

Background:

First-time flowering in poplars: minor genomic changes using new genomic techniques can change species-specific characteristics

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Abstract

Scientists in the US have successfully used new genetic engineering (new genomic techniques, NGTs) to radically shorten the juvenile phase in poplars from seven to ten years to just a few months, thus enabling premature flowering. It was shown that the fundamental species-specific characteristics of poplar can be changed with just a few minor genetic alterations and without adding new genes. Similar to annual arable plants, in theory, this makes it possible to cross and select NGT poplars within short periods of time, and thus significantly accelerate their release and marketing. However, if the poplars are released or escape into the environment, this trait may result in uncontrolled spread, with immense follow-on effects for the conservation of protected poplar species. For example, NGT poplars spreading in the environment could displace the black poplar, which is on the Red List of endangered species. In addition, complex ecosystems could be impacted or disrupted, as poplars interact with a large number of species, especially insects, including protected butterfly and beetle species.

Introduction

NGTs are molecular biological tools (e.g. the CRISPR/Cas9 gene scissors) and methods that enable targeted interventions in the genetic material of organisms. They allow individual genes to be specifically rewritten, to insert additional genes, or to simply remove or switch off endogenous genes via knockout (Jinek et al., 2012; Kawall, 2019). Currently, the criteria proposed by the EU Commission for the future regulation of NGT plants, i.e. arable and wild plants, including trees, only refers to the number and type of mutations, but not to their genomic context/location in the genome. The Commission suggests that the proposed criteria would be sufficient to verify the 'equivalence' of NGT plants and plants obtained from conventional breeding (including random mutagenesis), thereby exempting them from mandatory risk assessment. This backgrounder presents a case study on NGT poplar trees showing that the biological effects resulting from minor genome alterations can go far beyond what has ever been achieved by previous methods. Thus, the requirement for in-depth risk assessment is essential.

Biology and ecology of poplar trees

The genus of *Populus* (which belongs to the *Salicaceae* – willow family) includes about 35 species (Aas, 2006). They grow in the moderate climate of the northern hemisphere and can be found from Portugal in the west to China in the east, and across most regions throughout Eurasia, as well as in the US and Canada (De Rigo et al., 2016). In central Europe, poplars are the fastest growing tree species. Naturally occurring native species include white poplar (*Populus alba*), black poplar (*P. nigra*), European aspen (*P. tremula*) as well as hybrids such as grey poplar ($P. \times canescens = P. alba \times P. tremula$) (Aas, 2006). In addition, there are other subspecies which are grown for commercial purposes, e.g. balsam poplar (*P. balsamifera*) and black cottonwood (*P. trichocarpa*) as well as

hybrids of Canadian poplar (*P. x canadensis*), which stem from crossing the European *Populus nigra* with North American *P. nigra* varieties (Aas, 2006, Testbiotech, 2010).

Poplar species are regarded as pioneering plants that can grow and multiply even under unfavourable environmental conditions. They readily distribute via pollen and seeds, root suckers or even parts of broken branches and stick cuttings. Poplars grow almost anywhere – in old quarries, gravel pits, in sand or clay, in wetlands, mountains, plains etc. If they grow on river banks, seeds and broken branches can be carried over long distances in flowing water.

During their lifetime, poplar trees can produce huge amounts of seeds and pollen that are dispersed over large areas. A female poplar tree can, for example, produce 25 to 50 million seeds every year, which are dispersed from the tree as the characteristic and easily recognisable ‘poplar wool’ (Huber, 2010). The seeds will easily germinate wherever there is sufficient moisture, and where there is no ground vegetation. As soon as a young poplar has survived the first six months, it can resist adverse environmental conditions such as flooding, drought, erosion or glacial periods. In favourable conditions, the genetic material of a single tree can spread throughout a whole region. Taking into account the propagation biology of poplar and its huge biological potential for hybridisation, the Eurasian region from China up to Central Europe is an enormous coherent ecosystem for poplar species. If genetic material is introduced into this ecological system, gene flow can hardly be controlled, especially if it is associated with a fitness factor.

