Background:
The application of NGTs in *Brassicaceae*: environmental risk assessment scenarios

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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) and pennycress (Thlaspi arvense). The cultivated species of this plant family are global sources of nutrients and vegetable oil. The latter is mainly obtained from the oilseed crops, i. e. oilseed rape (Brassica napus) and camelina/false flax (Camelina sativa).

For many years, breeders have been working to improve the quality of both 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 these oilseed crops. The oil obtained from Brassicaceae oilseed crops is low in monounsaturated fatty acids (MUFAs), e. g. oelic acid or erucic acid, but it is high in polyunsaturated fatty acids (PUFAs), such as linoleic acid and linolenic acid, which are also known as omega-6 and omega-3 fatty acids, respectively. It is known that these PUFAs have numerous health benefits, but are also associated with oxidative instability. Depending on the intended purpose, attempts are being made to either increase (in food and feed) or decrease (industrial purposes) the content of PUFAs in Brassicaceae crops. In addition, there have been attempts to further reduce the erucic acid content. Besides altering oil content and composition, breeders are also trying to improve plant fitness by increasing yield, growth and resistance to abiotic stress, including resistance to plant pathogens and herbicides.

However, the genomic complexity of this plant family means that conventional breeding methods (including non-targeted mutagenesis) are somewhat restricted (Li et al., 2022). Several Brassicaceae crops are polyploid (up to allohexaploid), which means they have more than two sets of paired chromosomes and, therefore, more than two gene copies. 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 this can be achieved at all.

New genomic techniques (NGTs), such as CRISPR/Cas9, have been successfully applied 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 one gene, or even alter 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 developed within short periods of time. While some of the NGT-derived Brassicaceae genotypes were also achieved using conventional breeding (e. g. EMS (ethyl methane sulphonate)-breeding), others have been newly introduced into the plants. In future, combining and stacking NGT-derived genotypes of various genes is expected to lead to even more extensive overall genomic change in NGT plants (Raitskin and Patron, 2016; Zetsche et al., 2017; Kawall et al., 2020).

In the following an overview of current NGT applications in Brassicaceae oilseed crops, i. e. oilseed rape (B. napus), camelina (C. sativa) and pennycress (T. arvense) is provided. For this purpose, NGT applications in Brassicaceae oil plants in scientific
literature were identified and all NGT-plants without permanent transgene insertion were listed. Almost all of these NGT applications are expected to fall under Category 1 of the EU Commission proposal. According to the proposal, Category 1 plants would be exempt from risk assessment, including analysis of the intended traits and unintended genetic changes brought about by NGT processes. As specified in the proposal, these plants would only need to be registered. In legal terms, Category 1 NGT plants would be equated to conventionally-bred plants, even if they were biologically different. The resulting exemption from risk assessment and monitoring would, furthermore, extend to offspring and further crossings.

2. Overview of NGT applications in Brassicaceae oilseed plants

2.1. *Brassica napus* (oilseed rape)

*B. napus* (2n=38, AACC) is allotetraploid and arose from interspecific crosses between the diploid ancestors *B. rapa* (2n=20, AA) and *B. oleracea* (2n=18, CC) (An et al., 2019). The origin of oilseed rape cultivation lies in Europe and Asia. Originally, *B. napus* was grown as a vegetable, either for human consumption or as animal feed, and it was only after the Industrial Revolution that it was gradually domesticated as an oilseed plant (An et al., 2019). Oilseed rape is now grown globally and is economically one of the most important oil-seeds (Hu et al., 2021). As a double-low oilseed rape, also called canola, it now 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). Overall, more than 30 NGT applications in *B. napus* are described in scientific literature (see Table 1). Its agronomic importance as an oilseed crop has resulted in its genome being edited several times with NGTs, in various efforts to adapt the fatty acid composition and total oil content to individual intended uses (12 applications, marked in yellow in Table 1 and Figure 1). Attempts to reduce PUFAs and erucic acid by knocking out FAD and FAE 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 (marked in green in Table 1 and Figure 1).

