundaries of the known characteristics of a plant species, even if no additional genes are inserted and with very small genetic changes are made. Very often the results go beyond anything previously achieved in transgenic plants. At present, it appears that there is only one systematic overview, i. e. for *Brassicaceae* used in oil production (Koller & Cieslak, 2023a, see also Figure 3). No similar information seems to be available for plant species targeted in numerous NGT applications, such as rice, tomatoes, maize, soybean and wheat (Figure 1). This means that there is a data gap between the EFSA opinions on GMO regulation and the data provided in the abovementioned databases for many specific applications or other related publications. This data gap is largely impacting the EU Commission proposal on the future regulation of NGT plants.³ Consequently, the proposal needs to be thoroughly revised: data from both a larger number of case studies and the development of respective risk scenarios have to be taken into account, to ensure that future regulation is adequate to safeguard the environmental and health safety of NGT plants, including products derived thereof.

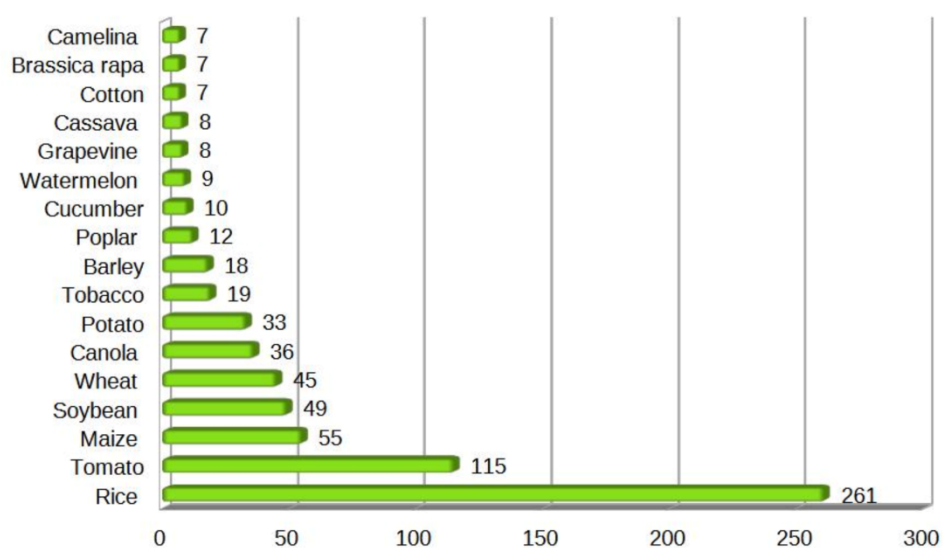


Figure 1: Number of published potential NGT applications in plants, taken from the EU-SAGE database at the end of 2023 (original data: <http://www.eu-sage.eu/genome-search>)

¹ <http://www.eu-sage.eu/genome-search>

² https://datam.jrc.ec.europa.eu/datam/mashup/NEW_GENOMIC_TECHNIQUES/index.html

³ COM(2023) 411 final 2023/0226 (COD) Proposal for a Regulation of the European Parliament and of the Council on plants obtained by certain new genomic techniques and their food and feed, and amending Regulation (EU) 2017/625, <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX%3A52023PC0411>

(2) Which criteria are suitable for making decisions on 'equivalence' to conventionally-bred plants?

The EU Commission suggests introducing criteria which only refer to the number⁴ and type of mutations⁵ in future regulation of NGT plants. The Commission believes that these criteria will be sufficient to verify the 'equivalence' of NGT plants and plants bred using conventional methods (including random mutagenesis), thus allowing them to be exempt from mandatory risk assessment. However, many governmental agencies believe that the proposed criteria are insufficient, including the French Agency for Food, Environmental and Occupational Health & Safety (ANSES, 2023), the German Federal Agency for Nature Conservation (BfN) (Bohle t et al., 2023) and experts from Environment Agency Austria (UBA) (Eckerstorfer & Heissenberger, 2023) as well as scientific organisations, e. g. the Ecological Society of Germany, Austria and Switzerland (GFOE, 2023) and experts working with civil society organisations⁶. It is evident that NGT applications in plants can result in genetic changes which are unlikely to occur in conventional breeding (Kawall, 2019). Several existing case studies show that NGTs can result in characteristics that go beyond the known boundaries of a species. Unless these fundamental differences to conventional breeding and random mutagenesis (see Figure 2) are taken into account, the safety of NGT plants in regard to health and the environment cannot be ensured. Therefore, the current proposal should be rejected or extensively revised. The obvious conclusion is that more information is needed on a case-by-case basis regarding specific biological effects (intended and unintended) on the geno- and phenotypes resulting from NGT processes (see also Testbiotech, 2023a and b).