Poplar habitats are central components of complex ecological systems and interact with many species, such as butterflies and beetles, including numerous protected species (Rotach, 2004; Reichholf, 2006). The complex interactions of poplar trees with the environment are also mirrored in versatile defence mechanisms above and below the ground (Biselli et al., 2022; Jiang et al., 2021; Philippe et al., 2009).

Despite being pollinated by wind, poplars are nevertheless important for bees: the buds of the black poplar, for example, are a key source of resins for propolis produced by honey bees (*Apis mellifera*) (Stanciauskaite et al., 2021). Propolis plays an important role for bees, it is both antibacterial and used as construction material in the beehive (Nader et al., 2021). Both willow and poplar trees function as pollen suppliers for honey bees in early spring, and the pollen can, therefore, also be found in the honey (Hofmann et al., 2005).

Current uses

Poplars are economically valuable and intensively grown forest trees. This is due to their advantageous breeding and agronomic properties, such as fast growth, low lignin content, easy vegetative propagation and a tendency to hybridise (Benje et al., 2021). Their wood properties make poplars an important source of raw materials for pulp, wood and fuel (Werner, 2012).

However, the long juvenile phase (7 to 10 years) of poplars is a major impediment in conventional breeding. It makes crossing and selection very time-consuming and severely limits breeding strategies.

Transgenic poplar

The first field trials with transgenic poplar trees producing insecticidal Bt-protein were conducted in China in 1994. By 2002, two poplar clones, *P. nigra* carrying the Cry1A Bt-toxin gene and a hybrid white poplar (clone 741) with a fusion of the genes for Cry1Ac and API (a proteinase inhibitor) had been approved for large scale cultivation. They are in the meantime being grown on an estimated area of 450 hm² (Wang et al., 2018). While no major environmental damage has so far been

reported, experts agree on the need to steadily monitor the release of the trees into the environment (Wang et al., 2018). However, it appears there is no available publication on a systematic investigation into uncontrolled spread. In many cases, the exact locations of releases seem to be unknown: as Wang (2004) reported, the trees (or cuttings) were also distributed via regional markets. Around 22 insect-resistant poplar varieties had been produced in China by 2018. These were approved for small-scale field testing, environmental release or pilot scale production, although no further commercial varieties were approved (Wang et al., 2018).

In the US, the company Living Carbon, has produced a transgenic poplar for future commercial planting. According to Tao et al. (2023) these trees are currently being tested in field trials. They exhibited a higher biomass under controlled growing conditions in the greenhouse, but with significant variations in overall growth and gene expression. The aim of the trials is to enhance the ability of carbon sequestration in plants to mitigate climate change. No data are available on the supply or input of water and fertilisers needed by the trees, and there is no data available on any changes in plant responses to environmental stressors and pathogens.

In the EU, several field trials with transgenic poplar were conducted or are currently ongoing.¹ The traits currently tested include drought stress tolerance, production of wood biomass by increased growth and changes in lignin composition. Two ongoing trials (in Sweden) involve poplar trees produced by NGT interventions.² Field trials with transgenic poplar were also conducted in Germany, Belgium, Poland, France and Spain.

New genetic engineering in poplars

The Western balsam poplar (*Populus trichocarpa*) was the first forest tree to have its genome sequenced (Tuskan et al., 2006), which is a prerequisite for the use of new genetic engineering. The first studies on applications of new genetic technology (NGT) in poplars were published in 2015 (Zhou et al., 2015).

NGTs can be used in poplars to investigate gene functions, introduce new traits and accelerate reproduction cycles. There are several examples of the use of NGTs in various poplar genomes (especially hybrid poplars), most of which are based on knock-outs. Recent goals, i.e. field of application, are changes in wood properties (reduction of lignin for paper production), yield (faster growth), flowering time and reaction to abiotic stress (salty and dry soils) (see Table 1).