2.2. *Camelina sativa* (camelina)

*C. sativa* is allohexaploid (2n = 6x = 40, AABBC) which emerged from a series of polyploidization events. Western Asia and Eastern Europe are considered regions of origin. As an oilseed crop, it is currently thought to have great potential to become an important raw material in the production of food and biofuel. Accordingly, its genome has already been genetically modified several times (9 applications, see Table 2). Similar to oilseed rape, attempts are being made to alter the fatty acid content and composition (7 applications, marked in yellow in Table 2 and Figure 1). NGT applications to reduce PUFAs and erucic acid (FAD and FAE genes) are particularly frequent. In addition, different knockouts (5 genes, multiple copies) were established

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in a multiplexing approach resulting in early-flowering plants (marked green in Table 2 and Figure 1).

2.3. Thlaspi arvense (pennycress)

Pennycress is diploid and found throughout Eurasia and North America. It is widely recognised as a weed. Currently, many attempts are being made to rapidly domesticate this wild plant for use as an oilseed crop, as it is cold tolerant, overwintering and has high oil and protein content. Cultivated as a winter cover crop ‘sandwiched’ between other crops, its oil could be used for biofuel, food and feed production. NGTs were used to knock out FAE1, thus making it possible to abolish erucic acid production (see Table 3 and Figure 1). This knockout strain was further combined with NGT-derived FAD2 and ROD1 knockouts to increase oleic acid content and decrease PUFAs.

![Figure 1. NGT applications in Brassicaceae oilseed plants B. napus, C. sativa and T. arvense. A total of 50 NGT applications were found in the three species that are expected to fall under Category 1 of the EU Commission proposal. Attempts to change the oil content or composition are marked in yellow. NGT applications that showed positive fitness effects in the plant are marked in green. Other traits marked in grey.](image-url)
3. Findings and scenarios relevant to risk assessment

3.1. Decreasing amounts of PUFA can negatively affect the health of pollinators

Several NGT applications in Brassicaceae oilseed crops focus on the alteration of the fatty acid composition in order 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 and they, therefore, have to be part of their diet (Hulbert et al., 2012; Arien et al., 2020).

Pollinators such as honey bees obtain PUFAs and other fatty acids from the pollen of plants (Manning, 2001). It was shown in B. napus that FAD genes are highly expressed in pollen, thus explaining their high levels of PUFAs (Piffanelli et al., 1997). Therefore, it can be assumed that the knockout of FAD genes will significantly decrease the PUFA content, including in pollen.

In habitats with extensive agricultural monocultures of plants with reduced PUFA content, bees may suffer from a deficiency of PUFAs due to a severely limited variety of pollen (Arien et al., 2015, 2020). It was shown that honey bees with an omega-3 dietary deficiency had reduced 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 their omega-6:3 ratio are also affecting brood development and adult longevity in honey bees. It was found that a balanced PUFA diet is necessary for maintaining proper colony development (Arien et al., 2020). In addition, PUFAs seem to have some antimicrobial properties that are able to inhibit honey bee pathogens, e. g. Paenibacillus larvae and fungal diseases (Ababouch et al., 1992; Feldlaufer et al., 1993; Manning, 2001).

As mentioned, changes in the 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 targeted editing of Brassicaceae genomes. In case of individual NGT traits, without in-depth research, it may be difficult to conclude if the specific genotype and phenotype could also be obtained by conventional breeding.

Beyond that, the overall number of NGT applications in Brassicaceae oilseed plants brings to light a new dimension in the scale (in terms of space and time) of potential releases of plants characterised by new genotypes and phenotypes. This will complicate any comparison with the impact of conventionally bred plants, since both spontaneous and intended crossings can lead to new combinations of traits causing unintended interactions without precedent (see below).
3.2. 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:

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

One example is oilseed rape (B. napus), which originated from a natural crossing of B. oleracea and B. rapa. Europe is the center of origin and genetic diversity for Brassica species to which oilseed rape belongs. Oilseed rape can form interspecific hybrids with cultivated species like B. rapa (turnip rape, field mustard, birdseed rape, etc.), B. juncea (different mustard cultivars) and others, but also with wild and weedy Brassicaceae. So far, spontaneous hybridization with a total of eight species native to or cultivated in Europe have been confirmed (Theenhaus et al., 2002; Breckling et al., 2003; FitzJohn et al., 2007; Devos et al., 2009; OECD, 2012; COGEM, 2019; Marotti et al., 2020).

