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Review

Environmental Risk Assessment Scenarios of Specific NGT Applications in *Brassicaceae* Oilseed Plants

Franziska Koller *, Michael Cieslak and Andreas Bauer-Panskus

Department of Genetic Engineering and Environment, Testbiotech eV, Frohschammerstraße 14, 80807 Munich, Germany
* Correspondence: franziska.koller@testbiotech.de

Abstract: Oilseed plants of the Brassicaceae plant family are cultivated for food, feed and industrial purposes on large-scale in Europe. This review gives an overview of current market-oriented applications of n*ew genomic techniques (NGTs)* in relevant *Brassicaceae* oilseed crops based on a literature survey. In this respect, changes in oil quality, *yield, growth and resistance to biotic and abiotic stress are* goals in oilseed rape *(B. napus)* and camelina *(C. sativa).* Environmental risk assessment scenarios are developed for specific NGT applications in *Brassicaceae* oilseed crops with either a changed oil composition or with fitness related traits. In case of a changed oil composition an increase or decrease of *polyunsaturated fatty acids (PUFA)* demonstrates risks for health and survival of pollinators. In case of fitness related traits other risks were identified, i. e. an increased risk of spread and persistence of NGT plants. Furthermore, there are indications for potential disturbance of interactions with the environment, involving signalling pathways and reaction to stress conditions. It is shown that for a comprehensive risk assessment the technological potential of NGTs, the plants' biology and the scale of releases have to be considered in combination. Therefore, the release of NGT plants into the environment for agricultural purposes will require risk assessment and monitoring of single traits as well as of combinatorial and long-term cumulative effects. In addition, risk management should develop concepts and measures to control and potentially restrict the scale of releases. This is especially relevant for NGT *Brassicaceae* in Europe which is a centre of diversity of this plant family.

Keywords: new genomic techniques (NGT); genetically engineered organisms; genome editing; risk assessment; unintended effects in NGT plants

1. Introduction

Brassicaceae is a diverse plant family and includes many domesticated plants as well as widespread wild plants, such as thale cress (*Arabidopsis thaliana), capsella (Capsella bursa-pastoris), wild radish (Raphanus raphanistrum) and pennycress (Thlaspi arvense). The cultivated species of this plant family are global sources of i. a. vegetables (cabbage, radish), seeds harvested for example to produce mustard and vegetable oil for food and industrial purposes. The latter is mainly obtained from the oilseed crops, i. e. oilseed rape (Brassica napus) and camelina/false flax (Camelina sativa).There is a strong possibility of cross-hybridisation between different species within the Brassicaceae plant family* (FitzJohn et al., 2007).

Breeders have been working for many years to improve both the quality of food and feed as well as raw material used in industry. *They have, for example, attempted to alter traits such as the oil content and the fatty acid composition of the oilseed crops. The oil obtained from Brassicaceae oilseed crops consists mainly of saturated fatty acids (palmitic, stearic and eicosanoic acids), monounsaturated fatty acids or MUFA (oleic, erucic and gondoic acids) and polyunsaturated fatty acids or PUFA (linoleic and linolenic acids)* (Sharafi et al., 2015; Rodríguez-Rodríguez et al., 2021)*. The latter are also known as omega-6 and omega-3 fatty acids, respectively. Both PUFAs are known to have numerous health benefits* (Abedi and Sahari, 2014)*, but are also associated with oxidative instability* (Kamal-Eldin, 2006)*. Depending on the intended purpose, some projects are aiming to either increase (in food and feed, see, e.g.* (Xie et al., 2020)*) or decrease (for food or industrial*

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purposes, see, e.g. (Knothe, 2008; Okuzaki et al., 2018; Esfahanian et al., 2021)*) the PUFA content in Brassicaceae crops. In addition, attempts are being made to further reduce the erucic acid content, as there is evidence that it is toxic* (Galanty et al., 2023). *Besides altering oil content and fatty acid composition, breeders are also focussing on improving yield, growth and resistance to biotic and abiotic stress, including resistance to plant pathogens and herbicides (see Section 3 and references in Tables 1 and 2 and A1–A3). Changes in these traits often imply positive fitness effects (See Section 3).*

Table 1. NGT *Brassicaceae* field trials in Europe (see (Testbiotech, 2023)).

Year of Publication	Notification Number	Company/Institute	Title	Plant	Country	Period of Release
2023	23/Q02	Rothamsted Research	Field assessment of gene edited Oilseed Rape with pod shatter resistance	*Brassica napus*	UK	2023-2028
2023	23/R08/01	Rothamsted Research	Synthesis and accumulation of seed storage compounds in *Camelina sativa*	*Camelina sativa*	UK	2023-2026
2023	B/SE/23/4198	Umeå University	Arabidopsis - photosynthesis and hormone biology	*Arabidopsis thaliana*	Sweden	2023-2027
2019	B/GB/19/R08/01	Rothamsted Research	Synthesis and accumulation of seed storage compounds in *Camelina sativa*	*Camelina sativa*	United Kingdom	2019-2023
2019	B/GB/19/52/01	John Innes Centre	Genetic regulation of Sulphur metabolism in *Brassica oleracea*	*Brassica oleracea (broccoli)*	United Kingdom	2019-2021

Table 2. NGT *Brassicaceae* deregulated in the US (USDA APHIS, 2022, 2024).

Year of Application	Plant	Applicant	Method	Trait*
2022	*Brassica juncea*	Pairwise Plant Services, Inc	CRISPR/Cas9	Reduced pungency and reduced trichome production
2022	Pennycress	Hjelle Advisors (CoverCress)	CRISPR/Cas9	Lowered erucic acid and lowered fiber in seeds
2020	Canola	Corteva Agriscience	CRISPR/Cas9	Altered Meal Qualities
2020	Pennycress	CoverCress	CRISPR/Cas9 (?)	CBI
2020	Pennycress	Illinois State University	CRISPR/Cas9 (?)	Development of pennycress as an oilseed-producing cover crop
2020	Canola	Yield10 Bioscience	CRISPR/Cas9	Altered Oil Content
2020	Pennycress	CoverCress Inc.	CRISPR/Cas9	CBI
2020	Camelina	Yield10 Bioscience	CRISPR/Cas9	CBI

2020	Pennycress	CoverCress Inc	CRISPR/Cas9	CBI
2019	Pennycress	Illinois State University	CRISPR/Cas9	CBI
2018	Camelina	Yield10 Bioscience	CRISPR/Cas9	CBI
2018	Pennycress	Illionois State University	CRISPR/Cas9	Altered Oil Content
2018	Camelina	Yield 10	CRISPR/Cas9	Altered Oil Content
2017	Camelina	Yield 10	CRISPR/Cas9	Altered Oil Content

*CBI: Confidential Business Information.