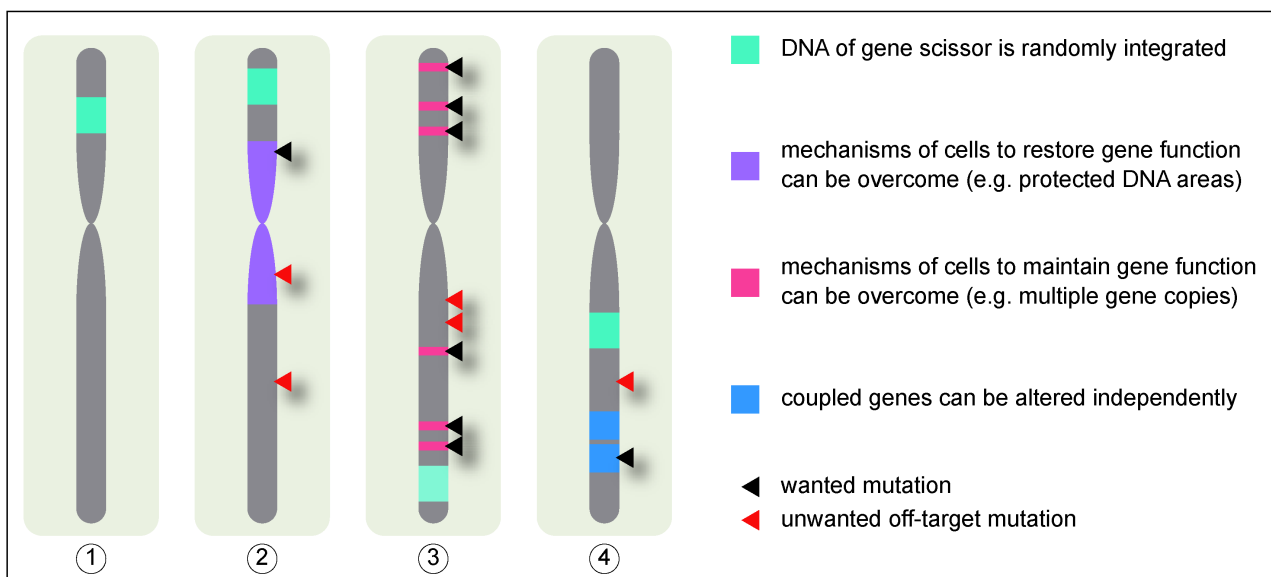


Figure 2: NGT applications in plants can result in genetic changes which are unlikely to occur with conventional breeding. Unlike conventional breeding (including non-targeted mutagenesis), new genetic engineering can overcome the limitations of naturally evolved genome organisation and go beyond the boundaries of species-specific characteristics (<https://www.testbiotech.org/en/node/3101>).

⁴ number of changes at different sites in the genome and number of changed nucleotides on each site

⁵ such as deletions and inversions

⁶ <https://www.testbiotech.org/en/content/joint-statement-scientists-future-eu-regulation-ngt-plants-perspective-protection-goals>

(3) Which examples of NGT plants show 'equivalence' to conventional breeding?

The aforementioned overview of *Brassicaceae* oil plants by Koller & Cieslak (2023a) shows that there may be examples of NGT oilseed rape (canola) plants that could be similar to conventionally-bred plants. However, no specific investigations were carried out to either prove or disprove this similarity. One example which is often used to claim 'equivalence' is the herbicide-resistant NGT oilseed rape produced by CIBUS⁷, as it seems to be similar to conventionally-bred plants (Fladung, 2016). However, no detailed genomic data (including intended and unintended genetic changes) have ever been published to either prove or disprove this 'equivalence'. It is also unclear which criteria or methodology should be applied, or even what data would be needed to come to reliable conclusions. Genomic data from NGT plants and molecular risk assessment will certainly be needed on a case-by-case basis to prove or disprove similarity or 'equivalence' to conventionally-bred plants, and to decide which further data will be needed to ensure both human health and environmental safety.

Current NGT applications in *Brassicaceae*

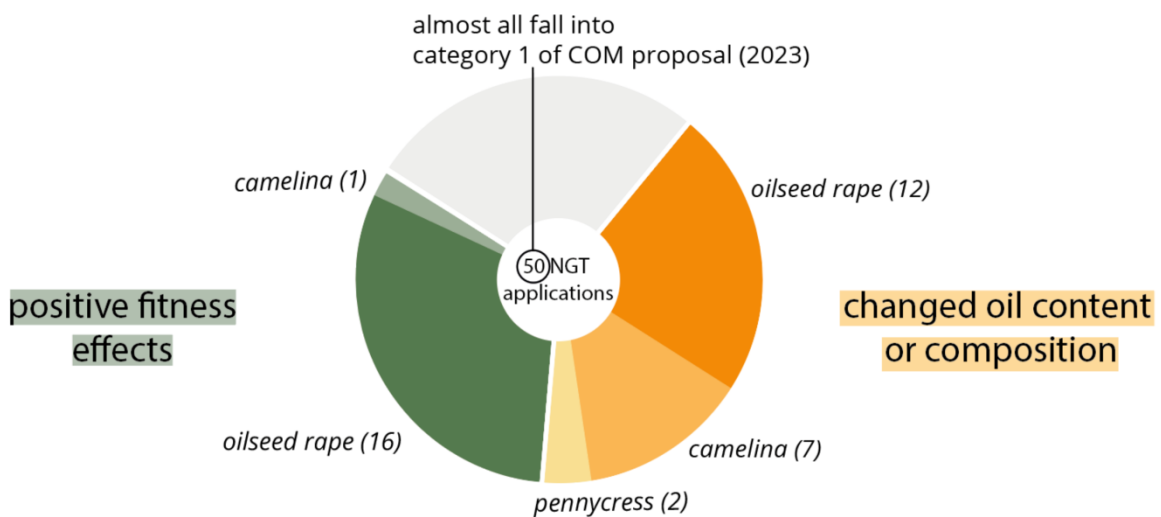


Figure 3: An initial overview of NGT applications in *Brassicaceae* used for oil production revealed differences and similarities to conventionally-bred plants (Koller & Cieslak, 2023a). As shown, more in-depth risk assessment is needed to prove or disprove similarity. As yet, no further overview has been published for other plant species, such as rice, tomato maize, soybean and wheat.

⁷ Derived from oligonucleotide directed mutagenesis, ODM

(4) Which examples of NGT plants show ‘non-equivalence’?

