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INFORMATIONEN ZUR UMWELTPOLITIK

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NEW GENETIC ENGINEERING – POSSIBLE UNINTENDED EFFECTS

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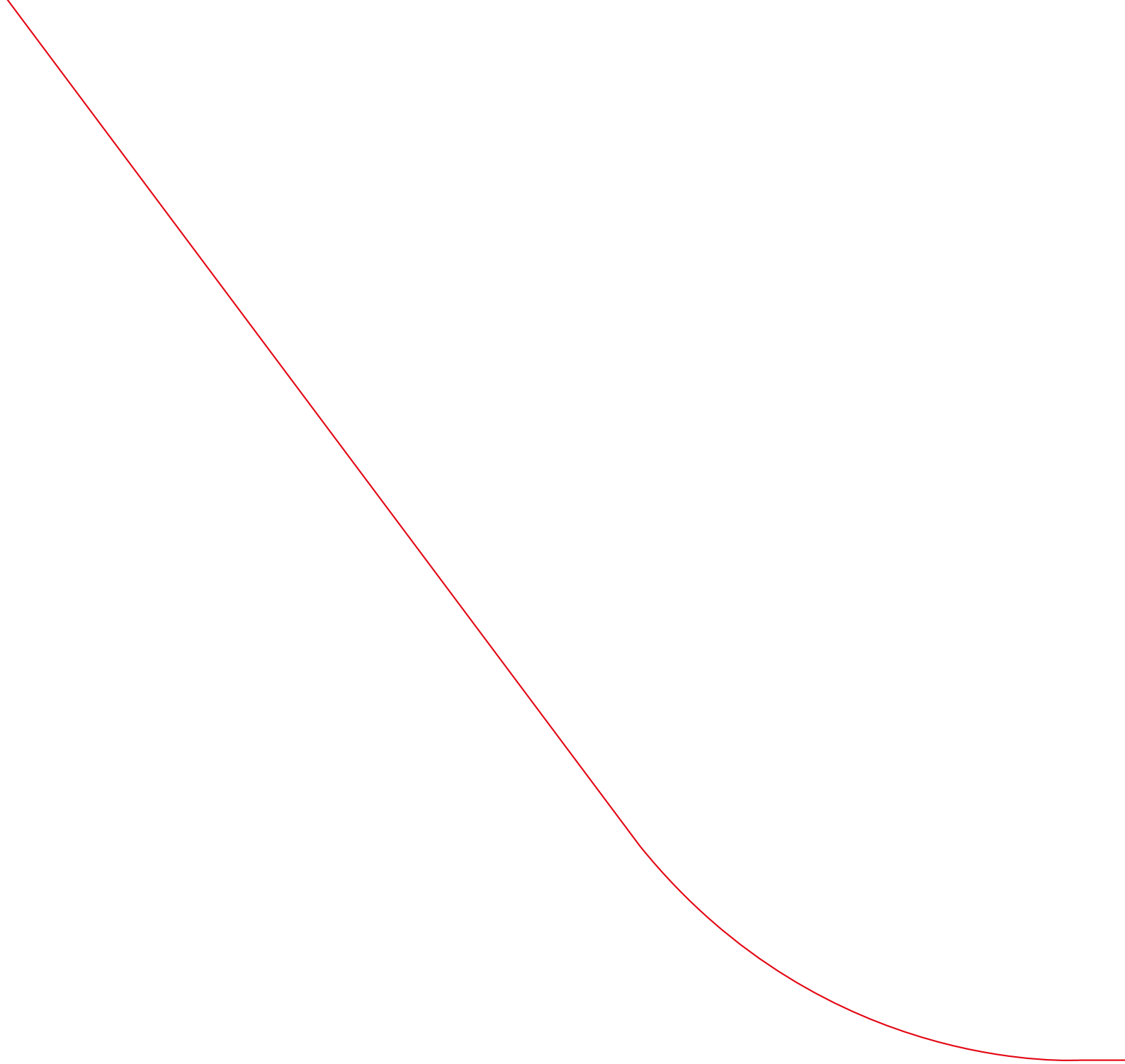

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SUMMARY

How potential risks of NGT products are dealt with is an essential aspect of the application of "new genetic engineering" techniques (or "new genomic techniques" or NGTs, as they are called by the European Commission) in agriculture and food production. Although the safety of NGT products is of considerable importance for developers, consumers and legislators, respectively, this aspect is not in the focus of the current debate concerning a proposal of the European Commission for a new regulation for NGT products. Therefore, this study examines this aspect on the basis of selected, representative examples of NGT products. The analysis intends to direct the focus on the issue of unintended effects, which may be associated with NGT products.

In public, the developers argue that new genetic engineering, in contrast to classical genetic engineering, is much more targeted than other approaches. They also emphasize that the genetic changes induced by such techniques would be comparable to changes that could also occur in conventionally bred varieties. However, such arguments ignore the fact that NGTs are also based on biological processes that cannot guarantee absolute precision. In addition, the level of precision for the introduction of genetic changes at specific locations in the DNA of the modified organisms bears no relation to the safety or the risk of the traits, which are developed in these NGT organisms. The present study therefore underlines previous statements of scientists and consumer organisations that unintended and not fully predictable effects can also occur with NGT products.

Such effects may be due to unintended genetic changes caused by cell culture and transformation methods, which are also used for the production of classical GMOs (genetically modified organisms containing transgenic DNA). For example, in the case of hornless cows which were produced by means of targeted mutagenesis (genome editing), a later independent review by experts from the US Food and Drug Administration (FDA) found that foreign DNA sequences, including an antibiotic resistance gene, had been unintentionally introduced into the DNA of the modified cows. Moreover, due to the technique used for the editing plant genomes mutations can also be induced at DNA sites that are similar, but not identical to the intended target site - so-called "off-target" mutations. However, the occurrence of such off-target mutations is rarely investigated comprehensively enough and at the right time of the development process of a NGT plant. Many of these

studies are conducted to rather optimize the genome editing approach than to monitor whether off-target mutations which may lead to adverse effects are still present in the NGT plant immediately prior to commercialisation of the NGT product. In any case, a growing body of scientific research indicates that a wide range of unintended genetic changes are possible through the application of NGTs. These include smaller or larger genetic changes adjacent to the target sequence, i.e. unintended "on-target" mutations. In extreme cases, the changes to the target sequence can trigger cascading rearrangements of parts of the affected chromosomes. Although unintended off- and on-target mutations with adverse effects do not occur in all genome editing approaches, the probability is high enough to conclude that the occurrence of unintended DNA changes needs to be addressed during a risk assessment.

In addition to these technology-related changes, unintended effects are also caused by the fact that the intended genetic changes will often not only result in the expression of a specific desired characteristic, but will simultaneously affect other functions or traits in the modified cells or organisms. These unintended "side effects" are often not predictable, mainly due to the limited knowledge regarding the complex interactions within higher organisms and the manifold interactions between different organisms in the environment. If a number of different, independent modifications is introduced simultaneously, the possibility for unintended side effects is even higher. Such multiplexing approaches which allow for the simultaneous targeted mutagenesis of several different DNA target sites are currently possible without too much technical difficulty and plants harboring complex modifications can be produced relatively quickly. However, plants with similar genetic modifications may not be produced by conventional breeding approaches at all or they could only be produced with a disproportionately high effort.

ARE LOWER SAFETY STANDARDS TO BE EXPECTED FOR NGT PRODUCTS?

Under the existing legislative framework for GMOs in the European Union, a risk assessment must be carried out prior to authorisation for deliberate release or commercialisation to determine whether GM products may cause adverse effects on human and animal health and the environment as a result of unintended modifications. According to a 2018 ruling by the European Court of Justice, such risk assessment is currently mandatory for NGT products as well.

However, in July 2023, the European Commission presented a draft regulation that would deviate significantly from the existing approach. The draft regulation would create two categories of NGT plants:

- NGT plants of the first category (NGT 1) – which includes more than 90% of all NGT plants according to the proposed definition – would no longer be subject to the obligations that currently exist for GMOs, e.g. with regard to risk assessment, post-authorisation environmental monitoring, labeling of all GM products, and cultivation requirements (coexistence and “opt-out measures” proposed by EU Member States).
- NGT plants of the second category (NGT 2) comprise a smaller group of NGT products with very complex modifications, i.e. plants with more than 20 different genetic modifications. In principle, similar provisions as for GMOs apply, but the respective requirements for NGT 2 plants may be less robust regarding risk assessment, monitoring, re-authorisation and the provision of detection methods by the developer.