The genomes of some *Brassicaceae* family members are particularly complex, which means that conventional breeding methods (including non-targeted mutagenesis) are somewhat restricted leading to difficulties in breeding (Li et al., 2022). Several *Brassicaceae* crops are polyploid (up to allohexaploid), i.e. they have more than two sets of paired chromosomes and, thus more than two copies of each gene. In addition, some have undergone genome triplication, which has resulted in a high number of duplicated genes (Wang et al., 2011). *In order to achieve a desired breeding characteristic, it is frequently necessary to knock out all homologous genes* (Wells et al., 2014)*, and from case to case it is doubtful whether is at all possible.*

New genomic techniques (NGTs), such as CRISPR/Cas9, have been successfully applied several times in Brassicaceae (Li et al., 2022; Tian et al., 2022; Ahmad et al., 2023)*. The technology has made it possible to alter multiple copies of a gene, or even alter several different genes simultaneously (multiplexing). Unlike conventional breeding, NGTs can be used to introduce genetic changes in genomic regions that are difficult to access and are highly protected* (Kawall, 2019)*. This technical potential allows genotypes and traits to be modified within short periods of time.*

While some of the Brassicaceae genotypes developed by NGTs were also achieved using conventional breeding (e. g. EMS (ethyl methane sulphonate) breeding), others are completely novel (see, e. g. (Bellec et al., 2022; He et al., 2023)*). In future, it is expected that different NGT-derived genotypes can be combined and stacked, thus leading to even more extensive overall genomic changes in NGT plants* (Raitskin and Patron, 2016; Zetsche et al., 2017; Kawall et al., 2020).

In addition to scientific publications dealing with basic and/or applied research on NGT *Brassicaceae*, which will be evaluated as part of this study, various projects are already at a more or less advanced stage of commercialisation and have been tested in field trials. For example, in 2023, the US company Pairwise brought CRISPR mustard plants (*B. juncea*) to the market, whose leaves are supposed to taste less pungent (Karlson et al., 2022), which is the only commercialised *Brassicaceae* NGT plant worldwide.

The following sections provide an overview of current NGT applications in *Brassicaceae* oilseed crops, i. e. oilseed rape (*B. napus*), camelina *(C. sativa)* and pennycress (*T. arvense*). For the purpose of this review, market-orientated NGT applications in *Brassicaceae* oil plants were identified in the scientific literature and all NGT plants without permanent insertion of transgenes were listed. Basic research projects and applications using oligonucleotide directed mutagenesis (ODM) methods were not considered.

2. Biological Basics

2.1. The Biology of Brassica Napus

Oilseed rape (*B. napus*) is allotetraploid (2n=38, AACC) (An et al., 2019) and the result of a natural cross between the diploid *Brassica* species *B. oleracea (2n=18, CC)* and *B. rapa (2n=20, AA)*. The evolutionary relationship between different *Brassica* species sharing three core genomes (A, B and C) is described in the 'Triangle of U' (Nagaharu, 1935). Europe is the centre of origin and genetic diversity for oilseed rape and several other *Brassica* species. Due to its origin, *B. napus* is well adapted to European climatic conditions. However, oilseed rape is grown globally and economically one of the most important oilseed crops (Hu et al., 2021). As 'double-low' oilseed rape (low erucic acid and low glucosinolate content in seeds), also known as canola, it provides a healthy and nutritionally balanced cooking oil; it is also an important source of biodiesel, industrial oil and protein for animal feed (Hu et al., 2021).

Weedy characteristics

All cultivated *Brassica* species have certain weedy characteristics, e.g. increased dispersal abilities, long seed dormancy, the ability to invade disturbed areas and natural plant communities. Additionally, several relatives of oilseed rape, e.g. *Sinapis arvensis, Raphanus raphanistrum* and *Hirschfeldia incana,* are regarded as weeds. Weedy forms of *B. napus*, *B. rapa* and *B. oleracea* also exist, e. g. oilseed rape has some wild (or weedy) plant characteristics, such as seed shedding and long secondary dormancy of the seeds in the soil. Although weedy oilseed rape is predominantly found on arable land, ruderal (feral) populations can persist over longer periods of time (Pascher et al., 2010, 2017; Banks, 2014): they have been found, for example, in various European countries (see, amongst others, (SCRI, 2004; Squire et al., 2011; Frieß et al., 2020)).

Interspecific hybridization

Oilseed rape has pronounced hybridization potential and can form hybrids with various congeneric (i.e. *Brassica)* species, such as *B. rapa* (turnip rape, field mustard, birdseed rape, etc.), *B. juncea* (various mustard varieties) and others, but also with wild and weedy *Brassicaceae* from different genera (Chèvre et al., 2004; Pascher et al., 2006). So far, spontaneous hybridization was confirmed in a total of eight native or cultivated species in Europe (Theenhaus et al., 2002; Breckling et al., 2003; FitzJohn et al., 2007; Devos et al., 2009; OECD, 2012; COGEM, 2019; Marotti et al., 2020).

Pollen flow

Oilseed rape is self-compatible and predominantly self-pollinated by wind and insects. The outcrossing rate is controversially discussed – estimates in literature range from between 12 and 55 percent (Becker et al., 1992; Darmency, 1997). It can thus be inferred that up to half of the pollen load may be dispersed by wind and, above all, by insects. Cross-pollination over very long distances has been demonstrated in oilseed rape (several kilometres) (Beckie et al., 2003; Ramsay et al., 2003). It is also a sought-after source of food, especially for bees and outcrossing can be expected in the collection area of both social and solitary bees (Pasquet et al., 2008).

Secondary dormancy

A considerable number of seeds are lost when oilseed rape is harvested, and end up back on the fields as volunteer rapeseed. According to Gulden et al. (Gulden et al., 2003), this loss can be up to around 6 % of the harvest; according to the EU research project SIGMEA, this can amount to a total of 6000 seeds per square meter (Gruber et al., 2007). Around 80 % of volunteers germinate during the first twelve months after harvest, but a considerable proportion of the seeds remain viable for up to 15 years in the soil (Lutman et al., 2003, 2005; D'Hertefeldt et al., 2008; Belter, 2016).

2.2. The Biology of Camelina Sativa

Camelina *(C. sativa)* is an annual *Brassicaceae* plant that is mainly cultivated in Europe and North America. *C. sativa* is allohexaploid (2n = 6x = 40, AABBCC). It is an ancient crop that has been used since prehistoric times (Zanetti et al., 2021). A recent study found that the plant was probably first cultivated in the Caucasus region (Brock et al., 2022). In contrast to other plants of the *Brassicaceae* family, camelina has not been widely used in plant breeding in the past, so only a small number of varieties are available for agricultural purposes (Vollmann and Eynck, 2015).