Previous evaluations of available data create the impression that the great majority of NGT plants with pending marketing authorisation differ in their biological characteristics compared to conventionally-bred plants (Testbiotech, 2019; Kawall, 2021b). There are certainly several examples of NGT plants and traits which are highly unlikely to be obtained from conventional breeding, including NGT tomatoes (Li et al., 2022, Nonaka et al., 2018; USDA, 2020; Zsögön et al., 2018), wheat (Sanchez-Leon et al., 2018, Raffan et al., 2021), rice (Zhang et al., 2019), poplar (Ortega et al., 2022), camelina (Kawall, 2021a) or plants with potential insect toxins (Bohle et al., 2023). Amongst the rare cases of NGT plants studied by EFSA (2020a? and 2022b), NGT wheat and NGT tomatoes were assumed to require in-depth risk assessment. The findings in these cases appear to be a suitable starting point for defining criteria and methodology to identify NGT plants that are ‘non-equivalent’ in comparison to plants obtained from conventional breeding.

These cases could also be used as a basis for the discussion on the amount of data needed for their overall risk assessment. However, this would require certain steps in risk assessment to be mandatory for all NGT plants in order to assess their safety, including a specific molecular assessment, e. g. genome sequencing, gene expression studies and so-called ‘omics’ (‘in-door risk assessment’, see Testbiotech 2023 a and b). The methods need to be suitable for detecting and assessing unintended and intended genetic changes, including all expected and unexpected, direct and indirect, immediate and delayed effects, which may constitute a risk to health or the environment.

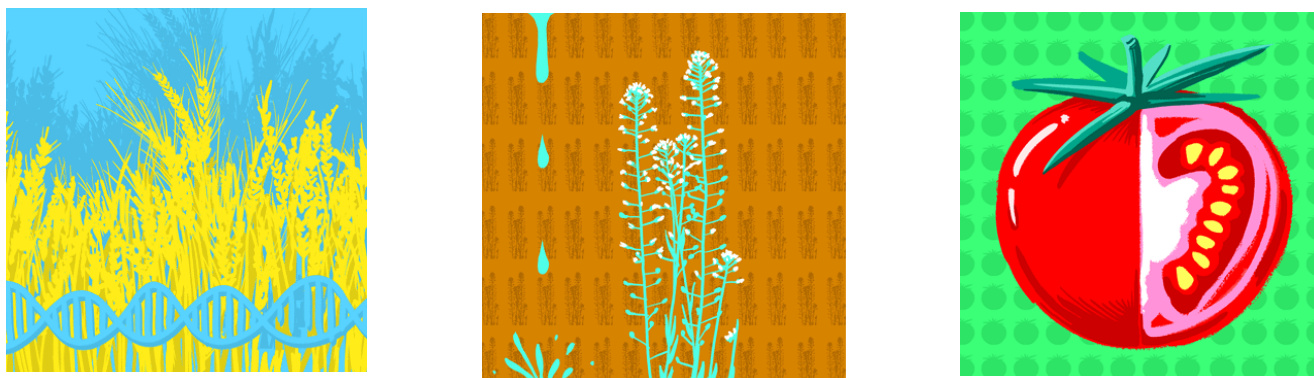


Figure 4: See short-cut examples of ‘non-equivalent’ camelina, tomato, wheat and others at: <https://www.testbiotech.org/en/limits-to-biotech>

(5) What do we know about unintended effects?

There are major uncertainties in relation to the impact of unintended effects resulting from NGT processes. EFSA, for instance, only considered the number and type of mutations that may occur as a result of the processes used in NGTs (for overview, see Koller & Cieslak 2023b). There was, however, no discussion about whether or how the resulting genotypes and phenotypes might differ from unintended effects which could be expected from random processes. Koller & Cieslak (2023b) introduced five categories of unintended genetic changes that may be relevant in the context of NGT risk assessment. Some of these are linked to the unintended insertion of transgenes. In addition, they consider that the likelihood of unintended genetic changes occurring at specific sites in the genome can be different compared to those that occur via random processes used in conventional breeding. One reason: the specific site of the unintended genetic changes (such as chromothripsis, delinvers or frame shift mutations, see also Testbiotech 2023a) and their resulting genomic pattern may be influenced by the targeted genomic sites. For example, the site and effects of unintended genetic changes may depend on the number of gene copies present in larger genomes.

However, the available data do not allow general conclusions on NGT plants with pending applications for market introduction in regard to their unintended effects. As explained above, certain steps in risk assessment must be required for all NGT plants in order to assess their safety (see also Koller & Cieslak, 2023b). The methods used must be suitable for detecting and assessing unintended and intended genetic changes as well as all their expected and unexpected, direct and indirect, immediate and delayed effects, that may constitute a risk to health or the environment.

Five categories of unintended genetic changes resulting from NGT processes

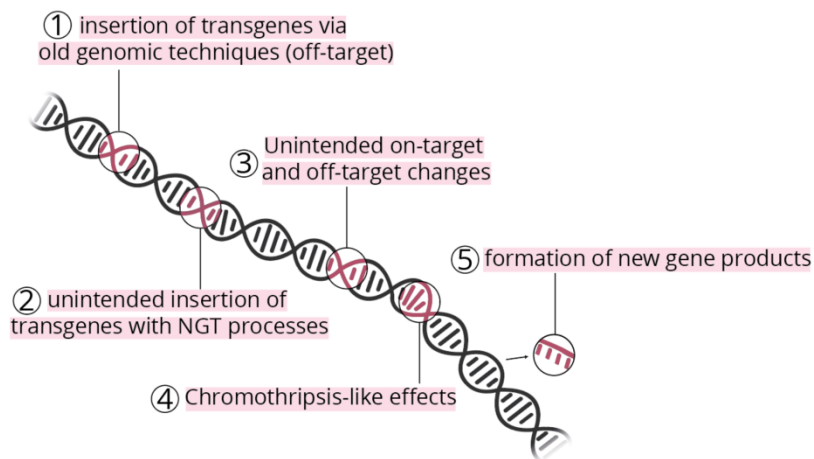


Figure 5: Five categories of potential unintended changes caused by NGT processes that warrant risk assessment (see Koller & Cieslak, 2023b).