Table1: Overview on publications on NGT applications on poplar trees (literature research)

Field of application	Author	Title
Abiotic stress tolerance (Improved drought and salt tolerance)	Zhang et al., 2023	A breeding strategy for improving drought and salt tolerance in poplar based on CRISPR/Cas9
Changed wood properties (More cellulose, reduced lignin)	Sulis et al., 2023	Multiplex CRISPR editing of wood for sustainable fibre production
Plant yield and growth (Increased formation of adventitious roots)	Ran et al., 2023	Long Non-Coding RNA lncWOX11a Suppresses Adventitious Root Formation of Poplar by Regulating the Expression of PeWOX11a
Plant yield and growth (Saccharification increase)	Guo et al., 2023	Manipulating microRNA miR408 enhances both biomass yield and saccharification efficiency in poplar
Changes in flowering	Klocko et al., 2023	Variation in floral form of CRISPR knock-outs of the poplar homologs of LEAFY and AGAMOUS after FT heat-induced early flowering
Metabolism of flavonoids	Liu et al., 2023	CRISPR/Cas9 Disruption of MYB134 and MYB115 in Transgenic Poplar Leads to Differential Reduction of Proanthocyanidin Synthesis

¹ https://webgate.ec.europa.eu/fip/GMO_Registers/GMO_Part_B_Plants.php

² See <https://www.testbiotech.org/en/field-trials-new-ge-plants> (detailed information on the field trials)

Field of application	Author	Title
		in Roots and Leaves
Exploration of multiplexing	Chen et al., 2023	Multiplex Editing of the Nucleoredoxin1 Tandem Array in Poplar: From Small Indels to Translocations and Complex Inversions
Exploration of transgene-free applications (basic research)	Hoengenaert et al., (2023)	Microparticle-mediated CRISPR DNA delivery for genome editing in poplar
Exploration of transgene-free applications (basic research)	Anders et al., (2023)	Accelerating wood domestication in forest treesthrough genome editing: Advances and prospects
Earlier flowering	Ortega et al., 2022	In vitro floral development in poplar: insights into seed trichome regulation and trimonoecy
Cellulose applications	Nayeri et al., 2022	CRISPR/Cas9-mediated P-CR domain-specific engineering of CESA4 heterodimerization capacity alters cell wall architecture and improves saccharification efficiency in poplar
CRISPR-mediated gene knock-in efficiency (HDR) (basic research)	Movahedi et al., 2022	Precise exogenous insertion and sequence replacements in poplar by simultaneous HDR overexpression and NHEJ suppression using CRISPR-Cas9
Reduced lignin	De Meester et al., 2021	Vessel- and ray-specific monolignol biosynthesis as an approach to engineer fiber-hypolignification and enhanced saccharification in poplar
Plant yield and growth	Fladung et al., 2021	Targeted CRISPR/Cas9-Based Knock-Out of the Rice Orthologs TILLER ANGLE CONTROL 1 (TAC1) in Poplar Induces Erect Leaf Habit and Shoot Growth
Reduced lignin	de Vries et al., 2021	CRISPR-Cas9 editing of CAFFEOYL SHIKIMATE ESTERASE 1 and 2 shows their importance and partial redundancy in lignification in <i>Populus tremula</i> × <i>P. alba</i>
Sterility	Azeez et al., 2021	CRISPR/Cas9-mediated single and biallelic knockout of poplar STERILE APETALA (PopSAP) leads to complete reproductive sterility
Exploration of transgene free applications (basic research)	Pilate et al., 2021	Toward transgene-free genome editing in poplar plants using Agrobacterium-mediated delivery of a CRISPR/Cas9 cytidine base editor
CRISPR-mediated gene knock-out efficiency (basic research)	Bae et al., 2021	Efficient knockout of the phytoene desaturase gene in a hybrid poplar (<i>Populus alba</i> × <i>Populus glandulosa</i>) using the CRISPR/Cas9 system with a single gRNA
CRISPR-mediated base editing efficiency (basic research)	Li et al., 2021	Highly efficient C-to-T and A-to-G base editing in a <i>Populus</i> hybrid
Reduced lignin	De Meester et al., 2020	Tailoring poplar lignin without yield penalty by combining a null and haploinsufficient CINNAMOYL-CoA REDUCTASE2 allele
CRISPR-mediated gene knock-out efficiency (basic research)	Wang et al., 2020	Efficient CRISPR/Cas9-Mediated Gene Editing in an Interspecific Hybrid Poplar with a Highly Heterozygous Genome
CRISPR-mediated gene knock-out efficiency (basic research)	Elorriaga et al., 2018	Variation in Mutation Spectra Among CRISPR/Cas9 Mutagenized Poplars
Reduced lignin	Zhou et al., 2015	Exploiting SNPs for biallelic CRISPR mutations in the outcrossing woody perennial <i>Populus</i> reveals 4-coumarate: CoA ligase specificity and redundancy
CRISPR-mediated gene knock-out efficiency (basic research)	Fan et al., 2015	Efficient CRISPR/Cas9-mediated Targeted Mutagenesis in <i>Populus</i> in the First Generation