The oil from camelina seeds is used as food and animal feed, but also for biofuels and industrial compounds (Shonnard et al., 2010; Iskandarov et al., 2014; Vollmann and Eynck, 2015). It contains large amounts of polyunsaturated fatty acids (PUFAs) such as essential linoleic and linolenic acids (Abramovic and Abram, 2005; Iskandarov et al., 2014).

Pollen flow

Camelina is primarily self-pollinating, but there is also evidence of cross-pollination by bees, bumblebees and other insects (see references in (CFIA, 2017)). Outcrossing in camelina decreases rapidly with distance. For example Walsh et al. (Walsh et al., 2015) demonstrate that the outcrossing rate in camelina populations was only 0.09 % at a distance of 20 meters. However, this does not rule out the possibility of rare but ecologically significant long-distance pollination events due to insects that are able to fly considerable distances (Pasquet et al., 2008).

Dormancy

Seed shattering and the small size of camelina seeds (seed rain from the combine harvester) contribute to harvest losses. Thus, due to primary or secondary dormancy the likelihood of volunteer plants and persistence may increase. There are differing statements in literature with regard to secondary dormancy, i.e. dormancy that is induced, for example, by non-germinated seeds buried in the soil. While Ehrensing & Guy (Ehrensing and Guy, 2008) found no dormancy in camelina seeds at all, Walsh et al. (Walsh et al., 2013) showed that volunteer populations disappear only after two years, even when herbicides are used, thus suggesting survival in the seed bank. Similarly another experiment conducted in south-eastern France found that 85.8 % of wild camelina seeds at a depth of 10 cm had survived after two and a half years (Saatkamp et al., 2009). Thus, while dormancy has been documented, the longevity of camelina seeds in the seed bank still needs to be investigated.

Hybridization potential

Camelina can hybridize with wild relatives such as *C. microcarpa* and *C. alyssum* (Séguin-Swartz et al., 2013; Julié-Galau et al., 2014; Zhang and Auer, 2020). According to results of a large number of studies, interspecific hybridization with other *Brassicaceae* such as oilseed rape, field mustard, *Arabidopsis* and others, seems unlikely (see overview in (CFIA, 2017)).

2.3. The Biology of Thlaspi Arvense

Pennycress (*T. arvense*) is another member of the *Brassicaceae* family currently being engineered with NGTs. It is closely related to the thale cress *Arabidopsis thaliana* and native to Eurasia, but can now be found worldwide as crop and weed (Ma et al., 2023). Uses include feed and the production of agrofuels (Keadle et al., 2023). Due to its high vitamin C content, it is also added to salads or cooked like spinach (Mitich, 1996). Pennycress is diploid, the plant's genome consists of seven chromosomes ($2n=14$) (McGinn et al., 2019). It has a high seed oil content and a favourable fatty acid composition that makes it suitable for use as oil crop for industrial purposes (Warwick et al., 2002). In agriculture, pennycress is mainly used as winter cover crop since it can tolerate very low temperatures (McGinn et al., 2019; López et al., 2021). As a cover crop, it provides important ecosystem services because it reduces soil erosion and the leaching of nutrients.

Weediness

Pennycress has only recently been domesticated and current cultivars still harbour many weed traits (McGinn et al., 2019). In North America, it is listed in several regions as a noxious weed which considerably reduces crop yields (Mitich, 1996).

Pollen flow

Pennycress is mainly wind-, but also self- or insect pollinated. In a study conducted in Germany, flowers were visited by different bee species, but not by honey bees (Groeneveld and Klein, 2014).

Seed dormancy

Due to its nearly non-domesticated history, *T. arvense* still inherits weedy characteristics, for example very long secondary seed dormancy. The seed bank can last 20 years or more (CABI, 2014).

Interspecific hybrids

Interspecific hybridization is not reported in *T. arvense* (Warwick et al., 2002).

Pollinator interaction

Pennycress has a high feed value for pollinating insects, and is especially valuable as early-season food source (Eberle et al., 2015).

3. Overview of NGT Applications in *Brassicaceae* Oilseed Plants

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3.1. *Brassica napus (Oilseed Rape)*

So far, around 45 market-orientated NGT applications in *B. napus* are described in scientific literature (see Table A1). The agronomic importance as an oilseed crop has resulted in its genome being edited several times with NGTs in order to adapt the fatty acid composition and total oil content to individual intended uses (14 applications, shown in orange in Table A1 and Figure 1). A*ttempts to reduce PUFAs and erucic acid by* knocking out FAD (fatty acid dehydrogenase) and FAE (fatty acid elongase) genes are particularly frequent. Several NGT applications are related to the survival and fitness of the plants, e. g. increased drought tolerance, superior growth, longer roots and a higher number of siliques and seeds (green in Table A1 and Figure 1).

3.2. *Camelina sativa (Camelina)*

Camelina has already been genetically engineered several times using NGTs (10 NGT applications, see Table A2). Similar to oilseed rape, attempts are being made to alter the fatty acid content and composition (8 applications, orange in Table A2 and Figure 1) and NGT applications to reduce PUFAs and erucic acid (FAD and FAE genes) are particularly frequent as well. In addition, different knockouts (5 genes, multiple copies) were established in a multiplexing approach resulting in early-flowering plants (green in Table A2 and Figure 1).

3.3. *Thlaspi arvense (Pennycress)*

In pennycress, NGTs were used to knock out FAE1, thus making it possible to abolish erucic acid production (see Table A3 and Figure 1). This knockout strain was further combined with NGT-derived FAD2 and ROD1 knockouts to increase oleic acid content and to decrease PUFA content.

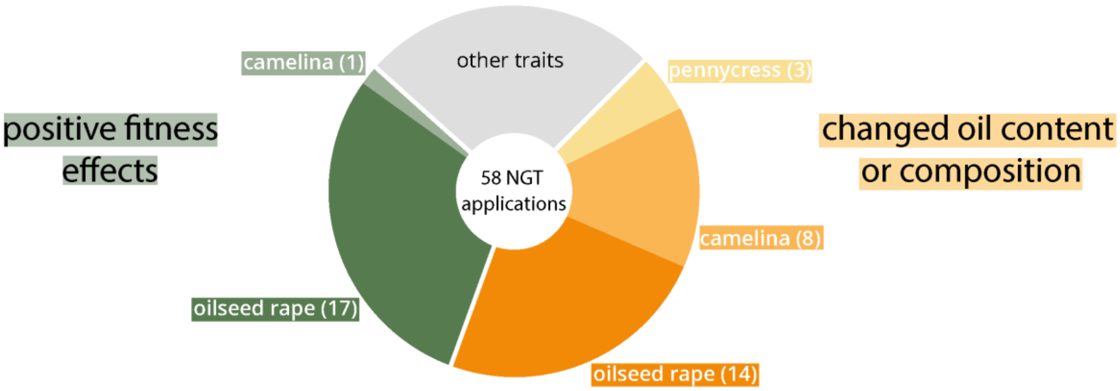

Figure 1. Categorized NGT applications in *Brassicaceae* oilseed plants *B. napus*, *C. sativa* and *T. arvense*. A total of 58 NGT applications were found in the three species in scientific literature. A*ttempts to change the oil content or composition are marked in orange. NGT applications that showed positive fitness effects (higher likelihood of persistence and spread) in the plant are marked in green. Other traits are marked in grey.*