EXAMPLES SHOW THAT THE ASSESSMENT OF UNINTENDED EFFECTS OF NGT PLANTS IS URGENTLY NEEDED

In order to assess how current standards for risk assessment of NGT products may be adversely impacted by the proposed new regulation, four NGT products were considered as examples. These examples include NGT plants that are already marketed in other non-EU countries, such as Japan, as well as plants whose development is described in the scientific literature, and which could be marketed in the future. The examples include three NGT 1 plants and one NGT 2 plant. They cover different areas of application, including two NGT plants with changes in their composition (NGT 1 and NGT 2), an NGT 1 plant with increased tolerance to environmental stress, and an example of a NGT 1 tomato plant, which was rapidly developed of from a wild tomato species. The selected examples only partially cover the wide variety of developments; however, the conclusions derived are relevant for all NGT plants.

- NGT Tomatoes with increased gamma-aminobutyric acid (GABA) content - NGT 1

This NGT tomato is an example of a "functional food"; the increased GABA content is thought to have beneficial effects in people suffering from high blood pressure.

It is however unclear, whether adverse effects on other populations, especially vulnerable groups, may be associated with the consumption of these tomatoes. In tomato plants, GABA also influences a variety of different metabolic processes, and exerts effects on microorganisms and insects; unintended effects of elevated GABA levels on plant shape and growth have been shown.

- NGT Wheat with lower gluten content - NGT 2

In these NGT wheat plants, more than 30 gluten genes were deactivated to reduce the total gluten content.

However, it is uncertain whether individuals suffering from gluten intolerance due to an autoimmune disease will tolerate the NGT wheat with reduced gluten content as well as other, gluten-free cereals. It must also be assessed whether gluten protein variants expressed as a result of the genetic changes are safe and whether an increased production of other wheat proteins with potentially allergic effects would occur in the NGT wheat. Gluten proteins also positively influence the tolerance of wheat plants to environmental stress, such as heat and drought stress. It is unclear whether the NGT wheat is as tolerant to such environmental stressors as other wheat varieties.

- NGT rice with increased tolerance to climate and salt stress - NGT 1

Genetic modification of a regulatory gene in NGT rice under controlled conditions results in increased resistance to increased salt concentrations in the soil, which will occur more frequently in the course of climate change.

However, it is not clear whether under changing field conditions these positive effects can also be realised without possible yield losses. Furthermore, it is unclear which indirect changes with regard to composition and food safety occur as a result of the genetic modification.

- *"De Novo Domesticated"* NGT tomatoes with increased resistance to plant diseases - NGT 1

Through introducing several independent genetic changes to genes that affect the shape and development or reproductive characteristics of tomato plants, a wild tomato can be turned into a plant with high similarity to cultivated tomato varieties in a single step - the conventional development of modern tomatoes by crossing and selection by comparison has taken almost 10,000 years. Desirable traits which are present in the wild plant such as lower disease susceptibility are retained.

A comprehensive risk assessment would have to assess whether the safety and wholesomeness of the NGT tomato may be adversely affected by other traits derived from the wild plant in comparison with tomato varieties cultivated currently for food production.

RECOMMENDATIONS

An intended objective of the proposed new regulation is to ensure a high level of safety for NGT plants, taking into account the precautionary principle. However, this objective is not compatible with the proposed waiver of a comprehensive risk assessment for NGT 1 plants as currently implemented for GMOs and the possible weakening of risk assessment requirements for NGT 2 plants.

- A uniform, robust risk assessment of all NGT plants is still urgently needed.

Even a cursory examination of a few examples of NGT plants illustrates that the safety of individual products can only be guaranteed by means of a case-specific assessment that focuses on plausible hazards, which may be associated with a respective NGT plant. In this risk assessment, all possible unintended effects on health and the environment must be taken into account – regardless of whether they are caused by the technology used to generate the NGT plant or by the characteristics of the developed traits.

In view of the existing knowledge gaps and uncertainties, general, theoretical considerations and the reference to existing requirements for conventional plants are absolutely not sufficient. Neither the requirements for variety registration nor the assessment of health risks of individual NGT products in the context of the novel food legislation are suitable nor comprehensive enough to ensure the safety of NGT plants concerning all risk issues.

In addition to a reduction of safety standards – especially for all NGT 1 plants and thus for the vast majority of NGT products, which may be expected in the future - the proposed regulation would have a variety of other undesirable effects:

- The implementation of the regulation would pose significant additional economic burdens on all agricultural production systems that are not allowed to use GMOs and NGT plants or refrain from using them, such as organic farming. It would thus jeopardise precisely those production systems that are important for a further sustainable development in agriculture.
- The abolition of labeling for food and feed products from NGT 1 plants would severely restrict consumers' freedom of choice.
- The rights of the Member States would be considerably weakened by the planned new regulation - however, they would have to assume additional responsibility, e.g. for the implementation of coexistence regulations for NGT 2 plants, without having the necessary preconditions at hands, e.g. the availability of detection methods.
- The proposed regulation does not contain measures that would ensure plant breeders' access to plant material for further development if this access is restricted by patents.

It is therefore important to ensure that the weaknesses of the proposed regulation are addressed by a comprehensive revision and that any further proposals aimed at an even more far-reaching deregulation are rejected.

ZUSAMMENFASSUNG

NEUE GENTECHNIK UND RISIKOABSCHÄTZUNG

Im Rahmen der laufenden Debatte um die Anwendung der „Neuen Gentechnik“ (bzw. der „Neuen genomischen Techniken“ oder NGTs, wie sie von der Europäischen Kommission bezeichnet werden) in Landwirtschaft und Lebensmittelproduktion, ist der Umgang mit den möglichen Risiken von Produkten der Neuen Gentechnik ein wesentlicher Aspekt. Obwohl die Sicherheit von NGT-Produkten von erheblicher Bedeutung sowohl für Entwickler:innen, Konsument:innen als auch für den Gesetzgeber ist, steht dieser Aspekt in der gegenwärtigen Diskussion über mögliche neue Regelungen für solche Produkte nicht im Zentrum der Debatte. Die vorliegende Kurzstudie beleuchtet daher diesen Aspekt anhand von ausgewählten, repräsentativen Beispielen für NGT-Produkte. Die Analyse soll vor allem den Blick dafür schärfen, welche unbeabsichtigten Effekte mit der Anwendung von solchen NGT-Produkten einhergehen könnten.

In der Öffentlichkeit wird von Entwicklerseite stark damit argumentiert, dass die Neue Gentechnik im Unterschied zur klassischen Gentechnik viel zielgerichteter arbeiten würde als andere Ansätze. Es wird auch betont, dass die damit erzeugten genetischen Änderungen vergleichbar wären mit Änderungen, die auch bei konventionell gezüchteten Sorten auftreten könnten. Solche Argumente lassen aber einerseits außer Acht, dass auch NGTs auf biologischen Prozessen beruhen, bei denen es nie eine absolute Präzision geben kann. Zudem sagt die Genauigkeit, mit der Änderungen an bestimmten Orten im Erbgut (in der DNA) der veränderten Organismen vorgenommen werden können, noch nichts über die Sicherheit bzw. das Risiko der damit hergestellten Organismen aus. Die vorliegende Studie unterstreicht daher nochmals die Aussagen von Wissenschaftler:innen und Konsumentenschützer:innen, dass auch bei NGT Produkten unbeabsichtigte und nicht immer vorhersehbare Effekte auftreten können.