The resulting biological characteristics (genotype and phenotype) of NGT poplars as well as the speed of development to introduce new traits in poplar populations may be very different in comparison to previous methods (see Ortega et al., 2022).

However, there are two main technical difficulties associated with NGT applications in forest trees. First, the genetic material of poplar trees is more heterogeneous in comparison to other crops. Consequently, the characteristics of the progeny of NGT poplars may be very different to the first generations. Second, reproductive cycles are much longer in poplar trees than in other plants, because it takes seven to ten years before poplars first flower. Therefore, crossing and selection take much longer than in annual plants, making it difficult to segregate non-desirable genetic elements (such as transgenes, see below) or to stabilise the intended traits.

Attempts have been made to develop transgene-free NGT poplar (e.g. Pilate et al., 2021, Hoengenaert et al., 2023, Anders et al., 2023). However, so far, this remains challenging, as the efficiency to apply more advanced NGT methods in poplars seem to require further improvements. Therefore, researchers have so far mostly used *Agrobacterium*-mediated transformation methods, in order to integrate the DNA for the synthesis of the gene scissors into the poplar genome. Insertion of the genes takes place randomly, and the molecular scissors are synthesised in the plant cells, thus bringing about the desired changes at specific genomic sites. Mendelian segregation (outcrossing) is used towards the end of the transformation process in order to obtain transgene-free poplars. This process is also typically used for arable crops. However, because of their long reproduction cycles, this segregation step is not feasible in genetically engineered poplar. That is why NGT poplar, in most cases, could only be marketed inheriting transgenes. The presence of these transgenic elements may become a hurdle for the introduction of NGT poplars, as for example Hoengenaert et al. (2023) explain: *“However, the presence of active Cas9-expression cassettes in the genome of the plant impedes their valorization, as the current regulatory framework complicates field testing and commercialization of plants harboring gene-editing reagents. In addition, the introgression of CRISPR-cassettes into wild relatives may be seen as a potential risk factor, as the dominant editing by the Cas9-nuclease and the gRNA might alter the frequency of the edited allele in natural populations. In sexually propagated plants, T-DNAs can be eliminated by Mendelian segregation, yielding transgene-free plants. However, plants that are asexually propagated and/or are highly heterozygous, e.g., poplar, grape vine, sugarcane, etc., lose their genetic constitution upon sexual reproduction.”*

Premature first-time flowering in NGT poplar

For the reasons given above, to shorten the time needed for reproduction, achieving early first flowering in young poplars is an important goal in all NGT applications in poplar. First attempts in transgenic poplar have been made to shorten the juvenile phase to less than 3 years (Mohamed et al., 2010) or 6 -10 months (Hoenicka et al., 2016). CRISPR/Cas knock-outs of genetic regulatory elements can now be used to induce first flowering even earlier: US scientists published a paper in 2022 reporting that it was possible to use NGTs to reduce the time until the first flowering to just 4 months (Ortega et al., 2022). After female plants were genetically engineered, the flowering of rooted plantlets was observed in laboratory conditions within 4 months after transformation. When cuttings were used to vegetatively propagate the NGT plants, flowering already occurred after 1 or 2 weeks. It can be assumed, although not specifically mentioned by Ortega et al. (2022), these cuttings could also grow in an open environment. In addition, the researchers found it was possible to turn female flowers into male flowers by using further NGT knock-outs, thus showing that crossing and selection may be performed using NGT plants of both sexes. The authors conclude: *“In sum, the in vitro flowering system bypasses the multiyear reproductive phase transition in poplar and fast-tracks the year-long floral development process to days and weeks. It offers a facile model for investigating floral traits and holds promise for rapid-cycle breeding and genomic selection in perennial trees.”*

If established successfully, this method could be used to segregate transgenes at an early stage of development, and thus lead to the accelerated development of transgene-free NGT poplars. It also can be used to produce offspring with more homozygous alleles, to stabilize the desired trait. In addition, the stacking of several NGT traits in one plant, or the transfer of NGT traits from one subspecies of poplar to another may also be enabled.