3.4. *Field Trials and Commercialisation Pipeline*

In the USA and also in Europe, various projects are underway to secure the market release of NGT-*Brassicaceae*. In Europe, there is currently no commercially viable NGT-*Brassicaceae* product. However, applications for field trials were filed in both the EU and the UK (Napier, 2021). All trials were carried out with plants modified with the CRISPR/Cas9 gene scissors (Table 1). After its withdrawal from the EU, the UK on a new legal basis and under simplified conditions under which NGT plants can be released into the fields. Current field trials in the EU and the UK include oilseed rape as well as camelina and broccoli (*B. oleracea*).

In the US, companies or research institutions can submit a deregulation request to the US Department of Agriculture (USDA). The Agency determines whether a transgenic or NGT plant that is to be placed on the market needs to legally regulated or can be marketed without restrictions. In the meantime, a number of NGT *Brassicaceae* have been deregulated in this way (see Table 2). However, deregulation does not automatically mean that a NGT plant will actually be commercialised. So far, only brown mustard (*B. juncea*) developed by the company Pairwise has been commercialised. In addition to oilseed rape, camelina and pennycress have been deregulated, most of which have had their oil content modified. In nearly half of the applications for deregulation, all the relevant information was declared to be confidential business information (CBI), so that no statements can be made here about the possible objectives of a project (Table 2).

4. Findings and Scenarios Relevant for Risk Assessment

4.1. Risks for Pollinators

Brassicaceae oilseed crops are highly attractive for pollinators: They offer easily accessible and highly abundant floral resources and are an important food resource (Eberle et al., 2015; Van Reeth et al., 2019). *Brassicaceae* pollen has lower protein:lipid values (Vaudo et al., 2020) and is particularly rich in PUFA linolenic acid (Manning, 2001).

It has been shown that fatty acids play key roles in the development, communication, reproduction and health of pollinators, including in their colonies (see, e. g. (Arien et al., 2015, 2020; Muth et al., 2018)). Studies indicate that the specific composition of fatty acids in pollen is important for host-plant choice and bee-flower interactions (Vaudo et al., 2020). Further, pollinators seem to regulate their dietary intake by relying on lipids as nutritional cue. This is likely to be caused by the impact of fatty acids have on their overall fitness (Ruedenauer et al., 2020).

Changes in the dietary composition of pollinators should be carefully examined for risks to the insects, as suboptimal nutrient balance is thought to be one of the threats to their populations (Vanbergen and Initiative, 2013).

4.1.1. Decreased Amounts of PUFA Can Negatively Affect the Health of Pollinators

Several NGT applications in *Brassicaceae* oilseed crops focus on fatty acid composition in efforts to obtain plants with a low PUFA content. Like other fatty acids, PUFAs have different important functions in many biological processes, both in plants and animals. Animals cannot synthesize PUFAs themselves so they need to be part of their diet (Hulbert et al., 2012; Arien et al., 2020).

The PUFA content of seeds is mainly reduced by knocking out FAD genes (Jiang et al., 2017; Morineau et al., 2017; Okuzaki et al., 2018; Huang et al., 2020; Jarvis et al., 2021; Lee et al., 2021; Liu et al., 2022; Shi et al., 2022). Another study successfully decreased the PUFA content by knocking out CIPK9 genes (Wang et al., 2023). In *B. napus* it was shown that FAD genes are highly expressed in pollen, thus explaining the high levels of PUFAs (Piffanelli et al., 1997). Therefore, it can be assumed that at least the knockout of FAD genes will significantly decrease the PUFA content, including pollen.

In habitats with extensive agricultural monocultures of plants with reduced PUFA content, bees may suffer from PUFA deficiency due to a severely limited variety of pollen (Arien et al., 2015, 2020). It was shown that honey bees with a linolenic acid dietary deficiency had smaller hypopharyngeal glands; they also showed poor performance in both olfactory and tactile associative learning assays (Arien et al., 2015). Results from experimental feeding studies showed that total lipid concentration and the linoleic:linolenic acid ratio are further factors affecting brood development and adult longevity in honey bees. A balanced PUFA diet is necessary for maintaining proper colony development (Arien et al., 2020).

In addition, PUFAs seem to have some antimicrobial properties capable of inhibiting growth of honey bee pathogens, e. g. *Paenibacillus* larvae and fungal diseases (Ababouch et al., 1992; Feldlaufer et al., 1993; Manning, 2001). PUFAs also have an important role as pheromones, e.g. in recognizing and discriminating between nestmates and non-nestmates (Breed, 1998), or in attracting workers to form the retinue group (Keeling et al., 2003).

Taken together, a sufficient supply of PUFAs appears to be very important for the health, colony development and communication of honey bees. Malnutrition resulting from dietary deficiency of certain fatty acids may therefore cause adverse effects in honey bees and other wild pollinators, and should be analysed in risk assessment before NGT crops with an altered fatty acid content are brought to market.

4.1.2. Increasing Amounts of Oil and Unsaturated Fatty Acids Can Negatively Affect the Health of Pollinators

In addition to approaches aiming to lower the PUFA content in oilseed crops, there are several studies that have used NGTs to successfully increase the total amount of oil and, in particular, unsaturated fatty acids in seeds.

For example, oleic acid contents were elevated by knocking out FAD genes (Jiang et al., 2017; Morineau et al., 2017; Okuzaki et al., 2018; Huang et al., 2020; Jarvis et al., 2021; Lee et al., 2021; Liu et al., 2022; Shi et al., 2022).

PUFA contents were elevated by knocking out different TT genes (Xie et al., 2020; Zhai et al., 2020; Li et al., 2023), SAD gene (Huang et al., 2023), KANT3 gene (He et al., 2023), GIF1 gene (He et al., 2023), *AGP11* gene (He et al., 2023) or EDA32 gene (He et al., 2023). These knockouts probably also have a considerable influence on the fatty acid composition in *Brassicaceae* pollen, and thus on the diet of pollinators.

Studies have shown that the reproduction and survival rate of bumble bees was significantly reduced when they ingested too many fatty acids (Vaudo et al., 2016; Ruedenauer et al., 2020). This may explain why bumble bees strongly avoided consuming pollen enriched with fatty acids in feeding experiments (Ruedenauer et al., 2020). In honey bees, feeding studies found significant increases in the mortality rate above a certain amount of oleic or linoleic acid (Manning et al., 2007).