(6) What do we know about long-term cumulative effects?

As summarised by Testbiotech (2023b), the release of NGT plants with different traits into a shared environment would require the establishment of clear criteria and methodologies to assess potential interactions and cumulative effects. This would be necessary in order to avoid disruption within ecosystem processes from organisms which had not adapted to existing biodiversity (Koller et al., 2023). Similar to climate change, it is the speed of developments that can overstretch the resilience of the ecosystems. This makes the introduction of measures to control and limit the overall scale of releases in terms of the number of organisms and traits vitally important. Experience gained from previous releases of transgenic plants also shows that interactions between the genetically engineered plants themselves may result in unpredictable effects (Koller et al., 2023). Furthermore, plants that persist and/or spread and propagate in the environment without spatiotemporal control, pose specific challenges for risk assessors and risk managers that can only be resolved by the introduction of cut-off criteria (Bauer-Panskus et al., 2020; Then et al., 2020).

It has to be assumed that long-term cumulative adverse effects will remain unpredictable. Therefore, the uncertainties can only be reduced by controlling and restricting the number, the scale and the duration of genetically engineered organisms (see also GFOE, 2023).

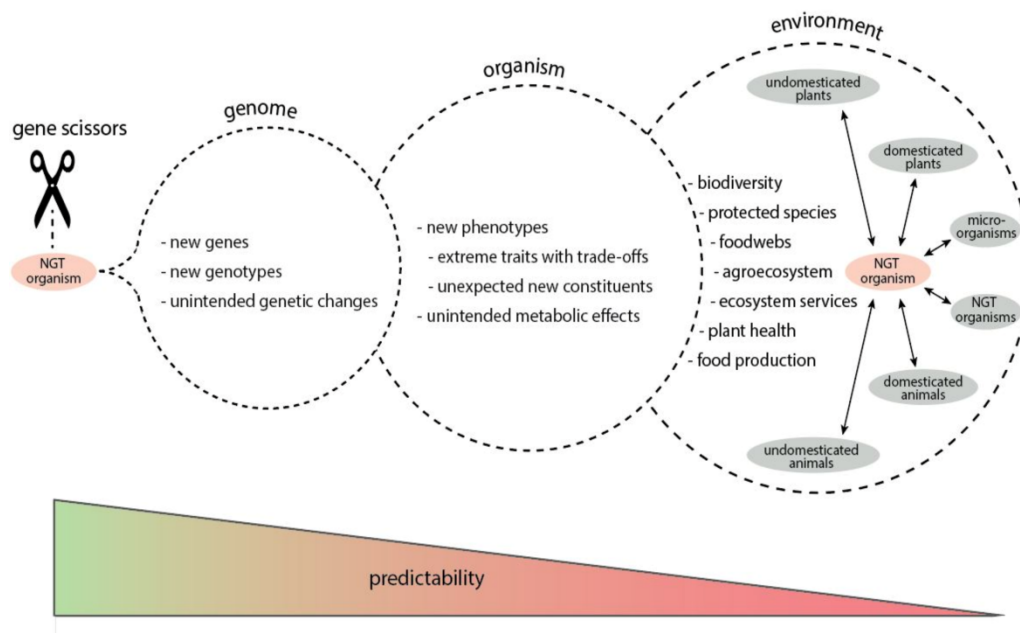


Figure 6: When predictability decreases, uncertainties increase (see Koller et al., 2023)

(7) What do we know about sustainability?

The EU Commission suggests introducing additional incentives such as labelling for plants that may contribute to sustainability. While this may be a commendable aim, it appears that the EU Commission is relying on databases such as the one provided by JRC⁸. However, the information that can be taken from these databases is rather vague and only refers to the intended traits. Therefore, although these data may nurture expectations of future trade benefits, they fail to provide any information on the factual degree of sustainability. At the same time, the EU Commission proposes using the information on possible benefits provided by producers within the framework of the variety protection system for its evaluations.⁹ However, the variety protection system is limited in its usefulness in regard to evaluating claims made by the producers on actual sustainability.

Unless a comprehensive technology assessment is carried out, there is a significant risk that short-term advantages in the cultivation of specific varieties will become disadvantages in the long-term, as is the case, for example, with transgenic, glyphosate-resistant plants: the initial effects of reducing herbicides in the cultivation of these plants were reversed in the medium- to long-term. Similar developments were observed in insect pests which became resistant to the insecticides produced by transgenic plants (Testbiotech, 2023c).



**Figure 7: For more information on genetically engineered plants and sustainability:
<https://www.testbiotech.org/en/impact-assessment>**

⁸ https://datam.jrc.ec.europa.eu/datam/mashup/NEW_GENOMIC_TECHNIQUES/index.html

⁹ https://food.ec.europa.eu/plants/plant-reproductive-material/legislation/future-eu-rules-plant-and-forest-reproductive-material_en

(8) Who is working on developing NGT plants and who will benefit?