Solche Effekte können auf unbeabsichtigten genetischen Änderungen beruhen, die durch Zellkultur und Transformationsmethoden hervorgerufen werden, welche unter anderem auch bei der Herstellung von klassischen GVOs (Genetisch veränderten Organismen die transgene DNA enthalten) verwendet werden. So wurde z.B. bei hornlosen Kühen die mittels gezielter Mutagenese (Genome Editing) hergestellt worden waren bei einer späteren unabhängigen Überprüfung durch Expert:innen der US-amerikanischen

Lebensmittelbehörde (FDA) gefunden, dass in die DNA der veränderten Kühe unbeabsichtigt auch Fremdsequenzen eingeführt worden waren, z.B. ein Antibiotika-Resistenzgen. Zudem können in NGT-Pflanzen - bedingt durch die verwendete Technik - Mutationen auch an bestimmten Stellen im Erbgut (d.h. der DNA) ausgelöst werden, die eine große Ähnlichkeit mit der intendierten Zielstelle aufweisen – sogenannte „off-target" Mutationen. Das Auftreten solcher off-target Mutationen wird aber nur in seltensten Fällen umfassend genug und zum richtigen Zeitpunkt untersucht. Viele dieser Untersuchungen werden zur Optimierung des gewählten Ansatzes für die Genomeditierung durchgeführt und dienen nicht der Kontrolle, ob unmittelbar vor einer Vermarktung eines NGT-Produkts noch off-target Mutationen mit nachteiliger Wirkung im NGT-Produkt zu finden sind. Eine wachsende Zahl wissenschaftlicher Untersuchungen deutet jedenfalls darauf hin, dass ein breites Spektrum von unbeabsichtigten genetischen Änderungen durch Anwendung von NGTs möglich ist, darunter auch kleine oder größere genetische Änderungen in der Nähe der Zielsequenz, d.h. unbeabsichtigte „on-target" Mutationen. Im Extremfall können durch die Änderungen an der Zielsequenz auch kaskadenartige Umlagerungen von Teilen der betroffenen Chromosomen ausgelöst werden. Obwohl nicht bei allen Ansätzen der Genomeditierung unbeabsichtigte off- und on-target Mutationen mit nachteiligen Wirkungen auftreten, ist die Häufigkeit doch so groß, dass das Vorkommen von unbeabsichtigten DNA-Änderungen in der Risikoabschätzung in Betracht gezogen werden sollte.

Neben diesen technikspezifischen Änderungen sind unbeabsichtigte Effekte vielfach auch dadurch bedingt, dass die beabsichtigten genetischen Veränderungen nicht nur die Ausprägung von erwünschten Merkmalen zur Folge haben, sondern gleichzeitig auch Auswirkungen auf andere Funktionen der modifizierten Organismen haben. Diese unbeabsichtigten „Nebenwirkungen" sind aufgrund des limitierten Wissens über komplexe Wechselwirkungen innerhalb höherer Organismen und über die vielfältigen Interaktionen zwischen verschiedenen Organismen in der Umwelt vielfach nicht vorhersehbar, können aber auch nicht ausgeschlossen werden. Die Möglichkeit für unbeabsichtigte Nebenwirkungen ist jedenfalls größer, wenn verschiedene, unabhängige Änderungen auf einmal eingeführt werden. Das ist bei NGTs die gleichzeitig eine zielgerichteten Mutagenese von mehreren verschiedenen DNA Zielstellen erlauben, aktuell ohne allzu große technische Schwierigkeiten möglich. Das Ergebnis sind Pflanzen, die mittels konventioneller Züchtung entweder gar nicht oder nur mit einem sehr hohen Aufwand erzeugt werden könnten.

SIND NIEDRIGERE SICHERHEITSSTANDARDS FÜR NGT-PRODUKTE ZU ERWARTEN?

Vor einer Zulassung zur Verwendung für die absichtliche Freisetzung oder die Vermarktung eines GVO in der Europäischen Union muss im Rahmen einer Risikoabschätzung überprüft werden, ob diese infolge von unbeabsichtigten Veränderungen nachteilige Effekten auf die menschliche und tierische Gesundheit sowie die Umwelt haben können. Einer Entscheidung des Europäischen Gerichtshofes aus 2018 zufolge, ist eine derartige Risikoabschätzung derzeit auch für NGT-Produkte verpflichtend.

Die Europäische Kommission hat im Juli 2023 aber einen Verordnungsentwurf vorgelegt, der eine recht deutliche Abkehr von der bestehenden Vorgangsweise mit sich bringen würde. Der Entwurf würde zwei Kategorien von NGT-Pflanzen schaffen:

- NGT-Pflanzen der ersten Kategorie (NGT 1) - zu der nach der vorgeschlagenen Definition mehr als 90% aller NGT-Pflanzen zählen - würden nicht mehr den Verpflichtungen unterliegen, die aktuell für GVOs bestehen, z.B. hinsichtlich Risikoabschätzung, Monitoring von Risiken nach der Zulassung, Kennzeichnung aller GV-Produkte, sowie Auflagen für den Anbau (Koexistenz- und so genannte nationale Opt-Out-Maßnahmen).
- NGT-Pflanzen der zweiten Kategorie (NGT 2) umfassen eine kleinere Gruppe von NGT-Produkten mit mehr als 20 unterschiedlichen genetischen Veränderungen, also sehr komplex veränderte Pflanzen. Für sie gelten zwar prinzipiell ähnliche Regelungen wie für GVOs, allerdings mit absehbar weniger robusten Auflagen betreffend Risikoabschätzung, Monitoring, Wiederzulassung und Nachweismethoden.

BEISPIELE ZEIGEN, DASS DIE ABSCHÄTZUNG VON UNBEABSICHTIGTEN EFFEKTEN BEI NGT-PFLANZEN DRINGEND NÖTIG IST

Um sichtbar zu machen, wo die vorgeschlagene Neuregelung nachteilige Auswirkungen auf die gegenwärtigen Standards für die Risikoabschätzung von NGT-Produkten hätte, geht die Studie beispielhaft auf vier NGT-Produkte ein. Diese Beispiele umfassen NGT-Pflanzen, die in anderen Ländern außerhalb der EU, z.B. in Japan, schon vermarktet werden, sowie Pflanzen deren Entwicklung in der wissenschaftlichen Literatur beschrieben ist und die bei weiterer Entwicklung in Zukunft auch auf den Markt gebracht werden könnten. Die Beispiele umfassen drei NGT 1 Pflanzen und eine NGT 2 Pflanze. Sie umfassen verschiedene Anwendungsgebiete, z.B. zwei NGT-Pflanzen mit Veränderung in ihrer stofflichen Zusammensetzung (NGT 1 und NGT 2), eine NGT 1 Pflanze mit erhöhter Toleranz gegen Umweltstress und ein Beispiel für die rasche Entwicklung von NGT 1 Tomaten-Pflanzen aus wilden Artverwandten. Die gewählten Beispiele decken die große Vielfalt von

Entwicklungen nur zu einem Teil ab; die abgeleiteten Schlussfolgerungen sind aber für alle NGT-Pflanzen relevant.

- NGT-Tomaten mit einem erhöhten Gehalt an ɤ-Aminobuttersäure (GABA) – NGT 1

Diese NGT-Tomate ist ein Beispiel für ein „funktionales Lebensmittel"; der erhöhte GABA Gehalt soll positive Auswirkungen bei Menschen haben, die unter hohem Blutdruck leiden. Ungeklärt ist, ob eventuell nachteilige Wirkungen auf andere Bevölkerungsgruppen, insbesondere vulnerable Gruppen mit dem Konsum dieser Tomaten verbunden sein könnten. In den Tomatenpflanzen beeinflusst GABA die Steuerung von Stoffwechselprozessen, sowie Wirkungen auf Mikroorganismen und Insekten; unbeabsichtigte Auswirkungen auf Pflanzengestalt und Wachstum wurden nachgelesen.

- NGT-Weizen mit einem niedrigeren Glutengehalt – NGT 2

In diesen NGT-Weizenpflanzen wurden mehr als 30 Glutenprotein-Gene deaktiviert, um den Gesamtgehalt von Gluten zu reduzieren.