The negative effects of fatty acids on survival and reproduction could be the result of intoxication with excessive amounts of fatty acid (Ruedenauer et al., 2020). Intake of high amounts of PUFAs, for example, might lead to lipid peroxidation and cell damage (Vaudo et al., 2016). On the other hand, a lack of other nutrients as a consequence of reduced overall pollen consumption due to fat avoidance, might further increase mortality (Ruedenauer et al., 2020). Hence, in habitats with extensive agricultural monocultures of plants with highly increased oil and PUFA content in pollen, pollinators, such as honey bees and bumble bees, may either over-consume fatty acids or suffer nutrient deficiencies due to fat avoidance.

As mentioned above, changes in oil composition of plants can also be achieved with conventional breeding (including random mutagenesis (see also (Havlickova et al., 2023)). However, NGTs offer new potential for the targeted editing of *Brassicaceae* genomes (He et al., 2023). In regard to individual NGT traits, it may be difficult without in-depth case-by case investigation to conclude on whether the specific genotype and phenotype could also be obtained from conventional breeding.

Furthermore, the overall number of NGT applications in *Brassicaceae* oilseed plants and the technological potential of NGTs (Kawall 2019) shows a new dimension in the scale (in terms of space and time) of potential releases of plants (Koller et al., 2023) with novel genotypes and phenotypes as for example shown by He et al. (He et al., 2023). This will complicate any prediction about future impact of NGT-plants, especially if compared with conventionally bred plants: Both spontaneous and intended crossings can lead to new combinations of traits causing unintended interactions without precedent (see Section 4.2.).

4.1.3. Further Observations Regarding Changes in Oil Composition

Changed oil content and composition may result in further unintended and undesirable effects. Fatty acids play important roles in plants, e. g. in the biosynthesis of secondary metabolites found in cellular membranes and phytohormones.

For example, the phytohormone jasmonate and its derivates have important roles as signalling molecules in plant defence, particularly against insect herbivores (Glazebrook, 2005; Howe and Jander, 2008). Linolenic acid is a precursor molecule of jasmonic acid (Gfeller et al., 2010). Changes in oil composition can therefore affect plant communication, signalling pathways and plant resistance to biotic or abiotic stressors (Kawall, 2021). In addition, there are further NGT applications with altered plant composition which could be relevant to interactions with pollinators, e. g. traits with a significant impact on hormone signalling (Sun et al., 2018; Wang et al., 2023) or flavonoid content (Xie et al., 2020; Zhai et al., 2020). In addition, changes in oil composition may also have effects on various other species which feed on plants, such as pest insects (Hixson et al., 2016) or other wild species (Colombo et al., 2018).

4.2. Persistence and Spread

It may not be easy to control the cultivation of NGT oilseed *Brassicaceae*, especially under European conditions. This is due to the biology of *Brassicaceae* species, such as oilseed rape (*B. napus*) and others. Some of the problems are:

- A broad range of *Brassica* species can hybridise with each other;
- Many *Brassicaceae* have weedy characteristics;
- Seeds can exhibit prolonged dormancy;
- Pollination by insects and wind, as well as dispersal, can occur over long distances.

Oilseed rape is also a good example of the difficulties associated with the cultivation of genetically engineered *Brassica* plants. Sohn et al. (Sohn et al., 2021) show that the uncontrolled spread of (transgenic) genetically engineered (GE) oilseed rape is already happening in different countries (Japan, Canada, USA, Switzerland, Argentina). The list includes countries that do not even allow the cultivation or import of transgenic plants, as transgenic populations near ports and along transport routes can originate from transgenic seeds that are imported legally or from contamination (Schulze et al., 2015).

GE herbicide-tolerant oilseed rape was recently shown to have hybridised with weedy *B. rapa* (bird rape mustard) in Canada (Laforest et al., 2022). Oilseed rape and *B. rapa* are intercrossable, and hybrids of both were previously confirmed (see, e g. (Jorgensen and Andersen, 1994; Warwick et al., 2008)). It was assumed that the hybrid plants had reduced fertility and were, therefore, unable to become permanently established in the environment. Contrary to this assumption, Laforest et al. showed that the genetically engineered trait is now detectable in purebred and weedy *B. rapa* plants in Canada (Laforest et al., 2022).

The oilseed rape example shows that genetically engineered *Brassicaceae* plants can persist in the environment as well as spread into wild or weedy populations. Assumptions of non-establishment should therefore be treated with caution. Other studies appear to indicate fitness advantages of GE oilseed rape or its hybrids with related species (see examples in (Bauer-Panskus et al., 2020)).

A closer look at NGT applications in *Brassicaceae* species shows there are several examples of fitness related traits (green in Tables A2 and A3). It can be hypothesised that these traits might further increase the risk of spread and persistence of genetically engineered *Brassicaceae* plants. Their potential to spread may be dependent on environmental conditions: This has been, for example, discussed for semi-dwarf phenotypes (Fan et al., 2021; Song et al., 2022) which might show improved survival in ruderal areas (Reuter et al., 2008).

4.3. Spontaneous Crossings and Stacking

NGTs make it possible to induce small, targeted changes in plant genomes to generate new properties. This is, in particular, the case with site-directed nuclease 1 applications (SDN 1, i. e. short deletions and/or insertions of a few base pairs), which also enable the alteration of several different DNA sequences – and thus several properties – simultaneously (multiplexing).

As shown in the overview of applications used in *Brassicaceae* oilseed plants, most traits are currently based on the knock out of single genes, gene copies, members of a particular gene family or genes associated with a distinct phenotype (e. g. (Kawall, 2021; Li et al., 2022)*). The two most prominent trait categories, i. e. altered fatty acid metabolism and positive fitness effects (orange and green in Tables A1–A3), comprise a variety of different traits, ranging from early/late flowering and the reduction of flavonoids to the increase/reduction of unsaturated fatty acids (see Tables A1–A3).*

These traits are influenced by many different genes that are frequently involved in multiple biological processes, which is why their alteration may lead to undesirable pleiotropic effects and unintended consequences in some circumstances. Therefore, the potential negative impacts on ecosystems, food webs and the health of wild species, livestock and humans need to be assessed on a case-by-case basis, i. e. individually for each event. In addition, possible combinations of the traits and impacts thereof need to be assessed and monitored in the affected areas and in wider environments. These potential combinations may occur through intentional crossings (stacked event) or in the case of Brassicaceae, also spontaneously (see for example (Zhang et al., 2021)*).* Consequently, it is likely that new combinations of geno- and phenotypes will emerge that were neither intended nor previously considered or tested for safety.