Furthermore, oilseed rape seeds can remain viable in the soil for more than ten years (D’Hertefeldt et al., 2008). Pollen from this plant is dispersed over very large areas by wind and insects, and in rare cases over distances of up to 26 km (Ramsay et al., 2003).

All cultivated Brassica species have weedy characteristics. For example, there are weedy forms of B. napus, B. rapa and B. oleracea. In addition, the wild Brassica species Sinapis arvensis, Raphanus raphanistrum and Hirschfeldia incana are also considered to be weeds. The same is true for Brassicaceae plants such as pennycress or camelina.

Oilseed rape is also a good example of the difficulties associated with the cultivation of genetically engineered Brassica plants. 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). Many of these countries do not even allow the cultivation of transgenic plants, but transgenic populations originating from imports have been found near ports and along transport routes.

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 had already been confirmed earlier. 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, the study shows that the genetically engineered trait is now detectable in purebred and weedy B. rapa plants in Canada.

The oilseed rape example shows that genetically engineered Brassicaceae plants can persist in the environment and spread into wild or weedy populations and that
assumptions of non-establishment should 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).

Looking at NGT applications in Brassicaceae species, it seems there are several examples of fitness related traits (marked in green in Tables 1 and 2). It can be hypothesised that these traits would further increase the risk of spread and persistence of genetically engineered Brassicaceae plants.

3.3. Spontaneous crossings and stacking

NGTs make it possible to induce small, targeted changes in plant genomes in order 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 prominent trait categories, i.e. altered fatty acid metabolism and increased fitness, comprise a variety of different traits, ranging from early/late flowering and the reduction of flavonoids to increased/reduction of unsaturated fatty acids (see Table 1).

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 have to be assessed on a case-by-case basis, i.e. individually for each event. In addition, combinations of the traits need to be assessed and monitored. These potential combinations may occur through intentional crossings (stacked event) or in case of Brassicaceae, also spontaneously. In consequence, it is likely that new combinations of geno- and phenotypes will emerge that were neither intended nor previously considered or tested for their safety.

If NGT Brassicaceae oilseed plants were to be released on a large scale, the likelihood of hybridisation within crossable species would increase. Furthermore, the chance of spontaneous and unintended combinations of different NGT traits would also increase. Similar outcrossing phenomena have already occurred in the cultivation of transgenic oilseed rape plants: spontaneous combinations of herbicide resistances have been observed, thus resulting in the occurrence of transgenic Brassica oilseed plants not approved for release (e.g. Aono et al., 2006; Schafer et al., 2011).

Stacking different events using technical means, conventional breeding or spontaneous crossings can all result in 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 compositions and have negative 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 events were individually classified as ‘safe’ 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 cause new combinations of traits, such as higher fitness and/or novel changes in oil composition.

In this context, unpredictable genomic interactions 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 processes of NGT (for overview, see Koller and Cieslak, 2023; Koller et al., 2023).

4. Conclusions

A closer look at NGT applications in Brassicaceae oilseed crops described in scientific literature (Table 1, 2 and 3), shows that various 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 these can, therefore, also be achieved using 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 comparable results using conventional methods. This is due, in particular, to the complexity of some Brassicaceae genomes. The use of NGTs opens up new possibilities, and new genotypes and phenotypes may be the result (Raitskin and Patron, 2016; Zetsche et al., 2017; Kawall et al., 2020). Furthermore, the scale of potential releases (in terms of space and time) of Brassicaceae NGT oilseed plants may also cause new combinations and interactions to occur that were neither intended nor assessed.