A wide range of stakeholders and scientists are working on NGT applications in plants, including academic institutions. It is evident that research and development can be considered to be a specialised business, driven by public research grants and/or contracts with industry. In many cases, it generates patents that are key to securing profits from the market introduction of, e.g. NGT seeds. Thus, creating a situation where breeders require legal and technical expertise to gain access to the patented technology. The patent portfolios of the individual companies can also play a decisive role. Having sufficiently large-scale production and distribution capacity is a further crucial factor for success in the seed business. Therefore, large companies, such as Bayer, Corteva (previously DowDupont), BASF and Syngenta/ChemChina, will have major advantages in the trading and marketing of NGT plants, while small and medium enterprises (SMEs) will very likely lose out. There is, however, a distinct possibility that China will cause a shift in the market: if EU-SAGE¹⁰ data are categorised by the number of patent applications per country/region, China is leading the way, followed at some distance by the USA. If, for example, Syngenta/ChemChina or the company Beijing DaBeiNong Biotechnology (DBN) are able to benefit from the ongoing research and development in Chinese research institutions, its future market share may increase rapidly. In general, future GMO regulation may influence the pace and the costs for market introduction, but it will not change the current situation in regard to SMEs or provide specific benefits to European breeders. The requirements for the mandatory approval processes would apply to all seed producers who want to sell their products on the European market. Therefore, the introduction of NGT plants into agriculture is highly likely to increase market concentration, with the biggest advantages going to producers in China and the USA.

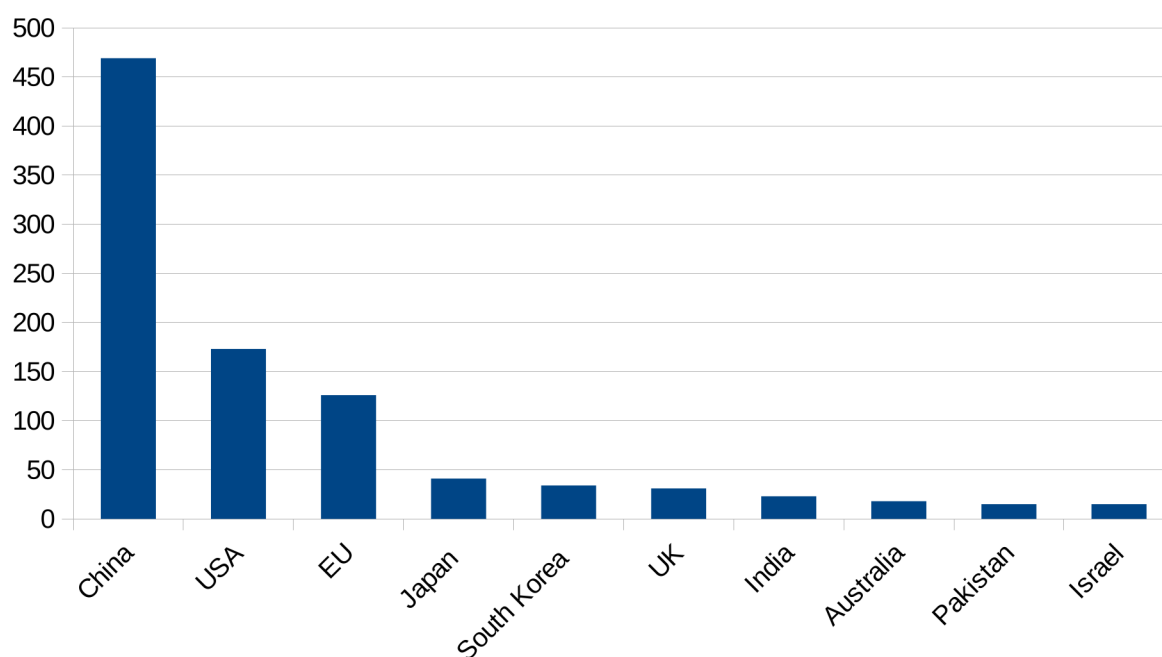


Figure 8: Number of published studies for NGT applications in plants categorised by country / regions. Note: several countries / regions can be involved in one publication (original data: <http://www.eu-sage.eu/genome-search>).

¹⁰ <http://www.eu-sage.eu/genome-search>

(9) What is the role of patents in controlling access to the technology and biodiversity?

Every year there is an increase in the number of patent applications filed for NGT plants. This development can, for example, be clearly seen in the number of international patent applications being filed at the World Intellectual Property Organisation (WIPO)¹¹. These patent applications are especially relevant for the EU, as in many cases, they may be converted to European patent applications. For example, if the search term 'CRISPR' is used (within the larger group of genetically engineered plants), it shows there were around 80 patent applications in 2023, while five years ago, there were about 50 (see Figure 9). However, this search only includes patent applications filed in English. From more detailed research (data not shown), we assume that the real figures should be about 25% higher. Thus, we can currently expect about 100 patent applications to be filed for NGT plants per year, with a clear upward trend.

In terms of companies, US-based Corteva (previously Dow Dupont) leads the way with around 100 international applications filed at the WIPO (see Figure 10) and around 30 patents granted by the European Patent Office (EPO) up until the end of 2022 (Testbiotech, 2023d). Patents on NGT plants are also associated with problems for conventional breeders, as in many cases, the scope of the patents is not restricted to the technology: randomly occurring gene variants are also claimed as inventions (see No Patents on Seeds!, 2023a).