Es ist aber fraglich, ob Personen, die durch eine Autoimmunerkrankung an Glutenunverträglichkeit leiden, den NGT-Weizen mit verringertem Glutengehalt genauso gut vertragen wie glutenfreie Cerealien. Ebenso muss geklärt werden, ob Glutenprotein-Varianten die infolge der genetischen Veränderungen gebildet werden, ungefährlich sind bzw. ob eine verstärkte Produktion anderer Weizenproteine mit potentiell allergischer Wirkung im NGT Weizen erfolgt. Glutenproteine beeinflussen in positiver Weise die Toleranz von Weizenpflanzen gegenüber Umweltstress, ausgelöst z.B. durch Hitze und Trockenheit. Unklar ist, ob der NGT Weizen daher gegenüber derartigem Stress genauso widerstandsfähig wie andere Weizensorten ist.

- NGT-Reis mit erhöhter Toleranz gegenüber Klima- und Salzstress – NGT 1

Durch die genetische Veränderung eines regulatorischen Gens wird im NGT-Reis unter kontrollierten Bedingungen eine erhöhte Widerstandsfähigkeit gegenüber erhöhte Salzkonzentrationen im Boden bewirkt, wie sie im Zuge des Klimawandels häufiger auftreten werden.

Es ist aber nicht geklärt, ob unter wechselnden Freilandbedingungen diese positiven Wirkungen ebenfalls – ohne dass mögliche Ertragsverluste auftreten – realisiert werden können. Weiters ist unklar, welche indirekte Veränderungen im Hinblick auf die Zusammensetzung und damit die Lebensmittelsicherheit auftreten.

- *„De Novo domestizierte“* NGT-Tomaten mit erhöhter Resistenz gegen Pflanzenkrankheiten – NGT 1

Durch mehrere unabhängige genetische Veränderungen in Genen, welche die Gestalt und die Entwicklung bzw. Reproduktionseigenschaften von Tomatenpflanzen beeinflussen, kann in einem Schritt aus einer Wildtomate eine Pflanze mit großer Ähnlichkeit zu Kulturtomatensorten hergestellt werden. Die konventionelle Entwicklung von modernen Tomaten durch Kreuzung und Selektion hat im Vergleich fast 10.000 Jahre benötigt. Erwünschte Wildpflanzeneigenschaften wie geringere Krankheitsanfälligkeit bleiben erhalten.

Eine umfassende Risikoabschätzung müsste klären, ob aufgrund von anderen nicht veränderten Wildpflanzeneigenschaften dieselbe Genussfähigkeit und Lebensmitteltauglichkeit gegeben ist, wie bei heutigen Kulturtomaten.

EMPFEHLUNGEN

Ein angestrebtes Ziel der beabsichtigten Neuregelung ist die Gewährleistung eines hohen Sicherheitsniveaus für NGT-Pflanzen unter Berücksichtigung des Vorsorgeprinzips. Eine solche Zielsetzung ist aber mit dem vorgeschlagenen Verzicht auf eine umfassende Risikoabschätzung bei NGT 1 Pflanzen gemäß der für GVOs geltenden Richtlinien und der möglichen Schwächung der Risikoabschätzung bei NGT 2 Pflanzen nicht kompatibel.

- Eine einheitliche, umfassende Sicherheitsprüfung von NGT-Pflanzen ist weiter notwendig.

Schon bei der oberflächlichen Betrachtung von nur wenigen Beispielen für NGT-Pflanzen wird klar, dass die Sicherheit einzelner Produkte nur mittels einer an der Praxis orientierten, fall-spezifischen Beurteilung garantiert werden kann, welche auf jeweils plausible Gefahrenpotentiale fokussiert. Bei dieser Risikoabschätzung müssen alle möglichen unbeabsichtigten Effekte auf Gesundheit und Umwelt berücksichtigt werden – unabhängig davon, ob sie durch die angewandte Technik bzw. die jeweils erzeugten Merkmale bedingt sind.

In Anbetracht der bestehenden Wissenslücken und Unsicherheiten sind allgemeine, theoretische Erwägungen und der Verweis auf Vorschriften, die für alle Nutzpflanzen bestehen, bei weitem nicht ausreichend. Weder die Sortenzulassung noch die Beurteilung von Gesundheitsrisiken im Rahmen der Novel Food Gesetzgebung bei einzelnen NGT-Produkten, sind geeignet bzw. umfassend genug, um die Sicherheit von NGT Pflanzen in allen wesentlichen Bereichen gewährleisten zu können.

Neben einer Reduktion der Sicherheitsstandards – insbesondere für alle NGT 1 Pflanzen und damit für die überwiegende Mehrzahl der zu erwartenden NGT-Produkte – hätte die geplante Neuregelung noch eine Vielzahl anderer unerwünschter Folgen:

- Die Umsetzung der Regelung würde eine große zusätzliche wirtschaftliche Belastung für alle landwirtschaftliche Produktionssysteme bedeuten, die GVOs und NGT-Pflanzen nicht einsetzen dürfen oder wollen, wie z.B. die biologische Landwirtschaft. Sie würde damit genau jene Bereiche belasten, die für eine weitere nachhaltige Entwicklung in der Landwirtschaft wichtig sind.
- Die Abschaffung der Kennzeichnung für Lebens- und Futtermittelprodukte aus NGT 1 Pflanzen würde die Wahlfreiheit von Konsument:innen empfindlich einschränken.
- Die Rechte der Mitgliedsländer würden durch die geplante Neuregelung erheblich geschwächt – dafür müssten sie zusätzliche Verantwortung z.B. für die Umsetzung von Koexistenzregelungen für NGT 2 Pflanzen übernehmen, ohne dass in allen Fällen die notwendigen Voraussetzungen, z.B. hinsichtlich der Verfügbarkeit von Nachweismethoden, gegeben sind.
- Die geplante Regelung enthält keine Maßnahmen, welche den Zugang von Pflanzenzüchtern zu Pflanzenmaterial für die Weiterentwicklung sicherstellen würden, wenn dieser von patentrechtlichen Bedingungen eingeschränkt wird.

Es muss daher rasch dafür gesorgt werden, dass die Schwächen des vorgeschlagenen Regelungsentwurfs durch eine umfassende Überarbeitung beseitigt werden und noch weitergehende Vorschläge, die auf eine noch weiter gehende Deregulierung abzielen zurückgewiesen werden.

1 INTRODUCTION

1.1 UNINTENDED EFFECTS ASSOCIATED WITH PRODUCTS ESTABLISHED BY NEW TECHNIQUES FOR GENETIC ENGINEERING – A CAUSE FOR PRECAUTION!

Since their introduction biotechnological methods to create genetically modified organisms (GMOs) were recognised as powerful tools to develop new plants, animals and microorganisms with novel traits e.g. for use in agriculture and food production. Similarly, the use of new techniques for genetic engineering such as genome editing, also called new genomic techniques (NGT), may enable the rapid development of a wide range of novel products with substantially new characteristics. Considering the limited knowledge on the overall biological effects of such powerful methods, adverse effects on health and the environment cannot be excluded. Indeed, possible hazards were identified for a number of NGT products, including plants, animals and micro-organisms (Then, 2022; Koller and Cieslak, 2023). Without a comprehensive risk assessment unintended genetic modifications such as the unexpected integration of transgenic DNA may be overlooked - as demonstrated by an independent study by the Food and Drug Administration, which detected the unintended integration of a transgenic antibiotic resistance gene in a hornless NGT cow (Norris et al. 2020). Thus, the use of NGTs to develop plants and animals e.g. for agricultural purposes needs to be accompanied by a strong commitment to ensure the safety of NGT products.

The concerns with regard to potential adverse effects by NGT products may be summarised as follows:

- Some newly developed traits may not only exhibit the intended beneficial effect(s), but they may also have additional unintended biological characteristics, that are less favourable or even adverse.
- All biotechnological methods can introduce unintended genetic changes in addition to the intended modifications. For NGT plants created by targeted mutagenesis this concerns several important issues: (i) effects resulting from the introduction of recombinant ("transgenic") DNA-elements to express the "genetic scissors" for genome editing and (ii) the unintended genetic changes ("off-target mutations") due to imprecise editing. Even without introduction of foreign DNA, such genetic changes can result in unintended biological effects that may compromise the safety of the NGT products.

Sometimes the occurrence of unintended genetic changes can be minimised, however they cannot be avoided completely. Unintended genetic changes associated with the methods to produce GMOs and NGT products should be subject to a risk assessment.