In addition to the intended alteration in the phenotype (and their combinations), unintended changes can also occur, with consequences for plant health and interaction with the environment. Especially in the case of changed oil content and composition, there may be numerous unintended and undesirable effects. Fatty acids play important roles in plants, e.g. in the biosynthesis of secondary metabolites such as phytohormones, and in cellular membranes. Therefore, alterations can affect plant communication, signaling pathways and plant resistance to biotic or abiotic stressors (Kawall, 2021). Furthermore, impairments to interaction with other organisms feeding on NGT plants have to be assumed in areas characterised by monocultures, e.g. bees may suffer from a deficiency of PUFAs (Arien et al., 2015). In addition, there are further NGT applications with altered traits which could be relevant for interactions with pollinators, e.g. traits with significant impact on hormone signalling (Sun et al., 2018) or flavonoid content (Xie et al., 2020; Zhai et al., 2020).

Numerous applications aiming to increase the fitness of domesticated plants may, in fact, further exacerbate the problems of spread, persistence and outcrossing of genetically modified Brassicaceae native to Europe. According to the characteristic of
the considered species, hazard mitigation would have to face high difficulties due to the hybridization potential, seed dormancy, persistence in the wild, and large pollen transfer distances.

5. Consequences for the future regulation of NGT plants

Most of the applications summarised in this fact-sheet would fall within the Category 1 of the new regulation of NGT plants proposed by the EU Commission. As already described, these plants would be exempt from risk assessment, including the analysis of the intended traits and unintended genetic changes brought about by NGT processes. In legal terms, Category 1 NGT plants could be equated to conventionally-bred plants, even if they are biologically different. The exemption from risk assessment and monitoring would also extend to the offspring and further crossings.

This means that further combinations and crossings are possible with no further restrictions or monitoring. It would, therefore, facilitate the production of NGT plants with considerably more genetic changes than specified in Category 1 criteria. The uncontrolled spread of a wide variety of stacked traits, which might also undergo further spontaneous crossings with potential negative consequences for health and the environment, may be biologically and environmentally relevant, difficult to trace and causing effects that are not reversible.

With increasing complexity and (intended or spontaneous) combinations of the new traits, it is to be expected that undesirable pleiotropic effects and unintended consequences will become more likely. At the same time, they may well be more difficult to predict. This may have serious consequences for ecosystems and could severely disrupt ecosystem services (such as insect pollination) and the food webs, especially if these NGT plants were to be released into the environment without risk assessment or monitoring.
6. Appendix

**Table 1.** *Brassica napus* NGT applications (green: increased plant fitness; yellow: changed oil content or fatty acid composition). Categorization based on EU-SAGE database (www.eu-sage.eu).