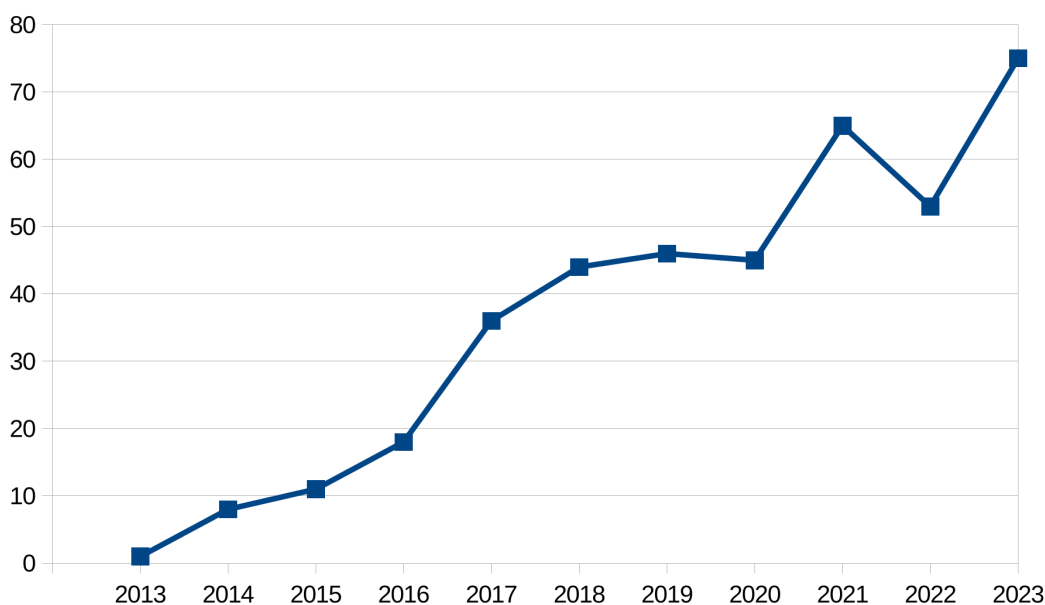


Figure 9: Number of international patent applications filed for genetically engineered plants (WIPO/WO) per year which include the term 'CRISPR' in the claims. Note: This online research includes only patent applications filed in English language (Source: <https://patentscope.wipo.int/search/en/search.jsf>, search within IPC C12N15/82)

¹¹ <https://patentscope.wipo.int/search/en/search.jsf>

(10) Can patents on NGT plants be prohibited in the EU?

Some proponents hope that NGT plants will eventually be exempted from patentability, especially if they are equated to conventionally-bred plants¹². The debate on patents appears to have in some ways become linked to a greater acceptance of deregulation for NGT plants. However, in the end, NGT plants will still be patented, regardless of whether they are deregulated or not. The European Patent Convention (EPC) is the decisive factor in this regard. There are 39 contracting states to the EPC, which forms the legal framework for granting European patents at the European Patent Office (EPO). The EPO is responsible for the granting of patents on inventive technical processes, but it excludes plants obtained from non-technical, non-targeted processes (No Patents on Seeds!, 2023b). However, in practice the EPO nevertheless grants patents on plants obtained from conventional breeding. The EU requested the exclusion of conventionally-bred plants from patentability as early as 2017. The EU parliament adopted several resolutions on the issue, thus prompting the EU Commission to issue a statement to this effect. In response, all EU member states demanded the EPO to stop issuing patents on plants obtained from crossing and selection. This initiative actually resulted in the EPO correcting its interpretation of the patent law. However, at the same time, a further loophole was established which still allows patents to be granted on genetic variants derived from random processes. In fact, the EPO has already granted several hundred patents on conventionally-bred plants, with patent claims covering more than 1000 European conventionally-bred varieties (No Patents on Seeds!, 2023a). The EU has so far been unable to resolve this problem, even though it would only require correct interpretation of existing patent law. Current demands (as voiced mainly within the EU parliament) that the EU should prohibit patents on NGTs lack plausibility and credibility, as this would require a change of the EPC, which would have to be agreed by all 39 contracting states of the EPO, and potentially take many years – even if it was possible. As clarified by the EPO¹³, those who grow NGT plants will also reap the patents.

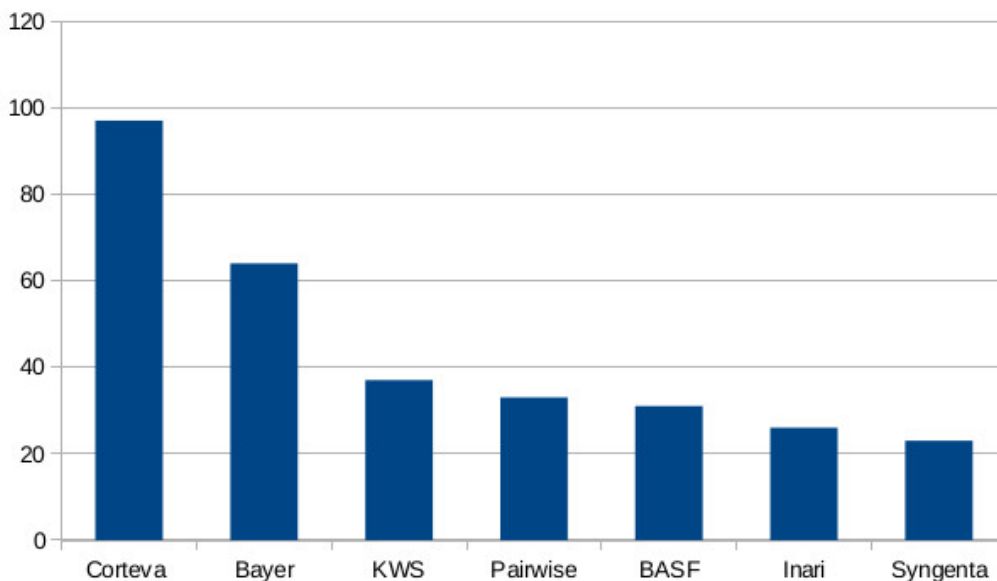


Figure 10: Number of international patent applications (WIPO /WO) for the usage of nucleases (CRISPR/Cas, TALEN, zinc finger- or meganucleases) in the food plant sector, categorised by companies, up until the end of 2022. Note: This online research includes only English language patent applications (Source: <https://patentscope.wipo.int/search/en/search.jsf>, search within IPC C12N15/82)