1.2 CONSIDERATION OF UNINTENDED EFFECTS IN THE CURRENT GMO LEGISLATION – AN IMPORTANT ISSUE FOR LEGISLATION AND RISK ASSESSMENT!

According to the national legislation in EU Member States, e.g. the Austrian Biosafety Law (Gentechnikgesetz), the existing EU legislation covering the use of GMOs, i.e. Directive 2001/18/EC, Regulation (EC) No 1829/2003 and relevant international obligations, e.g. the Cartagena Protocol on Biosafety under the Convention on Biological Diversity, a risk assessment needs to be conducted for GMOs prior to authorisation. These laws are based on the precautionary principle and require a risk assessment to address any intended and unintended changes in the characteristics of a GMO that have direct or indirect adverse effects.

As far as existing GMO regulations apply to NGT plants similar obligations to identify possible unintended adverse effects by a risk assessment are in place. However, the global regulatory landscape is more diverse for NGT products than for transgenic GMOs: In the last years a number of countries, among others the USA, South American and Asian countries, Australia and the UK, implemented special regulations for NGT products. These regulations exempt certain NGT products, in particular NGT plants which do not contain “foreign” DNA, from the GMO laws. Thus, in those countries a risk assessment is not required for such NGT plants.

1.3 THE 2023 EUROPEAN COMMISSION PROPOSAL FOR THE REGULATION OF NGT PRODUCTS – UNDERESTIMATING UNINTENDED EFFECTS?

According to a decision by the European Court of Justice in 2018 NGT products developed by targeted mutagenesis (i.e., genome editing) or GM technology are subject to existing GMO regulations in the EU. Therefore, the requirements of the GMO legislation apply to NGT plants. These requirements include a risk assessment to address possible unintended effects.

Subsequent to this court ruling the European Commission (EC) conducted a study and concluded that the current regulatory system is not supporting the development and use of NGT plants appropriately (European Commission, 2021). As a consequence, the EC tabled a proposal for a new regulation for NGT plants in July 2023. This proposal is currently discussed by the relevant EU bodies (EU Council and the European Parliament) and will be amended taking into account the views of Member States and the European Parliament.

The EC proposal introduces technical criteria to categorize NGT plants into two differently regulated groups and to exempt most NGT plants from requirements under the current GMO legislation. These proposed changes are based on policy decisions rather than on scientific arguments or risk considerations.

- Category 1 NGT plants that – according to the opinion of the EC – could also be produced by conventional breeding would be treated like conventional plants. Thus, the current requirements for authorisation, risk assessment, traceability and labelling of food and feed would not apply. A majority of NGT plants would fall under this category. The envisaged deregulation would compromise two important pillars of the current GMO legislation: a comprehensive assessment of risks and the freedom of choice for consumers.
- Category 2 NGT plants which do not meet the criteria for Category 1 NGT plants would be treated like "classical" GMOs, however with adaptations concerning requirements of the current GMO legislation. This could result in a less robust risk assessment and monitoring, particularly with respect to unintended effects.

Taken together, the EC proposal is a significant diversion from the current regulatory system implemented in the EU for GMOs. The elimination of a comprehensive assessment of potential unintended effects for both categories of NGT plants causes serious concerns:

- The overall objective of verification of the regulatory status of NGT plants is not fit to address safety aspects of the respective NGT plants or products.
- The data requirements for verification are likely not sufficient to identify whether unintended effects are associated with a particular Category 1 NGT plant.
- Whether unintended effects associated with Category 2 NGT plants will be adequately addressed will depend on the future design of the risk assessment for these products. The proposal outlines that this will be decided by delegated acts by the EC, which are not drafted or even outlined yet.

2 UNINTENDED EFFECTS OF GM CROPS - A COMPLEX ISSUE

"Unintended effects" regarding genetically modified organisms such as GM and NGT plants is commonly used to describe "side effects" of the alteration of the genome. Regarding these effects, genetically modified organisms are frequently compared with conventionally bred plants. The following sections will examine the issue in relation to both breeding approaches.

2.1 GENETICALLY MODIFIED ORGANISMS & UNINTENDED EFFECTS

Neither classical GM technology nor NGTs create products containing only specific, intended genetic modifications. Typically, a variety of differently modified individuals is created by these techniques containing different types of mutations and/or insertions of genetic elements.

Genetic changes, which result in a desired trait, are regarded as "intended modifications", e.g. the integration of a transgenic DNA construct, or the introduction of mutations at a specific location of the plant DNA. All other genetic changes, in particular changes resulting in undesirable characteristics of the GM or NGT product, are regarded as "unintended modifications". Looking at the overall development process of genetically modified plants unintended effects can be introduced at different steps:

- Unintended genetic chances can be the result of the specific techniques, such as the above mentioned off-target effects encountered with the techniques for genome editing or the additional integration of parts of the transgenic constructs.
- In addition, methods used for cultivation of plant cells and tissues as well as the regeneration of plantlets from single plant cells after transformation or genome-editing can induce additional mutations. In some cases the number of such mutations is higher than the number of off-target changes by genome editing itself (Sturme et al., 2022).

2.2 CONVENTIONAL PLANTS & UNINTENDED EFFECTS

Conventional breeding generally relies on the selection of plants with beneficial characteristics from populations showing some genetic variation and the crossbreeding of selected plants. Genetic diversity is introduced either through spontaneous genetic change occurring naturally, or by methods of classical mutagenesis using chemicals or radiation. Since such mutations are not deliberately targeted to specific locations in the genomic DNA or restricted to the integration of a specific transgenic DNA construct, the term "unintended modification" therefore does not make sense in this context. What is more important in this context is the ability of plant breeders to select desired trait(s) while removing all other genetic modifications from the plant genome, e.g. via a sufficient number of crossbreeding steps.

However, some advanced methods of conventional breeding[1] also employ cell and tissue culture techniques similar to the ones used in GM-technology. Therefore, a higher number of mutations can be detected in conventional plants developed with these methods.

2.3 ISSUES CONCERNING THE ASSESSMENT OF UNINTENDED EFFECTS

With regard to risk assessment of unintended effects, it is necessary to distinguish between two important levels. Adverse effects will only materialize when both levels are involved:

1. Unintended genetic changes are present in the genome of a modified plant
2. Unintended biological effects result from the genetic changes present in a modified plant

At the first level unintended hereditary genetic changes are considered. Such unintended genetic changes can be identified with methods of DNA analysis. Such an analysis can also establish whether unintended genetic changes are still present in the genome of a modified plant at the final development stage. The detection of unintended genetic changes, however, does not indicate that these mutations result in adverse biological effects.

The second level addresses whether relevant biological characteristics of a modified plant are adversely affected by the genetic changes present in the NGT plant. Unintended biological consequences may also be connected to an intended trait, if this trait is involved in multiple biological processes. As discussed below for a number of different NGT plants intended compositional changes, such as increased levels of γ-Aminobutyric acid (GABA)

[1] E.g. plants developed by embryo rescue techniques or methods to induce polyploidy.

or reduced amounts of gliadins, can impact the development of the respective plants and/or their interactions with the environment (responses to environmental stress such as draught, cold or pest insects). Also unintended health effects may arise from the consumption of plants with constituents that influence important physiological processes (e.g. GABA influences blood pressure and other neurological processes) or from consumption of De novo domesticated plants, which may contain a wide range of untested and untried constituents.

2.4 CURRENT STATE OF KNOWLEDGE CONCERNING UNINTENDED EFFECTS OF NGT PLANTS

The current state of knowledge concerning unintended effects of GMOs and particularly of NGT plants is still rather limited (Then, 2022). This is due to a number of technical and regulatory reasons (Sturme et al., 2022).

- A systematic analysis of unintended effects is not a specific focus of the ongoing research conducted for NGT plants.
- Most of the efforts to investigate off-target changes are conducted for method optimisation purposes rather than to identify potentially adverse effects.
- The focus of the research is centred on the “biased” identification of off-target mutations, occurring at DNA sequences with a very high sequence similarity to the intended target sites. Such methods do not detect the whole range of off-target mutations.
- From the available data on off-target modifications it is unclear whether unintended genetic changes are still present in NGT plants at the final development stage or whether adverse biological effects are connected with such changes.
- Most NGT plants developed in other countries than the EU are not regulated according to GMO laws, which limits the availability of information related to the safety of these NGT plants. The information needed for a review of the regulatory status of NGT applications is not primarily addressing safety aspects. Only a few NGT products are marketed to date, which explains why there is very limited practical experience with NGT plants from non-EU countries.

