

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. oleic 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.¹ 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, AABBCC) 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

¹ COM (2023) 411 final 2023/0226 (COD) Proposal for a Regulation of the European Parliament and of the Council on plants obtained by certain new genomic techniques and their food and feed, and amending Regulation (EU) 2017/625, <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX%3A52023PC0411>

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.

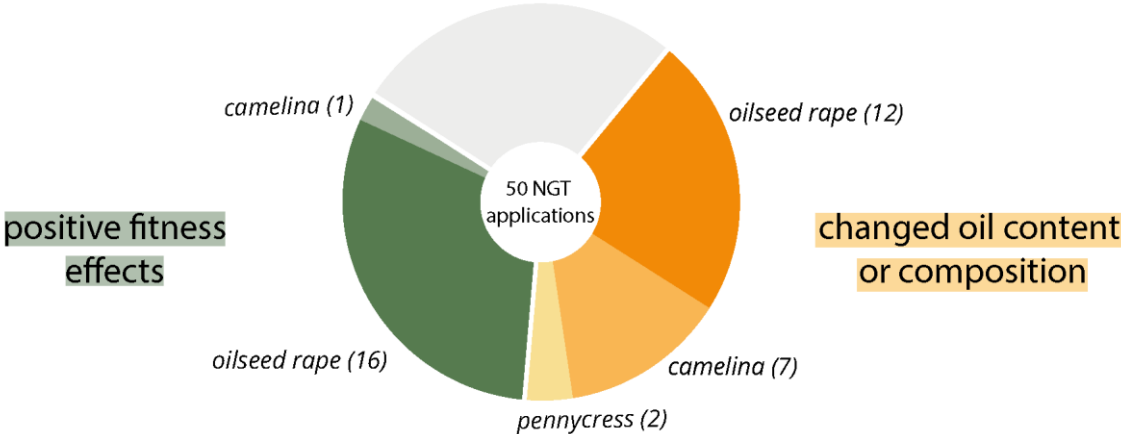


Figure 1. NGT applications in *Brassicaceae* oilseed plants *B. napus*, *C. sativa* and *T. arvense*. A total of 50 NGT applications were found in the three species that are expected to fall under Category 1 of the EU Commission proposal. Attempts to change the oil content or composition are marked in yellow. NGT applications that showed positive fitness effects in the plant are marked in green. Other traits marked in grey.

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).

Field of application	Edited gene(s)	Trait category	Trait	Reference
Abiotic stress tolerance	<i>BnCUP1</i> (<i>Cd uptake-related gene</i>)	Cadmium tolerance	Reduced 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	<i>CUP1</i> (<i>Cd uptake-related</i>)	Cadmium tolerance	Reducing Cd accumulation. Displayed superior growth and longer roots.	https://doi.org/10.3390/cells11233888
Abiotic stress tolerance	<i>BnPUB18</i> and <i>BnPUB19</i> (<i>Plant U-box</i>)	Drought tolerance	Significant improvements to drought tolerance.	https://doi.org/10.1016/j.indcrop.2023.116875
Abiotic stress tolerance. Harvesting processing	<i>BraRGL1</i> (<i>DELLA protein</i>)	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	<i>BnIDA</i> (<i>Inflorescence Deficient in Abscission</i>)	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 <i>Sclerotinia sclerotiorum</i> (<i>Sclerotinia stem rot</i> (SSR)). Longer flowering period.	https://doi.org/10.1093/plphys/kiac364
Biotic stress tolerance	<i>WRKY70</i> (<i>WRKY transcription factors</i>)	Fungal resistance	Enhanced resistance to <i>Sclerotinia sclerotiorum</i> (<i>Sclerotinia stem rot</i> (SSR)).	https://doi.org/10.3390/ijms19092716
Biotic stress tolerance	<i>BnCRT1a</i> (<i>calreticulin</i>)	Fungal resistance	Activation of the ethylene signalling pathway, which may contribute to reduced susceptibility towards <i>Verticillium longisporum</i> (<i>V143</i>).	https://doi.org/10.1111/pbi.13394
Biotic stress tolerance. Harvest properties. Storage properties	<i>BnF5H</i> (<i>Ferulate-5-hydroxylase gene</i>)	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 <i>Sclerotinia sclerotiorum</i> resistance.	https://doi.org/10.1111/pce.14208
Biotic stress tolerance. Yield	<i>BnaIDA</i> (<i>inflorescence deficient in abscission</i>)	Fungal resistance. Changed flowering time.	Reduced floral organ abscission, silique dehiscence (diverge), and disease severity caused by <i>S. sclerotiorum</i> . 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 process	<i>BnS6-Smi2</i> (<i>S locus</i>)	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 process	<i>BnaDMP</i> (<i>domain of unknown function 679 membrane protein</i>)	Doubled haploid induction	Establishment of maternal haploid induction.	https://doi.org/10.1111/jipb.13244
Breeding process	<i>BnaDMP</i> (<i>domain of unknown function 679 membrane protein</i>)	Doubled haploid induction	Higher haploid induction rate.	https://doi.org/10.1111/jipb.13270
Breeding process	<i>BnARC1</i> (<i>E3 ligaseARM-Repeat-Containing protein</i>)	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	<i>BnaSAD2</i>	Changed fatty acid content	Higher stearic acid content.	https://doi.org/10.1007/s00122-023-04414-x
Food quality. Feed quality	<i>BnITPK (inositol tetrakisphosphate kinase)</i>	Changed protein value	Reduced phytic acid, an increase of free phosphorus, an increase in protein value and no adverse effects on oil contents.	https://doi.org/10.1111/pbi.13380
Food quality. Feed quality. Industrial properties	<i>BnFAD2 (fatty acid desaturase 2)</i>	Changed fatty acid composition	Increase in the content of oleic acid.	https://doi.org/10.1016/j.plaphy.2018.04.025
Food quality. Feed quality. Industrial properties	<i>BnTT8 (transparent testa 8)</i>	Changed fatty acid composition	Modification of FA 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	<i>BnFAD2 (fatty acid dehydrogenase 2)</i>	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	<i>BnFAD2 (fatty acid desaturase 2) and BnFAE1 (fatty acid elongase1)</i>	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	<i>KASII (canola β-ketoacyl-ACP synthase II)</i>	Changed fatty acid composition. Changed oil content	Decreases in palmitic acid, 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. Industrial properties	<i>BnTT2 (transparent testa 2)</i>	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	<i>BnaFAE1 (fatty acid elongase 1)</i>	Changed fatty acid composition. Changed oil content	Decreased erucic acid content.	https://doi.org/10.3389/fpls.2022.848723
Food quality. Feed quality. Industrial properties	<i>BnCIPK9 (Calcineurin B-like (CBL)-interacting protein kinase 9)</i>	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	<i>BnSFAR4 and BnSFAR5 (seed fatty acid reducer)</i>	Changed oil content	Increase of 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	<i>BnLPAT2 and BnLPAT5 (Lyso-phosphatidic acid acyltransferase)</i>	Changed oil content	Increased seed oil content.	https://doi.org/10.1186/s13068-022-02182-2
Food quality. Feed quality. Industrial properties	<i>BnFAD2 (fatty acid desaturase 2)</i>	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 properties	<i>BnaSBE (starch branching enzymes)</i>	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	<i>BnaCOL9 (CONSTANS-like 9)</i>	Changed flowering time	Early-maturing breeding.	https://doi.org/10.3390/ijms232314944
Harvest properties	<i>BnBRI1 (leucine-rich repeat receptor-like protein kinase)</i>	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. Yield	<i>BnALC (ALCATRAZ)</i>	Changed plant architecture	Increased shatter resistance to avoid seed loss during mechanical harvest.	https://doi.org/10.1104/pp.17.00426