More generally, this example shows that essential species-specific characteristics of trees can be changed just by a few minor changes in the genome. In this regard, new genetic engineering appears to be more successful and powerful than earlier approaches in transgenic poplars (Mohamed et al., 2010). This enormous technical potential is associated with potentially far reaching environmental risks.

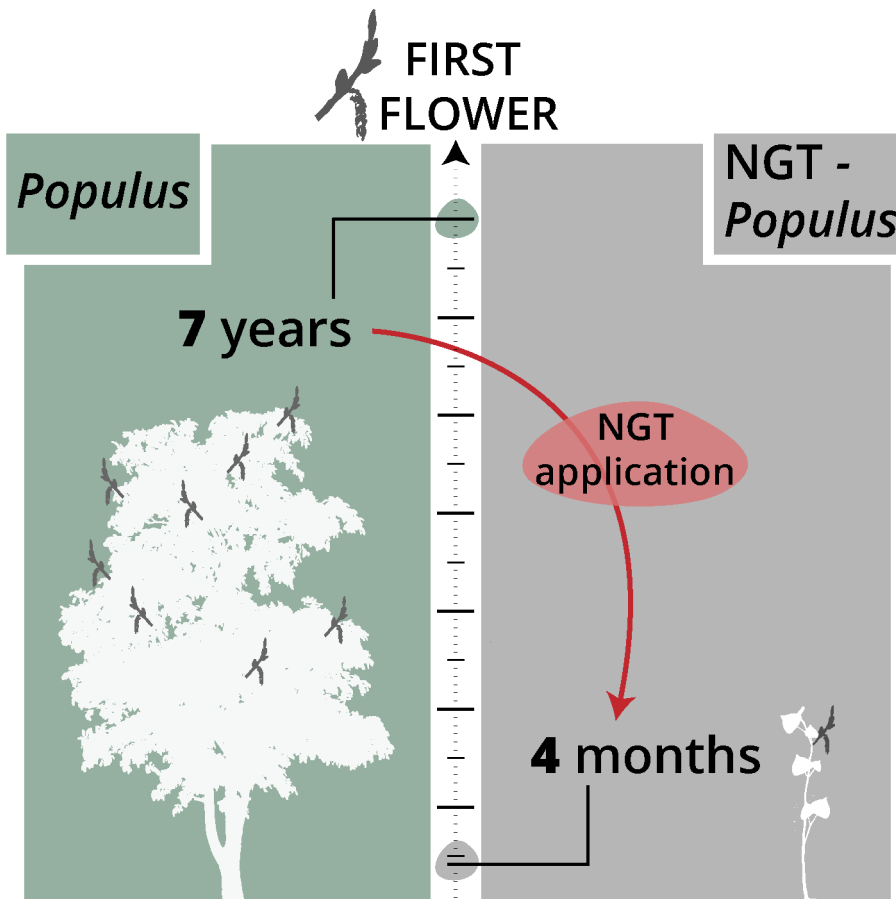


Figure 1: Ortega et al. (2022) show that species-specific characteristics of poplar trees can be changed just by a few minor genomic alterations.

Risk scenarios

NGT poplars have the potential to hybridise with wild poplar populations, possibly leading to displacement. For example, due to fitness advantages resulting from NGT traits (such as early flowering, higher growth, resistance to stressors), NGT poplars could spread invasively. The extremely short juvenile phase and premature flowering in NGT poplars is particularly relevant in this context. Shortening the development time of genetically modified trees means that numerous NGT poplars with different traits could be released into the environment in shorter periods of time, thus greatly increasing the probability of unwanted interactions (see Koller et al., 2023). It also has to be considered that, after crossing with wild populations, hybrids or other genetically engineered

poplars, the offspring may have characteristics and risks that would not have originally been expected (Bauer-Panskus et al., 2020). In general, the use of NGTs may change the characteristics of species much faster than the ecosystems are able to adapt.