2.4.1 RESULTS FROM AVAILABLE META-ANALYSES ADDRESSING THE OCCURRENCE OF UNINTENDED GENETIC CHANGES IN NGT PLANTS

Most of the scientific publications describing newly developed NGT plants focus on the technical development of these NGT plants and their traits. Only some of these reports address the issue of unintended genetic changes introduced during the overall

development process. In most cases off-target activity is investigated to evaluate the precision of a particular method. However, data on the initial frequencies of off-target mutations do not provide information whether unintended genetic changes are still present in the final NGT products developed for marketing. Thus, these data are not sufficient for risk assessment regarding unintended effects of NGT plants.

Some recent reviews analysed the available data on off-target changes in NGT plants (Modrzejewski et al., 2019; Modrzejewski et al., 2020; Chu and Agapito-Tenfen, 2022; Sturme et al., 2022). Most of the analysed studies addressed a commonly used method for genome editing where the CRISPR-Cas nuclease is expressed from transgenic constructs, which were previously inserted into the plant genome. This approach ensures a high mutation rate at the intended target site(s), but it is also associated with an increased occurrence of off-target mutations.

The overwhelming majority of the available studies did not investigate the whole range of off-target changes in the genome-edited plants, but only off-target mutations occurring at a small number of genomic sequences with a high similarity with the target site. This approach must be considered insufficient since it disregards information from less biased studies which found unintended genetic changes in NGT plants at DNA sequences which were not predicted as possible off-target sites (Sturme et al. 2022).

Further evidence is available from studies in mouse and human cells indicating the widespread occurrence of unintended modifications in the vicinity of the target sites for genome editing, including large deletions and other major modifications of the structure of the targeted chromosome, e.g. Chromothripsis, which is describing the reassortment of multiple neighboring chromosome regions (Park et al., 2023). Using appropriate testing methods such extensive chromosome aberrations were recently detected in the vicinity of target sequences in genome edited tomato plants for the first time (Samach et al., 2023). This emerging information on unexpected unintended modifications, which would be retained during further breeding, needs to be considered for the risk assessment to avoid overlooking relevant hazards. The proposal of the EC on the contrary is not addressing the emerging information concerning the presence of such unintended modifications in NGT products properly (Then, 2023).

2.4.2 KNOWLEDGE GAPS REGARDING UNINTENDED EFFECTS IN NGT PLANTS

Significant knowledge gaps and uncertainties still exist concerning the identification and assessment of unintended effects:

The mentioned scientific reviews and the opinions published by the European Food Safety Authority (EFSA) (Naegeli et al., 2020; Paraskevopoulos and Federici, 2021) focus on the information on off-target changes and argue that genome editing introduces fewer off-

target mutations than the number of genetic changes occurring during conventional breeding. Since GM- and conventional methods are also involved in the development of NGT plants this comparison is not reasonable. Rather the overall development process of NGT plants needs to be considered to address all sources for unintended genetic changes and their effects (Eckerstorfer et al., 2023).

Most conclusions which dismiss the relevance of unintended effects are based on general rather than case-specific considerations. However, genome editing approaches are serving different purposes, which in turn impacts their off-target activity (Modrzejewski et al., 2020): Some methods are optimised for a unique genomic target site and designed to minimize unintended off-target mutations. However, less specific methods are used to simultaneously mutate several target sequences that are similar, but not identical. These methods cannot be optimised for precision without compromising the approach. Such case-specific differences are not considered sufficiently by EFSA (Eckerstorfer et al., 2021).

The available reviews also indicate a lack of evidence concerning the biological effects of unintended genetic changes. According to Sturme et al. (2022) relevant information to assess off-target modifications is missing, e.g. information on the biological effects of off-target modifications. In addition, few information is available for unintended effects of different types of on-target mutations. The lack of such evidence is highly relevant since these modifications are retained in NGT plants.

To address these challenges several experts and institutions have provided suggestions how to assess unintended genetic changes (Haut Conseil des Biotechnologies, 2017; Eckerstorfer et al., 2019; Kawall et al., 2020; Lema, 2021). These suggestions can serve as a starting point to establish appropriate guidance for risk assessment.

3 UNINTENDED EFFECTS OF NGT PLANTS – EXAMPLES FROM RECENT DEVELOPMENT

The following chapters present some examples for recently developed NGT plants and discuss which unintended adverse effects should be considered for NGT plants.

The discussion of these NGT plants in the following chapters is focused on possible unintended effects associated with the introduced trait(s) and the overall characteristics of the particular NGT plants. The identification of unintended genetic changes during risk assessment could lead to further plausible hypotheses concerning potential adverse effects. The comparative assessment of composition, as well as agronomic and phenotypic characteristics can facilitate a case-by-case risk assessment of NGT plants with a similar level of confidence as currently accomplished for GMOs.

3.1 NGT TOMATOES WITH INCREASED LEVELS OF GABA

NGT tomato plants with an increased level of gamma-Aminobutyric acid (GABA) are available for commercial use in Japan since 2021. The Japanese marketing approval was not based on a risk assessment following the requirements for GMOs, as NGT plants such as the GABA tomato are not regulated as GMOs in Japan. According to the current regulatory proposal by the EC the GABA tomato would be considered a Category 1 NGT plant and thus exempt from risk assessment.

The GABA tomato is an example for a NGT product with a modified composition. Unlike other NGT plants of this category the GABA tomato is a “functional food” product, i.e. a food with expected or demonstrated specific health effects: The developers claim that the consumption of the GABA tomato will lower the blood pressure in humans which suffer from hypertension while people with normal blood pressure would not be affected (Nonaka et al., 2017). However, as with other “functional” substances, such effects are dose-dependent and may be different in specific groups of the population (e.g. children, adults or old people). The amount of GABA may also be dependent on the way in which GABA tomatoes are processed in different food products.

Furthermore, unintended effects due to the increased GABA level in the modified plants are plausible, since GABA impacts a range of different plant characteristics such as growth and development, resistance to a range of environmental stressors, including draught, cold, lack of oxygen and plant pathogens and pests. In addition, GABA is involved in the regulation of the production of secondary metabolites involved in the response of tomato plants to environmental stress. A permanent overproduction of GABA may have unintended effects on the level of certain metabolites produced in tomato plants, the ability to respond to stressors and could impact the interactions with other organisms, such as microorganisms, nematodes and insects, including pollinating insects.

Thus, a number of unintended effects need to be assessed:

- Unintended effects on the health of specific population groups, including the most vulnerable, due to increased consumption and unintended use of tomatoes with higher levels of GABA.
- Unintended effects on the morphology and growth characteristics of tomato plants as described by Nonaka et al. (2017), due to an inhibition of cell wall growth caused by higher levels of GABA.
- Unintended effects on the level of secondary metabolites which are involved in plant-plant and plant-insect interactions.
- Unintended effects on the ability of tomato plants to properly respond to abiotic environmental stress conditions.
- Inhibitory effects on other organisms including beneficial microorganisms and insects (e.g. butterfly larvae) and a possible influence of elevated GABA levels on the interaction with pollinators such as bumblebees.
- Unintended outcrossing of the trait to other tomato varieties may be possible.

Without a comprehensive risk assessment, no robust evidence is available concerning the possible health benefits for all or groups of the population nor the above mentioned potential adverse effects.

3.2 NGT WHEAT WITH A LOWER AMOUNT OF GLUTEN

The presence of gluten-proteins in certain cereals such as wheat, can cause serious health effects, including celiac disease (Marín-Sanz et al., 2023). To develop wheat varieties with a lesser amount of gluten, CRISPR-Cas technology was used to reduce the expression of gluten genes in bread wheat by modifying a large number (up to 35 out of 45) alpha-gliadin genes (Sánchez-León et al., 2018). This wheat is an example for an NGT plant containing extensive and complex genetic modifications, which cannot be achieved by

conventional methods. The NGT wheat would likely be classified as a Category 2 NGT plant.