<table>
<thead>
<tr>
<th>Field of application</th>
<th>Edited gene(s)</th>
<th>Trait category</th>
<th>Trait</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abiotic stress tolerance</td>
<td>BnCUP1 (Cd uptake-related gene)</td>
<td>Cadmium tolerance</td>
<td>Reduced Cd accumulation without a distinct compromise in yield, also for agricultural production in Cd-contaminated soils.</td>
<td><a href="https://doi.org/10.3390/cells11233888">https://doi.org/10.3390/cells11233888</a></td>
</tr>
<tr>
<td>Biotic stress tolerance</td>
<td>BnIDA (Inflorescence Deficient in Abscission)</td>
<td>Changed flowering time. Fungal resistance</td>
<td>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 <em>Sclerotinia sclerotiorum</em> (<em>Sclerotinia</em> stem rot (SSR)). Longer flowering period.</td>
<td><a href="https://doi.org/10.3390/jmgs19092716">https://doi.org/10.3390/jmgs19092716</a></td>
</tr>
<tr>
<td>Biotic stress tolerance</td>
<td>WRKY70 (WRKY transcription factors)</td>
<td>Fungal resistance</td>
<td>Enhanced resistance to <em>Sclerotinia sclerotiorum</em> (<em>Sclerotinia</em> stem rot (SSR)).</td>
<td><a href="https://doi.org/10.1093/plphys/kiac364">https://doi.org/10.1093/plphys/kiac364</a></td>
</tr>
<tr>
<td>Biotic stress tolerance</td>
<td>BnCRT1a (calreticulin)</td>
<td>Fungal resistance</td>
<td>Activation of the ethylene signalling pathway, which may contribute to reduced susceptibility towards <em>Verticillium longisporum</em> (Vl43).</td>
<td><a href="https://doi.org/10.1111/pbi.13394">https://doi.org/10.1111/pbi.13394</a></td>
</tr>
<tr>
<td>Biotic stress tolerance. Harvest properties. Storage properties</td>
<td>BnFSH (Ferulate-5-hydroxylase gene)</td>
<td>Fungal resistance. Changed plant architecture.</td>
<td>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 <em>Sclerotinia sclerotiorum</em> resistance.</td>
<td><a href="https://doi.org/10.1111/pce.14208">https://doi.org/10.1111/pce.14208</a></td>
</tr>
<tr>
<td>Biotic stress tolerance. Yield</td>
<td>BnIDA (inflorescence deficient in abscission)</td>
<td>Fungal resistance. Changed flowering time.</td>
<td>Reduced floral organ abscission, silique dehiscence (diverge), and disease severity caused by <em>S. sclerotiorum</em>. Improved yield by reducing seed loss due to premature siliques dehiscence during mechanical harvesting and losses due to stem rot. Longer flowering period.</td>
<td><a href="https://doi.org/10.1016/j.xplc.2022.100452">https://doi.org/10.1016/j.xplc.2022.100452</a></td>
</tr>
<tr>
<td>Breeding process</td>
<td>BnS6-Sm12 (S locus)</td>
<td>Avoiding self-fertilization</td>
<td>Self-incompatibility to prevent inbreeding in hermaphrodite angiosperms via the rejection of self-pollen.</td>
<td><a href="https://doi.org/10.1111%2Fpbi.13577">https://doi.org/10.1111%2Fpbi.13577</a></td>
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<tr>
<td>Food quality</td>
<td>Feed quality</td>
<td>Industrial properties</td>
<td>BnTPK (inositol tetrakisphosphate kinase)</td>
<td>Changed protein value</td>
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<tr>
<td>Food quality</td>
<td>Feed quality</td>
<td>Industrial properties</td>
<td>BnFAD2 (fatty acid desaturase 2)</td>
<td>Changed fatty acid composition</td>
</tr>
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<td>Food quality</td>
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<td>Industrial properties</td>
<td>BnTT8 (transparent testa 8)</td>
<td>Changed fatty acid composition</td>
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<td>Food quality</td>
<td>Feed quality</td>
<td>Industrial properties</td>
<td>BnFAD2 (fatty acid dehydrogenase 2)</td>
<td>Changed fatty acid composition</td>
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<tr>
<td>Food quality</td>
<td>Feed quality</td>
<td>Industrial properties</td>
<td>BnFAD2 (fatty acid desaturase 2) and BnFAE1 (fatty acid elongase 1)</td>
<td>Changed fatty acid composition</td>
</tr>
<tr>
<td>Food quality</td>
<td>Feed quality</td>
<td>Industrial properties</td>
<td>KASII (canola β-ketoacyl-ACP synthase II)</td>
<td>Changed fatty acid composition. Changed oil content</td>
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<tr>
<td>Food quality</td>
<td>Feed quality</td>
<td>Industrial properties</td>
<td>BnTT2 (transparent testa 2)</td>
<td>Changed fatty acid composition. Changed oil content</td>
</tr>
<tr>
<td>Food quality</td>
<td>Feed quality</td>
<td>Industrial properties</td>
<td>BnaFAE1 (fatty acid elongase 1)</td>
<td>Changed fatty acid composition. Changed oil content</td>
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<tr>
<td>Food quality</td>
<td>Feed quality</td>
<td>Industrial properties</td>
<td>BnCIPK9 (Calcineurin B-like (CBL)-interacting protein kinase 9)</td>
<td>Changed fatty acid composition. Changed oil content</td>
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<tr>
<td>Food quality</td>
<td>Feed quality</td>
<td>Industrial properties</td>
<td>BnSFAR4 and BnSFAR5 (seed fatty acid reducer)</td>
<td>Changed oil content</td>
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<td>Food quality</td>
<td>Feed quality</td>
<td>Industrial properties</td>
<td>BnLPAT2 and BnLPAT5 (Lyso-phosphatic acid acyltransferase)</td>
<td>Changed oil content</td>
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<tr>
<td>Food quality</td>
<td>Feed quality</td>
<td>Industrial properties</td>
<td>BnFAD2 (fatty acid desaturase 2)</td>
<td>Changed fatty acid composition. Changed oil content</td>
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<td>Harvest properties</td>
<td>Harvest properties</td>
<td>Yield</td>
<td>BnaCOL9 (CONS-TANS-like 9)</td>
<td>Changed flowering time</td>
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<td>Harvest properties</td>
<td>Yield</td>
<td>BnBR11 (leucinerich repeat receptor-like protein kinase)</td>
<td>Changed plant architecture</td>
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<tr>
<td>Harvest properties</td>
<td>Harvest properties</td>
<td>Yield</td>
<td>BnALC (ALCAT-RAZ)</td>
<td>Changed plant architecture</td>
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Table 2. *Camelina sativa* NGT applications (green: increased plant fitness; yellow: changed oil content or fatty acid composition). Categorization based on EU-SAGE database (www.eu-sage.eu).