The feralisation of NGT poplars or the outcrossing of NGT traits into wild poplar populations would be especially hazardous for ecology. Pollen-mediated gene flow, in particular, can spread NGT traits into wild populations and displace their alleles from the natural gene pool. This will reduce the genetic diversity of a population and could jeopardize its general adaptability and its long-term fitness, with unpredictable consequences for future population dynamics of, e.g. the Eurasian black poplar.

In the case of NGT poplar with premature flowering, the trait may go along with fitness costs. Therefore, it is not clear if these NGT plants could become invasive. However, there is also a risk for 'outbreeding depression' if maladapted genotypes introgress into locally adapted populations (Montalvo & Ellstrand, 2001). Effects like these may turn out to be context dependent and contingent on the genetics of the specific wild populations and the local biotic and abiotic environmental conditions (Mercer et al., 2006). Crosses with low-performing NGT plants may reduce the fitness of hybrids compared to the local plants thus decreasing the overall fitness of the natural plant populations (Crémieux et al., 2010). Thus, also NGT plants with reduced fitness may become a threat for the population of e.g. the Eurasian black poplar.

Conventional poplar plantations with hybrid poplars or non-native poplars are already seen as a serious threat to native poplar populations (Vanden Broeck et al., 2021). The European black poplar (*P. nigra*) is gradually being displaced by the Euro-American (*P. × canadensis*) and Inter-American (*P. × generosa*) hybrid poplars, but also by the *P. nigra* cultivars, such as the male Lombardy poplar (*Populus nigra* cv. '*Italica*' *Duroi*) (Arens et al., 2002; Cagelli & Lefevre, 1995; Heinze, 1998; Janssen, 1997; Vanden Broeck et al., 2021). In fact, native poplars have for some time been seen as probably the most threatened forest tree species in the old natural floodplain forests in the temperate zone (Lefèvre et al., 2001).

Research shows the relevance of both short and long-distance dispersal of seeds and pollen. The pollen and seeds of poplar are dispersed by the wind; a high proportion of which are deposited in the surrounding countryside within a distance of 1000 meters (Rathmacher et al., 2010). In their investigations along the river Elbe (Germany) Rathmacher et al. (2020) reported that 4 percent of poplar pollen and 1 percent of the poplar seeds travelled over distances of more than 2 kilometres. Considering the enormous number of seeds – 25 to 50 million – these percentages are highly biologically relevant. Fertilisation was found to occur at distances of over 8 kilometres from the trees releasing the pollen; seed was found to travel over distances of more than 6 kilometres. A case study of DiFazio et al., (2004) on the invasiveness of transgenic poplars concluded that long-distance transfer of seed and pollen is even more important; although rare, it has a high statistical chance of occurring. These single events may be decisive for true invasiveness in the long-term (see also Testbiotech, 2010). In addition, poplars also spread vegetatively over long distances, e.g. through the uncontrolled spread of cuttings by humans or via broken top shoots in streams or rivers (Arens et al., 2002; Ziegenhage et al., 2008).

Using NGTs to distort inflorescence and pollen (Ortega et al., 2022) in order to reduce the likelihood of transmission or fertilisation cannot completely eliminate outcrossing. This is problematic because the stability of these traits cannot be predicted over the entire lifetime of a poplar.

Releases of NGT poplars or NGT traits that outcross into other poplar populations can also have fatal consequences for organisms that interact with them. For example, unintended changes in bud formation, e.g. by knocking out genes that regulate flower initiation, may have consequences for propolis production, and thus for the bee population. In general, abrupt changes in the composition of poplars, which also affect their interactions with other organisms, can destabilise ecosystems and accelerate species extinction. Many species, especially insects interacting with poplars, including black poplar, are already under considerable environmental stress due to environmental toxins, habitat fragmentation, loss of habitats or biodiversity and climate change (Rotach 2004; Reichholf, 2006).

To some extent, safety for human health might become an issue for risk assessment: poplar pollen is known to produce several allergens that may, for example, trigger hay fever (Guo et al., 2023). Therefore, we must also consider the extent to which NGT poplars in the environment may in future unintentionally expand the allergen spectrum.