The extensive editing poses a number of substantial risk assessment challenges regarding unintended effects:

- The many edited gliadin genes were modified in different ways. A characterisation of the different outcomes of editing (effective silencing of expression of the modified gene, production of a truncated gliadin protein or unintended creation of a new hybrid protein with unknown effects) needs to be conducted to be able to assess the overall effects of all modified gliadins.
- Potential unintended effects leading to the expression or production of new compounds or altered levels of other constituents. Reduction in the gliadin content in wheat leads to a compensatory increase in glutenins (Mullins et al., 2022). Thus, a comprehensive comparative analysis should be conducted to provide a basis for the food safety assessment.
- An allergenicity and toxicological assessment of newly created proteins or altered levels of constituents (i.e. gliadins and glutenins as well as other potentially allergenic non-gluten proteins) needs to be conducted.
- Gliadins are also involved in plant responses to environmental stress, such as drought and heat (Marín-Sanz et al., 2023). The reduction in the content of different gliadins may affect the ability of the NGT wheat plant to cope with such conditions.

The combination of a multitude of different genetic modifications in a single NGT plant is creating substantial challenges for the assessment of possible environmental and health effects as acknowledged by EFSA (Naegeli et al., 2021). Thus a risk assessment according to the existing EFSA guidance was recommended (Mullins et al., 2022). A risk assessment based on vaguely defined “risk profiles” and general considerations as suggested by the EC would not be an appropriate alternative, in particular for NGT products, such as this NGT wheat plant.

3.3 NGT RICE WITH INCREASED TOLERANCE AGAINST SALT STRESS

The EC assumes that NGT approaches may offer a sustainable way to address the negative effects of increased climate stress on crop production. However, the complex nature of abiotic stress response in plants is a major obstacle to the development of plants with enhanced resistance, as seen by the limited number of reports on plants with increased resistance to abiotic stress (Kawall, 2021). Most research in this field is conducted in China with a strong focus on rice as an important crop, which is particularly sensitive to salt stress (Nascimento et al., 2023). A substitution of NGT rice with other crop species that are

generally less sensitive to salt stress may not be an option since rice is a traditional staple food in parts of the world and switching to alternative crop species may not be feasible for that reason.

NGT rice lines with an increased resistance to salt were e.g. developed by the targeted mutation of the osRR22 gene, a regulator of gene expression in rice (Zhang et al., 2019). osRR22 induces the production of antioxidants in salt-stressed rice plants and increases the levels of pigment production, photosynthesis and transpiration. The osRR22 gene also plays a role in the development of leafs, roots and flowers. Recent research shows that the mutated osRR22 leads to a range of different developmental and compositional changes (Aycan et al., 2023). The NGT rice would also be another example of a Category 1 NGT plant.

NGT rice seedlings could tolerate higher amounts of salt in small scale experiments in the greenhouse, but were not yet investigated in large scale open field trials and a comprehensive evaluation of the biological effects of the NGT plant was not conducted. The broad range of plant characteristics affected by the modification of osRR2 suggests that different unintended effects on environmental interactions or nutritional quality are plausible. Issues which need to be considered during an ERA include the following:

- It is unclear whether the genome edited rice will perform under field conditions as expected or show other unintended reactions. Field trials with proper control are needed to assess the performance of different cultivars under stress or non-stress conditions.
- Enhanced environmental stress resistance may result in a yield burden – the initial test results indicating that no yield depression in NGT rice occurs under no-stress conditions need to be confirmed under a variety of environmental conditions.
- If the modification is indeed increasing the fitness of rice plants under salt stress, it needs to be considered whether the trait could increase the environmental fitness of weedy relatives upon hybridisation and outcrossing.
- It unclear whether physiological or metabolic changes are initiated by the modified regulatory gene, that may have an impact on the composition and possibly on the food safety of NGT rice.

The NGT rice was developed based on information from a conventionally developed rice variant with a mutation in the osRR22 gene. Nevertheless, the complex environmental interactions of such NGT plants pose challenges to the assessment of possible risks.

3.4 DE NOVO DOMESTICATED TOMATOES WITH INCREASED DISEASE RESISTANCE

Historically the "domestication" of wild plants for agricultural food and feed production, i.e., to render them nutritious and safe to eat, was a very long process involving many consecutive steps of selection and breeding. In the case of tomato plants, it took approximately 10.000 years to turn the sturdy wild tomato plants with just a few small and basically unedible fruits, into the currently cultivated (domesticated) tomato varieties, which produce many big and nutritious tomatoes. Genetic research has identified some key regulatory genes which can be modified with NGTs to fast-track the transformation of wild tomato plants into plant varieties which resemble domesticated tomato plants (Li et al., 2018; Zsögön et al., 2018). The targeted mutation of four or six genes, respectively, resulted in modified wild tomato plants with growth patterns and fruit characteristics comparable to domesticated tomato varieties. Other traits of the wild plants, such as resistance to pathogens or abiotic stress (e.g. draught or salinity) are retained. "De novo domestication" could be used to develop traits, which may not be established by conventional breeding. According to the regulatory proposal of the EC *de novo domesticated* tomatoes would be considered Category 1 NGT plants and "equivalent" to modern varieties. This disregards that these NGT tomatoes are genetically similar to the parental wild plants, which are not consumed as food and have no "history of safe use".

As *de novo domesticated* tomatoes are not directly comparable to conventional GM tomato varieties, the developers (Zsögön et al., 2018), EFSA (Mullins et al., 2022) and others (Kawall, 2021; Eckerstorfer et al., 2023) agree, that the current approach for a risk assessment using a conventional bred plant with a history of safe use as a comparator is not applicable.

The risk assessment of such NGT plants would need to focus primarily on the many untried and untested characteristics of the parental wild plants and consider further unintended changes of these characteristics resulting from the introduced mutations. The following issues need to be addressed:

- A comprehensive assessment of the environmental effects of the cultivation of *de novo domesticated* plants including effects on exposed wildlife.
- This assessment needs to be based on a robust characterisation of the phenotype of the parental wild tomato plants and on a characterisation of the phenotypic changes induced by the genetic modifications developed by targeted mutagenesis.
- A comprehensive assessment of the health effects of *de novo domesticated* plants due to their consumption as food. Such an assessment needs to take into account the

guidance for and the experiences with the assessment of novel foods according to the EU regulations for novel foods (Regulation (EU) 2015/2283).

- If *de novo domesticated* plants would be used for feed production an assessment of the feed safety of such products is needed, which may be based on similar approaches as the food risk assessment.
- This assessment needs to be based on the identification and characterisation of new compounds and altered levels of endogenous constituents of the *de novo domesticated* plant in comparison to the parental wild plant and currently used tomato varieties.
- Based on the findings of the compositional/comparative assessment any toxicological, allergenic and nutritional hazards need to be identified.

EFSA acknowledged the challenges that are associated with the risk assessment of such *de novo domesticated* plants and concluded that the existing guidelines may not be sufficient and need to be further developed (Mullins et al., 2022). *De novo domesticated* plants are of special concern, due to the higher likelihood for unintended effects as compared to NGT plants, which are developed from plant lines with a history of safe use: Their complete parental genetic background could be a source of unintended effects, e.g. hazards in relation to food safety. In addition, the introduced genetic changes could result in further unintended physiological changes. EFSA stated that – even based on a risk assessment - it may not be possible to conclude that such plants are as safe and nutritious as a conventional crop (Mullins et al., 2022).

4 CONCLUSIONS – HOW TO DEAL WITH UNINTENDED EFFECTS OF NGT PLANTS?

4.1 THE PROTECTION OF HEALTH AND THE ENVIRONMENT SHOULD NOT BE LESS IMPORTANT THAN THE PROMOTION OF ECONOMIC INNOVATION!

A general objective of the legislative proposal for NGT plants according to the EC is to "maintain a high level of protection of human and animal health and of the environment, in accordance with the precautionary principle". A similar objective in the current GMO legislation is implemented by a risk assessment according to the case-by-case principle and an environmental monitoring for GM products. According to the GMO legislative framework a core data set for a comparative risk assessment needs to be provided to identify unintended genetic modifications and to assess unintended effects. In addition, monitoring is conducted to identify unintended long-term outcomes of commercial use, which could not be anticipated and assessed during the risk assessment. The GMO regulations also acknowledge the responsibility of individual Member States for the cultivation of GM plants, based on the structural differences of the respective agricultural systems. Even though the GMO risk assessment framework still needs further improvement, in general it follows a precautionary approach to ensure a high level of protection.