If there were to be large scale releases of NGT *Brassicaceae* oilseed plants, the likelihood of hybridisation within crossable species would increase. Furthermore, the chances of spontaneous and unintended combinations of different NGT traits occurring would also increase. Similar outcrossing phenomena have already been reported in the cultivation of transgenic oilseed rape plants where spontaneous combinations of herbicide resistances have been observed. These spontaneous combinations resulted in the occurrence of transgenic *Brassica* oilseed plants that were not approved for release (e. g. (Aono et al., 2006; Schafer et al., 2011)).

The stacking of different NGT events via conventional breeding or spontaneous crossings can all result in NGT offspring with new biological characteristics that are absent in the parental plants (see (Bauer-Panskus et al., 2020)). These biological characteristics could, for example, affect plant species composition with unintended and possibly undesirable consequences for ecosystems, food webs, the health of wild species and consumers. They may also cause NGT plants to become invasive. Even if each of the initial NGT events were individually classified as ‘safe’ or ‘unlikely to become invasive’ in risk assessment, their offspring may show next-generation effects associated with unexpected risks caused by genomic interactions. As with transgenic plants, spontaneous crossings of NGT plants may result in new combinations of traits, such as higher fitness and/or novel changes in oil composition.

In this context, unpredictable interactions at the genome level may also depend on the genetic background (including cryptic gene variants, see for example (Aguirre et al., 2023)) and the accumulation of unintended genetic changes caused by the NGT processes (for overview, see (Koller and Cieslak, 2023; Koller et al., 2023)).

5. Conclusions

A closer look at NGT applications in *Brassicaceae* oilseed crops described in scientific literature, shows that various, often fitness-relevant traits have been altered, e. g. growth and yield, oil and protein composition, abiotic and biotic resistance and fertility.

Currently, most of these traits are conferred by knocking out single genes or gene copies. Some of the traits can, therefore, also be achieved with conventional breeding methods. However, the increasing complexity of NGT applications, which may involve several different genes (Bellec et al., 2022), means that it is becoming increasingly difficult to achieve the same or ‘equivalent’ results using conventional methods. This is due, in particular, to the complexity of some *Brassicaceae* genomes, but also to the specific technical potential of NGTs (Kawall, 2019) that may rapidly evolve further (He et al., 2023).

The use of NGTs opens up new possibilities to modify the genome (Raitskin and Patron, 2016; Zetsche et al., 2017; Kawall, 2019; Kawall et al., 2020), and often results in new genotypes and phenotypes that are unlikely to result from conventional breeding even if no additional DNA sequences are inserted and no new proteins are produced by the plants (ANSES, 2023; Bohle et al., 2023; Eckerstorfer and Heissenberger, 2023). Furthermore, the spatio-temporal scale of potential releases of *Brassicaceae* NGT oilseed plants may also cause new combinations and interactions to occur that were neither intended nor assessed – all of which could have inherently unpredictable ecological consequences (Bauer-Panskus et al., 2020; Koller et al., 2023).

In case of a changed oil composition an increase or decrease of *PUFAs* demonstrates risks for health and survival of pollinators. The likelihood of outcrossing and spread, and environmental risks would increase depending on a couple of factors: a) the number of released new genotypes, b) the spatio-temporal scale of release if new genotypes, c) the fitness relevance of the new traits and d) the novelty of traits compared to the wildtype. Persistence and spread of novel genotypes can hardly be controlled and also hazard mitigation will be extremely difficult due to the hybridization potential, seed dormancy, persistence in the wild, and large pollen transfer distances.

Environmental risk assessment scenarios of specific NGT applications in *Brassicaceae* oilseed crops as outlined in this review, show that the technological specificity (with the potential for increasing trait complexity) as well as the plants' biology plus the scale of releases have to be seen in combination. Therefore, the potential release of NGT plants for agricultural purposes requires risk assessment and monitoring not only of singular traits, but also of combinatorial and long-term cumulative effects as foreseen in Directive 2001/18/EC. In addition, risk management will require new concepts and measures to control and potentially restrict the scale of releases. This is especially relevant for potential releases of NGT *Brassicaceae* in Europe which is a centre of diversity of this plant family.

Appendix A

Table A1. *Brassica napus* NGT applications (green: positive fitness effects (higher likelihood of persistence and spread)); orange: changed oil content or fatty acid composition). Categorization based on EU-SAGE database (www.eu-sage.eu).

Field of Application	Edited Gene(s)	Trait Category	Trait	Reference
Abiotic stress tolerance	*BnCUP1 (Cd uptake-related gene)*	Cadmium tolerance	Reduced Cadmium (Cd) accumulation without a distinct compromise in yield, also for agricultural production in Cd-contaminated soils.	https://doi.org/10.3390/cells11233888
Abiotic stress tolerance	*CUP1 (Cd uptake-related)*	Cadmium tolerance	Reducing Cd accumulation. Displayed superior growth and longer roots.	https://doi.org/10.3390/cells11233888
Abiotic stress tolerance	*BnPUB18 and BnPUB19 (Plant U-box)*	Drought tolerance	Significant improvements to drought tolerance.	https://doi.org/10.1016/j.indcrop.2023.116875
Abiotic stress tolerance. Harvesting processing	*BraRGL1 (DELLA protein)*	Changed flowering time. Changed plant architecture	Early maturing varieties. Promotes the flower bud differentiation without affecting the stalk quality. Improved breeding of early maturing varieties (bolting and flowering).	https://doi.org/10.1093/hr/uhad119
Biotic stress tolerance	*BnIDA (Inflorescence Deficient in Abscission)*	Changed flowering time. Fungal resistance	Floral abscission-defective phenotype in which floral organs remained attached to developing siliques, and dry and colourless senesced floral parts remained attached to mature siliques. Enhanced resistance against *Sclerotinia sclerotiorum*	https://doi.org/10.1093/plphys/kiac364