Consequences for the regulation of NGT plants

The paper published by Ortega et al. (2022) reports that new traits which exceed the spectrum of known species traits can be achieved with NGTs without adding genes and by simply altering a few short gene segments. In addition, the time genetically modified trees need to develop can be accelerated, thus allowing many trees with different characteristics to be introduced into a shared receiving environment within short periods of time. If Ortega et al (2022) successfully establish their approach, then NGT poplars could be propagated, crossed and selected very quickly to produce new gene combinations – similar to arable crops. At the same time, releasing these trees into the environment could increase their potential to spread in the environment if fertile pollen and seeds are produced much earlier than would normally be expected.

For future regulation of NGT plants, the EU Commission suggests introducing criteria which only refer to the number³ and type of mutations⁴. The Commission believes that these criteria are sufficient to verify the ‘equivalence’ of NGT plants and plants obtained from conventional breeding (including random mutagenesis), therefore allowing them to be exempt from mandatory risk assessment.⁵ Further-reaching specifications prepared by the EU Parliament suggest that only those NGT plants should be submitted to mandatory risk assessment which may produce new, altered or chimeric proteins.⁶

However, several governmental agencies have come to very different conclusions, e.g. the French Agency for Food, Environmental and Occupational Health & Safety (ANSES, 2023), the German Federal Agency for Nature Conservation (BfN) (Bohle et al., 2023) and experts from the Environment Agency Austria (UBA)(Eckerstorfer & Heissenberger, 2023) as well as scientific organisations, such as the Ecological Society of Germany, Austria and Switzerland (GFOE, 2023) and experts working with civil society organisations⁷.

Indeed, there are several examples of NGT plants and traits that are unlikely to be obtained from conventional breeding. These examples include NGT tomatoes (Li et al., 2022, Nonaka et al., 2017; USDA, 2020; Zsögön et al., 2018), wheat (Sanchez-Leon et al., 2018, Raffan et al., 2021), rice (Zhang et al., 2019), camelina (Kawall 2021) or plants with potential insect toxicity (Bohle et al.,

³ Number of changes at different sites in the genome and number of changes nucleotides on each site

⁴ Such as deletions and inversions

⁵ <https://data.consilium.europa.eu/doc/document/ST-14204-2023-INIT/EN/pdf>

⁶ https://www.europarl.europa.eu/doceo/document/AGRI-AD-757371_EN.pdf

⁷ <https://www.testbiotech.org/content/joint-statement-scientists-future-eu-regulation-ngt-plants-perspective-protection-goals>

2023). Amongst the NGT plants studied by EFSA (2020b and 2022b), NGT wheat and NGT tomatoes were assumed to require in-depth risk assessment.

Ortega et al. (2023) are adding a further example to this list of NGT plants with biological characteristics that go beyond those known for these species. These plants, i.e. poplars, are highly relevant to complex ecosystems. It would be irresponsible to release them into the environment without detailed risk assessment and without adequate methods to prevent their uncontrolled spread. This is particularly relevant for poplar species that are perennial and can spread within the ecosystems not being limited to arable land.

Several steps in risk assessment must be required for all NGT plants in order to assess their safety. These requirements should include a specific molecular risk assessment (Koller & Cieslak 2023) comprising genome sequencing, gene expression studies and so-called 'omics'. 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.

In addition, the release of NGT plants with different traits into a shared environment would necessitate the establishment of clear criteria and methodologies to assess potential interactions and cumulative effects. It should be borne in mind that NGT plants have not adapted through evolutionary processes and may not be readily ecosystem-compatible (Koller et al., 2023). Ultimately, it is the speed and scale of introduced NGT organisms that could overwhelm the resilience and balance of our natural ecosystems.

Finally, plants that persist and / or spread and propagate in the environment without spatio-temporal control pose particular challenges for risk assessors and risk managers, which can only be resolved by 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. The uncertainties can only be reduced by controlling and restricting the number, the scale and the duration of genetically engineered organisms (GFOE, 2023).⁸

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⁸ See also: https://gfoe.org/sites/default/files/ngt_gfoe_final.pdf

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