The EC regulatory proposal for NGT plants is based on a very different approach. In spite of the overall objective to ensure a high level of protection, other objectives, e.g. to support the development of innovative biotechnologies and to strengthen economic competitiveness, significantly influenced the EC proposal. The proposed approach deviates significantly from the precautionary principle, omitting a case-by-case risk assessment of individual NGT products, and thus from an approach that would appropriately address specific unintended effects.

For all Category 1 NGT products – comprising more than 90% of all NGT plants - no case-specific risk assessments according to the guidelines implemented for GMOs would be required; whether an assessment of food safety of certain NGT 1 plants, among others the GABA tomato and *de novo domesticated* plants, would need to be conducted is uncertain at the moment. Such a decision would be up to case-specific considerations, which may not be straightforward and thus hard to predict. For Category 2 NGT products, which contain

highly complex genetic modifications, the risk assessment requirements could be less comprehensive than for GMOs with transgenic modifications.

As described in the previous chapters, the possibility of unintended effects cannot be excluded in a general way. In spite of the lack of robust scientific knowledge regarding unintended effects of NGT products, the proposed NGT regulations are based on theoretical comparisons of general characteristics of NGT plants with a hypothetical range of plants, which might be generated by conventional breeding methods. This, however, does not take into account the technical feasibility of such developments or the practical existence of the respective conventional plants.

4.2 ARE UNINTENDED EFFECTS OF NGT PLANTS PLAUSIBLE? – LESSONS TO BE DRAWN FROM EXISTING EXAMPLES OF NGT PLANTS

The overview on a number of NGT plants indicates crucial deficiencies of the regulatory approach proposed by the EC.

Three of the four examples (GABA tomatoes, salt tolerant rice, de novo domesticated tomato) would be considered Category 1 NGT plants according to the proposed NGT regulations – with severe consequences for safety related requirements. The current proposal does not include provisions for a case-specific risk assessment, nor for monitoring or labelling of NGT food products. The EC proposal would also not require traceability and coexistence measures.

In contrast the available evidence concerning possible unintended effects of these Category 1 NGT plants and their traits is rather limited. According to the current approaches for GMOs and Novel Foods, possible adverse effects need to be assessed prior to placing on the market! For the GABA tomato which contains an elevated level of a substance inducing health effects and for the *de novo domesticated* tomato the existing knowledge base is far from sufficient to exclude possible adverse effects without a risk assessment. In particular, the *de novo domesticated* tomato poses extremely difficult assessment challenges: Without a history of safe use for the parental plant at hands, a no-risk classification seems to be ill-advised.

The available information concerning unintended genetic changes is insufficient in terms of an assessment of possible adverse effects. Available data typically do not address all types of such unintended modifications but focus mostly on off-target mutations at genomic sequences homologous to the respective target site(s). Also, the question whether or which unintended off- or on-target modifications are still present in marketable NGT plants is not sufficiently addressed by the current approaches to detect unintended genetic modifications. A thorough investigation of such unintended modifications is however warranted, as shown e.g. in the case of genome-edited hornless cattle. These NGT cattle were

developed in 2013 and screened for off-target modifications using common, but insufficient methods. A subsequent, independent investigation revealed that foreign DNA was inserted unintentionally into the cattle genome, including a bacterial antibiotic resistance gene from the transgenic DNA repair template which was used to direct genome editing (Norris et al., 2020). The experts from the US Food and Drug Administration (FDA) concluded that such hereditary on-target modifications are underreported in NGT animals and may have been overlooked in some cases. These unintended modifications are not compatible with an NGT status and would raise plausible concerns during risk assessment. Sturme et al. (2022) indicate that similar issues may arise for NGT plants.

The low gluten NGT wheat would likely be regarded as a Category 2 NGT food product according to the proposed regulation and may be subjected to a shortened and weakened risk assessment. This, however, would not be in line with the earlier conclusions by EFSA with regard to the assessment challenges identified for such products (Naegeli et al., 2021). Other proposed regulatory changes (e.g. the possibility to waive the requirements for monitoring or the interdiction of national restrictions etc.) could further lower the current level of protection. In addition, even a low gluten content could endanger humans suffering from celiac disease. Thus, this NGT wheat may not be a sustainable solution, but rather an obstacle to the increased use of other truly gluten-free cereals.

A look on the uncertainties regarding unintended effects of the discussed NGT plants suggests that a risk assessment as required for GMOs should be conducted to ensure the safety and wholesomeness of these NGT plants and to meet the pursued high level of protection.

EFSA concluded that the current guidance for the risk assessment of GM plants is in principle adequate and sufficient for many NGT plants (Naegeli et al., 2020; Naegeli et al., 2021), but in certain cases, e.g. for *de novo domesticated* plants, additional guidance needs to be established (Mullins et al., 2022). However, the limited amount of investment (1,6 % of the total research budgets) available for safety-related research in EU countries may impede improvements (Arbeiterkammer, 2022).

4.3 UNINTENDED EFFECTS OF A DEREGULATION OF NGT PLANTS

Adequate risk assessment and monitoring needs to be ensured for all NGT plants

As discussed above the available scientific knowledge on unintended effects of NGT plants as well as the practical experience with such plants is very limited – partly due to the novelty of the methods and partly due to the complexity and the variety of changes that can be introduced by these techniques (Koller and Cieslak, 2023).

To address risk issues of NGT plants comprehensively a precautionary, case-specific approach, taking into account the available experience with comparable products, is needed. The risk assessment for NGT products should be based on similar principles as adopted for GMOs and should address nutritional safety and quality, as well as effects on the environment. The assessment also needs to address off-target modifications and other unintended genetic changes, which remain in the final NGT product and might result in adverse effects.

Currently no other legislative requirements for agricultural products in the EU, such as requirements for variety registration and Novel Foods provide similar comprehensive assessment and protection standards as the EU regulations for GMOs. To ensure that NGT plants and food and feed products are safe a precautionary risk assessment of all NGT plants needs to be guaranteed.

A substantial revision of the current legislative proposal by the EC is required

The current regulatory proposal by the EC would result in significant undesirable effects on a whole range of aspects including, but not limited to safety and consumer rights:

"Unintended effects" regarding the protection of health and the environment due to the lack of risk assessment and monitoring requirements for Category 1 NGT plants and a weakened risk assessment for Category 2 NGT plants.

To address any plausible unintended effects on human and animal health and the environment it needs to be ensured that a risk assessment for all NGT plants is implemented according to the principles adopted for GMOs!

"Unintended economic effects" for other agricultural production systems - Producers of GM-free products and organic farmers will bear the whole burden to ensure the integrity of their products, while relevant tools will be missing (e.g. traceability information and analytical methods for detection).

The insufficient requirements for transparency of use of NGT plants need to be revised to ensure that GM-free production can observe their responsibilities to keep their products free from NGT plants, as included in the proposed regulation!

"Unintended effects" for consumers - No labelling and traceability for food and feed products for Category 1 NGT plants is foreseen, which is jeopardising the rights of consumers and farmers for making informed choices for personal or business reasons.

Transparency concerning NGT food and feed products and requirements for labelling to provide information that is vital for making informed choices need to be upheld!

"Unintended effects" for EU Member States and their competent authorities - The rights of Member States concerning the use of NGT plants within their territories are significantly

weakened, yet they will have to bear the burden to implement coexistence measures for Category 2 NGT plants, in spite of a significant uncertainty whether necessary enforcement tools are available.

The current balance between obligations for Member States and their responsibilities for implementing national measures and participating fully in decision-making needs to be maintained!

“Unintended effects” for plant breeders - The proposed regulation does not address the issue of patentability of NGT products, in spite of concerns that the access of plant breeders to breeding materials may be restricted in the future.

The freedom of plant breeders to operate without restrictions by the far-reaching scope of patents on seeds produced with NGTs needs to be safeguarded!

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