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</thead>
<tbody>
<tr>
<td>Food quality,</td>
<td>CsFAD2 (fatty</td>
<td>Changed fatty acid composition</td>
<td>Increased oleic acid content (proportional decrease in linoleic and linolenic acid content).</td>
<td><a href="https://doi.org/10.1111/pbi.12671">https://doi.org/10.1111/pbi.12671</a></td>
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<td>Industrial properties</td>
<td>acid desaturase 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food quality,</td>
<td>CsFAD2 (fatty</td>
<td>Changed fatty acid composition</td>
<td>Increased oleic acid content (proportional decrease in linoleic and linolenic acid content). Reduced monounsaturated fatty acids.</td>
<td><a href="https://doi.org/10.1111/pbi.12663">https://doi.org/10.1111/pbi.12663</a></td>
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<td>Industrial properties</td>
<td>acid desaturase 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industrial properties</td>
<td>CsFAD2 (fatty acid desaturase 2)</td>
<td>Changed fatty acid composition</td>
<td>Enhanced monounsaturated fatty acid levels, partially bushy phenotype.</td>
<td><a href="https://doi.org/10.3389/fpls.2021.702930">https://doi.org/10.3389/fpls.2021.702930</a></td>
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<tr>
<td>Food quality,</td>
<td>CsCRUC (cruciferin C)</td>
<td>Changed protein composition, Changed fatty acid composition</td>
<td>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.</td>
<td><a href="https://doi.org/10.1186/s12870-019-1873-0">https://doi.org/10.1186/s12870-019-1873-0</a></td>
</tr>
<tr>
<td>Feed quality,</td>
<td>FAE1 (fatty acid elongase 1)</td>
<td>Changed fatty acid composition</td>
<td>Decreased erucic acid content, increased levels of omega-3 fatty acids such as ALA.</td>
<td><a href="https://doi.org/10.1111/pbi.13876">https://doi.org/10.1111/pbi.13876</a></td>
</tr>
<tr>
<td>Industrial properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. *Thlaspi arvense* NGT applications (yellow: changed oil content or fatty acid composition). Categorization based on EU-SAGE database (www.eu-sage.eu).

<table>
<thead>
<tr>
<th>Field of application</th>
<th>Edited gene</th>
<th>Trait category</th>
<th>Trait</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food quality, Feed quality</td>
<td>FAD2 (fatty acid desaturase 2), ROD1 (reduced oleate desaturation 1) and FAE1 (fatty acid elongation 1)</td>
<td>Changed fatty acid composition. Changed oil content.</td>
<td>Increased oleic acid amount in seed oil. Reduction of PUFAs.</td>
<td><a href="https://doi.org/10.3389/fpls.2021.652319">https://doi.org/10.3389/fpls.2021.652319</a></td>
</tr>
<tr>
<td>Food quality</td>
<td>FAE1 (fatty acid elongation 1)</td>
<td>Changed fatty acid composition.</td>
<td>Abolishing erucic acid production and creating an edible seed oil comparable to that of canola.</td>
<td><a href="https://doi.org/10.1111/pbi.13014">https://doi.org/10.1111/pbi.13014</a></td>
</tr>
</tbody>
</table>
7. References