			(Sclerotinia stem rot (SSR)). Longer flowering period.	
Biotic stress tolerance	*WRKY70 (WRKY transcription factors)*	Fungal resistance	Enhanced resistance to *Sclerotinia sclerotiorum* (Sclerotinia stem rot (SSR)).	https://doi.org/10.3390/ijms19092716
Biotic stress tolerance	*BnCRT1a (calreticulin)*	Fungal resistance	Activation of the ethylene signalling pathway, which may contribute to reduced susceptibility towards *Verticillium longisporum* (*Vl43*).	https://doi.org/10.1111/pbi.13394
Biotic stress tolerance. Harvest properties. Storage properties	*BnF5H (Ferulate-5-hydroxylase gene)*	Fungal resistance. Changed plant architecture.	Decreased S/G lignin compositional ratio (ratio of syringyl (S) and guaiacyl (G) units in lignin). Stem strength dependence on lignin composition / stem lodging. More tightly packed stem structure, probably leading to a lower stem lodging index. Improves *Sclerotinia sclerotiorum* resistance.	https://doi.org/10.1111/pce.14208
Biotic stress tolerance. Yield	*BnaIDA (inflorescence deficient in abscission)*	Fungal resistance. Changed flowering time.	Reduced floral organ abscission, silique dehiscence (diverge), and disease severity caused by *S. sclerotiorum*. Improved yield by reducing seed loss due to premature silique dehiscence during mechanical harvesting and losses due to stem rot. Longer flowering period.	https://doi.org/10.1016/j.xplc.2022.100452
Breeding processing	*BnS6-Smi2 (S locus)*	Avoiding self-fertilization	Self-incompatibility to prevent inbreeding in hermaphrodite angiosperms via the rejection of self-pollen.	https://doi.org/10.1111%2Fpbi.13577
Breeding processing	*BnaDMP (domain of unknown function 679 membrane protein)*	Doubled haploid induction	Establishment of maternal haploid induction.	https://doi.org/10.1111/jipb.13244
Breeding properties	*BnaDMP (domain of unknown function 679 membrane protein)*	Doubled haploid induction	Higher haploid induction rate.	https://doi.org/10.1111/jipb.13270
Breeding properties	*BnCYP704B1 (cytochrome P450)*	Male sterility	Establishment of male sterility: pollenless, sterile phenotype in mature anthers.	https://doi.org/10.3390/plants12020365

Breeding properties	*BnARC1 (E3 ligaseARM-Repeat-Containing protein)*	Enables self-fertilization	Complete breakdown of self-incompatibility response. Promoting outcrossing and genetic diversity.	https://doi.org/10.1016/j.xplc.2022.100504
Food quality	*BnaSAD2*	Changed fatty acid content	Higher stearic acid content.	https://doi.org/10.1007/s00122-023-04414-x
Food quality. Feed quality	*BnITPK (inositol tetrakisphosphate kinase)*	Changed protein value	Reduced phytic acid, increase of free phosphorus, increase in protein value and no adverse effects on oil contents.	https://doi.org/10.1111/pbi.13380
Food quality. Feed quality. Industrial properties	*BnFAD2 (fatty acid desaturase 2)*	Changed fatty acid composition	Increased oleic acid content.	https://doi.org/10.1016/j.plaphy.2018.04.025
Food quality. Feed quality. Industrial properties	*BnTT8 (basic helix-loop-helix, bHLH)*	Changed fatty acid composition	Modification of fatty acid composition, including increases in palmitic acid, linoleic acid and linolenic acid and decreases in stearic acid and oleic acid.	https://doi.org/10.1111/pbi.13281
Food quality. Feed quality. Industrial properties	*BnFAD2 (fatty acid dehydrogenase 2)*	Changed fatty acid composition	Modification of fatty acid composition. The oleic acid content in the seed increased significantly, while linoleic and linolenic acid contents decreased accordingly.	https://doi.org/10.1007/s00122-020-03607-y
Food quality. Feed quality. Industrial properties	*BnFAD2 (fatty acid desaturase 2) and BnFAE1 (fatty acid elongase1)*	Changed fatty acid composition	Increased content of oleic acid, reduced erucic acid levels and slightly decreased polyunsaturated fatty acids content.	https://doi.org/10.3390/genes13101681
Food quality. Feed quality. Industrial properties	*KASII (canola β-ketoacyl-ACP synthase II)*	Changed fatty acid composition. Changed oil content	Decreased palmitic acid content, increased total C18 and reduced total saturated fatty acid contents.	https://doi.org/10.1111/j.1467-7652.2012.00695.x
Food quality. Feed quality.	*BnaTT7, BnaTT18, BnaTT10,*	Changed fatty acid composition.	Elevated seed oil content and decreased pigment and lignin accumulation. Decreased oleic acid and increased linoleic and linolenic acid contents.	https://doi.org/10.1111/pbi.14197

Industrial properties	*BnaTT1, BnaTT2 or BnaTT12 (transparent testa)*	Changed oil content	Down-regulation of key genes in flavonoid synthesis.	
Food quality. Feed quality. Industrial properties	*BnTT2 (transparent testa 2)*	Changed fatty acid composition. Changed oil content	Reduced flavonoids and improved fatty acid composition with higher linoleic acid and linolenic acid.	https://dx.doi.org/10.1021/acs.jafc.0c01126
Food quality. Feed quality. Industrial properties	*BnaFAE1 (fatty acid elongase 1)*	Changed fatty acid composition. Changed oil content	Deacreased erucic acid content.	https://doi.org/10.3389/fpls.2022.848723
Food quality. Feed quality. Industrial properties	*BnCIPK9 (Calcineurin B-like (CBL)-interacting protein kinase 9)*	Changed fatty acid composition. Changed oil content	Regulate seed oil metabolism. Increased levels of monounsaturated fatty acids and decreased levels of polyunsaturated fatty acids.	https://doi.org/10.1093/plphys/kiac569
Food quality. Feed quality. Industrial properties	*BnSFAR4 and BnSFAR5 (seed fatty acid reducer)*	Changed oil content	Increased seed oil content without pleiotropic effects on seed germination, vigour and oil mobilization. Improving oil yield.	https://doi.org/10.1111/pbi.13381
Food quality. Feed quality. Industrial properties	*BnLPAT2 and BnLPAT5 (Lysophosphatidic acid acyltransferase)*	Changed oil content	Increased seed oil content.	https://doi.org/10.1186/s13068-022-02182-2
Food quality. Feed quality. Industrial properties	*BnFAD2 (fatty acid desaturase 2)*	Changed fatty acid composition. Changed oil content	Enhanced seed oleic acid content.	https://doi.org/10.3389/fpls.2022.1034215
Food quality. Feed quality. Industrial	*BnKANT3, BnGIF1, BnAGP11 or BnEDA32*	Changed fatty acid composition. Changed oil	Increased linoleic or linolenic acid content.	https://doi.org/content/33/5/798.full