¹² See for example https://www.europarl.europa.eu/doceo/document/AGRI-AD-757371_EN.pdf

¹³ <https://www.euractiv.com/section/agriculture-food/news/europe-patent-office-gene-edited-plants-will-be-patented-under-gmo-rules/>

Summary and recommendations

The above Q&A show that further information, data, methodology and criteria are needed to identify NGT plants that may be considered to be similar or 'equivalent' to those obtained from conventional breeding. Contrary to the proposal of the EU Commission, these data can only be acquired on a case-by-case basis with a step-by-step risk assessment (see Testbiotech, 2023a and b). Many more case studies with larger groups of plants need to be completed before any decisions are made. In addition, risk management measures need to be introduced in order to control and limit the scale and duration of NGT releases into shared receiving environments (Koller et al., 2023). Furthermore, a technology assessment should be established to assess sustainability claims and the impact of NGT patents on plant breeding and agriculture (Testbiotech, 2023c).

As pointed out in previous reports (Testbiotech, 2023 a and b), Testbiotech is calling for the continuation of mandatory risk assessment for all genetically engineered organisms. Certain steps in risk assessment must be required for all NGT plants in order to assess their safety ('in-door risk assessment'). These requirements should include a specific molecular assessment, such as genome sequencing, gene expression studies and so-called 'omics' (such as transcriptomics, proteomics and metabolomics). The methods used must be suitable for detecting and assessing unintended and intended genetic changes as well as all their expected and unexpected, direct and indirect, immediate and delayed effects, that may constitute a risk to people, the environment and nature. The overall amount of data needed in each case can only be determined after these first steps of 'in-door risk assessment' are completed. NGT plants which are then designated 'similar' to conventionally-bred plants should still be regulated to maintain traceability, retrievability and oversight of further crossings (stacked events) and/or the further introduction of genetic changes via NGTs in order to be able to remove them in case of a possible emergency.

References

ANSES (2023) Avis relatif à l'analyse scientifique de l'annexe I de la proposition de règlement de la Commission européenne du 5 juillet 2023 relative aux nouvelles techniques génomiques (NTG) – Examen des critères d'équivalence proposés pour définir les plantes NTG de catégorie 1 (autosaisine n° 2023-AUTO-0189). Maisons-Alfort : Anses, 34 p, <https://www.anses.fr/en/system/files/BIOT2023AUTO0189.pdf>

Bauer-Panskus A., Miyazaki J., Kawall K., Then, C. (2020) Risk assessment of genetically engineered plants that can persist and propagate in the environment. *Environ Sci Eur*, 32, 32. <https://doi.org/10.1186/s12302-020-00301-0>

Bohle F., Schneider R., Mundorf J., Zühl L., Simon S., Engelhard M. (2023) Where Does the EU-Path on NGTs Lead Us?, Preprint, <https://doi.org/10.20944/preprints202311.1897.v1>

Eckerstorfer M. & Heissenberger A. (2023) New Genetic Engineering – possible unintended effects, Umweltbundesamt und Arbeiterkammer Wien, Informationen zur Umweltpolitik, Vol 208, https://emedien.arbeiterkammer.at/viewer/image/AC16982244/1/LOG_0003/

EFSA (2020a) Applicability of the EFSA Opinion on site-directed nucleases type 3 for the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide directed mutagenesis. *EFSA J* 18(11): 6299. <https://doi.org/10.2903/j.efsa.2020.6299>

EFSA (2020b) Scientific Opinion on the evaluation of existing guidelines for their adequacy for the microbial characterisation and environmental risk assessment of microorganisms obtained through synthetic biology. *EFSA J*, 18(10): 6263. <https://doi.org/10.2903/j.efsa.2020.6263>

EFSA (2021) Scientific Opinion on the evaluation of existing guidelines for their adequacy for the molecular characterisation and environmental risk assessment of genetically modified plants obtained through synthetic biology. *EFSA J* 19(2): 6301. <https://doi.org/10.2903/j.efsa.2021.6301>

EFSA (2022a) Draft updated opinion on plants developed through cisgenesis and intragenesis, <https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0l7U0000011Zb2/pc0176>

EFSA (2022b) Scientific Opinion on the evaluation of existing guidelines for their adequacy for the food and feed risk assessment of genetically modified plants obtained through synthetic biology. *EFSA J*, 20(7): 7410. <https://doi.org/10.2903/j.efsa.2022.7410>

Fladung M. (2016) *Cibus'* herbicide-resistant canola in European limbo. *Nat Biotechnol* 34(5): 473-474, <https://doi.org/10.1038/nbt.3558>

GFOE (2023) New genomic techniques from an ecological and environmental perspective: science-based contributions to the proposed regulations by the EU Commission. Expert Group “New Genomic Techniques”, Ecological Society of Germany, Austria and Switzerland (GFÖ). https://gfoe.org/sites/default/files/ngt_gfoe_final.pdf

Kawall K. (2019) New possibilities on the horizon: genome editing makes the whole genome accessible for changes. *Front Plant Sci*, 10: 525. <https://doi.org/10.3389/fpls.2019.00525>

Kawall K. (2021a) Genome-edited *Camelina sativa* with a unique fatty acid content and its potential impact on ecosystems. *Environ Sci Eur*, 33(1), 1-12. <https://doi.org/10.1186/s12302-021-00482-2>