properties		content		
Food quality. Feed quality. Industrial properties	*BnaSBE (starch branching enzymes)*	Changed plant architecture. Changed carbohydrate composition.	Higher starch-bound phosphate content and altered pattern of amylopectin length pattern. Thick main stem.	https://doi.org/10.1093/plphys/kiab535
Harvest properties	*BnaCOL9 (CONSTANS-like 9)*	Changed flowering time	Early-maturing breeding.	https://doi.org/10.3390/ijms232314944
Harvest properties	*BnBRI1 (leucine-rich repeat receptor-like protein kinase)*	Changed plant architecture	Semi-dwarf lines without decreased yield in order to increase harvest index.	https://doi.org/10.3389/fpls.2022.865132
Harvest properties.	*BnJAG (jagged)*	Changed plant architecture	Changes in pod dehiscence zone with potential to increase shatter resistance.	https://doi.org/10.3390/biom9110725
Harvest properties.	*BnIND (INDEHISCENT)*	Changed plant architecture	Increased shatter resistance to avoid seed loss during mechanical harvest.	https://doi.org/10.1007/s00122-019-03341-0
Harvest properties. Yield	*BnALC (ALCATRAZ)*	Changed plant architecture	Increased shatter resistance to avoid seed loss during mechanical harvest.	https://doi.org/10.1104/pp.17.00426
Harvesting processing	*BnaSVP (Short Vegetative Phase)*	Changed flowering time	Early-flowering phenotypes.	https://doi.org/10.1016/j.cj.2021.03.023
Seed quality	*BnPAP2 (production of anthocyanin pigment 2)*	Changed seed pigments	Yellow seed coat and reduced proanthocyanidins. Reduced expression of various flavonoid biosynthesis genes.	https://doi.org/10.1016/j.jia.2023.05.001
Visual appearance	*BnaCRTISO (carotenoid isomerase)*	Changed ornamental plant properties	Altered colour of petals and leaves in order to improve the ornamental value of rapeseed and promote the development of agriculture and tourism.	https://doi.org/10.3389/fpls.2022.801456
Yield	*BnaSDG8 (Methyltransferase SDG8)*	Changed flowering time	Early-flowering varieties influenced by epigenetic modification.	https://doi.org/10.1111/tpj.13978
Yield	*BnCLV3*	Changed	Increased silique and seed number and higher	https://doi.org/10.1111

	(CLAVATA3)	plant architecture	seed weight.	/pbi.12872
Yield	*BnaMAX1 (more axillary growth (max))*	Changed plant architecture	Increased branching phenotypes with more siliques in order to increased yield.	https://doi.org/10.1111/pbi.13228
Yield	*BnD14 (strigolactone receptor BnD14)*	Changed plant architecture	Shoot architectural changes. Increase of total flowers.	https://doi.org/10.1111/pbi.13513
Yield	*BnaA03.BP (BREVIPEDICELLUS)*	Changed plant architecture	Optimizing rapeseed plant architecture, semi-dwarf and compact architecture.	https://doi.org/10.1111/pbi.13703
Yield	*BnaEOD3 (ENHANCER OF DA1)*	Changed plant architecture	Shorter siliques, smaler seeds, and an increased number of seeds per siliques. Increased seed weight per plant.	https://doi.org/10.1002/jcp.29986
Yield	*BnEOD1 (Enhancer of DA1)*	Changed plant architecture	Increased seed size and weight.	https://doi.org/10.21203/rs.3.rs-3204656/v1

Table A2. *Camelina sativa* NGT applications (green: positive fitness effects (higher likelihood of persistence and spread)); orange: changed oil content or fatty acid composition). Categorization based on EU-SAGE database (www.eu-sage.eu).

Field of Application	**Edited Gene(s)**	**Trait Category**	**Trait**	**Reference**
Food quality. Industrial properties	*CsFAD2 (fatty acid desaturase 2)*	Changed fatty acid composition. Changed oil content	Increased oleic acid content (proportional decrease in linoleic and linolenic acid content).	https://doi.org/10.1111/pbi.12671
Food quality. Industrial properties	*CsFAD2 (fatty acid desaturase 2)*	Changed fatty acid composition	Increased oleic acid content (proportional decrease in linoleic and linolenic acid content).	https://doi.org/10.1111/pbi.12663
Industrial properties	*CsFAD2 (fatty acid desaturase 2)*	Changed fatty acid composition	Enhanced monounsaturated fatty acid levels, partially bushy phenotype.	https://doi.org/10.3389/fpls.2021.702930
Food quality. Feed quality.Industrial properties	*CsCRUC (cruciferin C)*	Changed protein composition. Changed fatty acid composition	Changed seed amino acid content (increased proportion of alanine, cysteine and proline, and decrease of isoleucine, tyrosine and valine). Increased relative abundance of all saturated fatty acids.	https://doi.org/10.1186/s12870-019-1873-0

Industrial properties	*CsDGAT1 or CsPDAT1 (acyl-CoA:- or phospholipid:diacylglycerol acyltransferase)*	Changed triacylglycerols content. Changed fatty acid composition. Changed oil content	Produce triacylglycerols (TAGs) that are valuable as industrial feedstocks. Reduced oil content, partially higher levels of linoleic acid.	https://doi.org/10.1093/pcp/pcx058
Food quality	*FAE1 (fatty acid elongase 1)*	Changed fatty acid composition	Decreased erucic acid content, increased levels of omega-3 fatty acids such as linolenic acid as well as eicosapentaenoic and docosahexaenoic acid in transgenic camelina.	https://doi.org/10.1111/pbi.13876
Food quality	*FAE1 (fatty acid elongase 1)*	Changed fatty acid composition	Increased oleic and linolenic acid content by blocking eicosenoic and erucic acid synthesis in transgenic camelina.	https://doi.org/10.1038/s41598-023-34364-9
Food quality	*FAE1 (fatty acid elongase 1)*	Changed fatty acid composition	Reduction of C20-C24 very long-chain fatty acids (VLCFAs).	https://doi.org/10.1016/j.plaphy.2017.11.021
Yield	*FLC (flowering locus C), SVP (short vegetative phase), LHP1 (like heterochromatin protein 1), TFL1 (terminal flower 1) and EFL3 (early flowering locus 3)*	Changed flowering time	Early-flowering, shorter stature and/or basal branching. Different combinations of mutations had a positive or negative impact on yield.	https://doi.org/10.3390/agronomy12081873
Food quality. Feed quality	*CsGTR1 and CsGTR2 (glucosinolate transporter)*	Changed glucosinolate content	Decreased and eliminated glucosinolate content in order to improve quality of oil and press cake.	https://doi.org/10.1111/pbi.13936

Table A3. *Thlaspi arvense* NGT applications (orange: changed oil content or fatty acid composition). Categorization based on EU-SAGE database (www.eu-sage.eu).

Field of Application	Edited Gene	Trait Category	Trait	Reference

Food quality. Feed quality. Industrial properties	*FAD2 (fatty acid desaturase 2), ROD1 (reduced oleate desaturation 1) and FAE1 (fatty acid elongation 1)*	Changed fatty acid composition. Changed oil content.	Increased oleic acid amount in seed oil. Reduction of PUFAs.	https://doi.org/10.3389/fpls.2021.652319
Food quality	*FAE1 (fatty acid elongation 1)*	Changed fatty acid composition.	Abolishing erucic acid production and creating an edible seed oil comparable to that of canola.	https://doi.org/10.1111/pbi.13014
Industrial properties	*FAE1-3 (fatty acid elongation)*	Changed fatty acid composition.	Abolishing erucic acid production, further crossing with transgenic pennycress.	https://doi.org/10.3389/fenrg.2021.620118

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