Kawall K. (2021b) The generic risks and the potential of SDN-1 applications in crop plants. *Plants*, 10(11), 2259. <https://doi.org/10.3390/plants10112259>

Koller F., Schulz M., Juhas M., Bauer-Panskus A., Then C. (2023) The need for assessment of risks arising from interactions between NGT organisms from an EU perspective. *Environ Sci Eur* 35: 27. <https://doi.org/10.1186/s12302-023-00734-3>

Koller F. & Cieslak M. (2023a) Background: The application of NGTs in Brassicaceae: environmental risk assessment scenarios. Fachstelle Gentechnik und Umwelt, https://fachstelle-gentechnik-umwelt.de/wp-content/uploads/Brassicaceae_Background.pdf

Koller F. & Cieslak M. (2023b) A perspective from the EU: Unintended genetic changes in plants caused by NGT – their relevance for a comprehensive molecular characterisation and risk assessment. *Front Bioeng Biotechnol* 11: 1276226. <https://doi.org/10.3389/fbioe.2023.1276226>

Li J., Scarano A., Gonzalez N.M., D’Orso F., Yue Y., Nemeth K., Saalbach G., Hill L., de Oliveira Martins C., Moran R., Santino A., Martin C. (2022) Biofortified tomatoes provide a new route to vitamin D sufficiency. *Nat Plants*, 8: 611-616. <https://doi.org/10.1038/s41477-022-01154-6>

No Patents on Seeds! (2023a) The future of plant breeding is under threat in Europe. Current interpretation of patent law is insufficient to stop patents on conventional breeding, <https://www.no-patents-on-seeds.org/en/report2023>

No Patents on Seeds! (2023b) Correct legal interpretation of Article 53(b), EPC, within the context of the EU patent directive 98/44, Legal analysis provided by No Patents on Seeds!, March 2023, [https://www.no-patents-on-seeds.org/sites/default/files/news/Interpretation%20Art%2053%20\(b\)%20_NPoS_0.pdf](https://www.no-patents-on-seeds.org/sites/default/files/news/Interpretation%20Art%2053%20(b)%20_NPoS_0.pdf)

- Nonaka S., Arai C., Takayama M., Matsukura C., Ezura H. (2017) Efficient increase of γ -aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. *Sci Rep*, 7: 7057. <https://doi.org/10.1038/s41598-017-06400-y>
- Ortega M.A., Zhou R., Chen M.S.S, Bewg W.P. Simon B., Tsai C.-J. (2022) *In vitro* floral development in poplar: insights into seed trichome regulation and trimonoecy. *New Phytol* 237: 1078-1081. <https://doi.org/10.1111/nph.18624>
- Raffan S., Sparks C., Huttly A., Hyde L., Martignago D., Mead A., Hanley S.J., Wilkinson P.A., Barker G., Edwards K.J., Curtis T.Y., Usher S., Kosik O., Halford N.G. (2021) Wheat with greatly reduced accumulation of free asparagine in the grain, produced by CRISPR/Cas9 editing of asparagine synthetase gene TaASN2. *Plant Biotechnol J*, 19(8): 1602-1613. <https://doi.org/10.1111/pbi.13573>
- Sanchez-Leon S., Gil-Humanes J., Ozuna C.V., Gimenez M.J., Sousa C., Voytas D.F., Barro F. (2018) Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. *Plant Biotechnol J*, 16: 902-910. <https://doi.org/10.1111/pbi.12837>
- Testbiotech (2019) Am I Regulated? The US example: why new methods of genetically engineering crop plants need to be regulated. <https://www.testbiotech.org/node/2345>
- Testbiotech (2023a) How to ensure a science-based and up-to-date regulation of NGT plants. Testbiotech Background 07-12-2023, <https://www.testbiotech.org/node/3175>
- Testbiotech (2023b) New genetic engineering: EU Commission proposal for new regulation endangers nature, the environment and our future livelihoods. Testbiotech Background 31-08-2023, <https://www.testbiotech.org/node/3104>
- Testbiotech (2023c) Genetic engineering in agriculture: between high flying expectations and complex risks The use of genetic engineering in agriculture requires a comprehensive technology assessment, <https://www.testbiotech.org/en/content/genetic-engineering-agriculture-between-high-flying-expectations-and-complex-risks>
- Testbiotech (2023d) New GE patents 2022: ‘Second-hand GE’ plants claimed as inventions – How CRISPR/Cas & Co are used to create new seed monopolies. Testbiotech Background 5-7-2023, <https://www.testbiotech.org/node/3082>
- Then C., Kawall K., Valenzuela N. (2020) Spatio-temporal controllability and environmental risk assessment of genetically engineered gene drive organisms from the perspective of EU GMO Regulation. *Integr Environ Assess Manag*, 16(5): 555-568. <https://doi.org/10.1002/ieam.4278>
- USDA (2020) Regulatory status of genome edited J2 knockout tomato lines, https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/19-282-01-a3-air-inquiry-cbidel.pdf, https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/19-282-01-air-response-signed.pdf
- Zhang A., Liu Y., Wang F., Li T., Chen Z., Kong D., et al. (2019) Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. *Mol Breed*, 39: 47. <https://doi.org/10.1007/s11032-019-0954-y>
- Zsögön A., Cermak T., Naves E.R., Notini M.M., Edel K.H., Weigl S., Freschi L., Voytas D.F., Kudla J., Peres L.E.P (2018) De novo domestication of wild tomato using genome editing. *Nat Biotechnol*, 36: 1211-1216. <https://doi.org/10.1038/nbt.4272>