Harvesting processing	<i>BnaSVP (Short Vegetative Phase)</i>	Changed flowering time	Early-flowering phenotypes.	https://doi.org/10.1016/j.cj.2021.03.023
Seed quality	<i>BnPAP2 (production of anthocyanin pigment 2)</i>	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	<i>BnaCRTISO (carotenoid isomerase)</i>	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	<i>BnaSDG8 (Methyltransferase SDG8)</i>	Changed flowering time	Early-flowering varieties influenced by epigenetic modification.	https://doi.org/10.1111/tpj.13978
Yield	<i>BnCLV3 (CLAVATA3)</i>	Changed plant architecture	Increased silique and seed number and higher seed weight.	https://doi.org/10.1111/pbi.12872
Yield	<i>BnaMAX1 (more axillary growth (max))</i>	Changed plant architecture	Increased branching phenotypes with more siliques in order to increased yield.	https://doi.org/10.1111/pbi.13228
Yield	<i>BnD14 (strigolactone receptor BnD14)</i>	Changed plant architecture	Shoot architectural changes. Increase of total flowers.	https://doi.org/10.1111/pbi.13513
Yield	<i>BnaA03.BP (BRE-VIPEDICELLUS)</i>	Changed plant architecture	Optimizing rapeseed plant architecture, semi-dwarf and compact architecture.	https://doi.org/10.1111/pbi.13703
Yield	<i>BnEOD1 (Enhancer of DA1)</i>	Changed plant architecture	Increased seed size and weight.	https://doi.org/10.21203/rs.3.rs-3204656/v1

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).

Field of application	Edited gene(s)	Trait category	Trait	Reference
Food quality. Industrial properties	<i>CsFAD2 (fatty acid desaturase 2)</i>	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	<i>CsFAD2 (fatty acid desaturase 2)</i>	Changed fatty acid composition	Increased oleic acid content (proportional decrease in linoleic and linolenic acid content). Reduced monounsaturated fatty acids.	https://doi.org/10.1111/pbi.12663
Industrial properties	<i>CsFAD2 (fatty acid desaturase 2)</i>	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	<i>CsCRUC (cruciferin C)</i>	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
Food quality	<i>FAE1 (fatty acid elongase 1)</i>	Changed fatty acid composition	Decreased erucic acid content, increased levels of omega-3 fatty acids such as ALA.	https://doi.org/10.1111/pbi.13876

Industrial properties	<i>CsDGAT1</i> or <i>CsPDAT1</i> (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	<i>FAE1</i> (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	<i>FLC</i> (flowering locus C), <i>SVP</i> (short vegetative phase), <i>LHP1</i> (like heterochromatin protein 1), <i>TFL1</i> (terminal flower 1) and <i>EFL3</i> (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	<i>CsGTR1</i> and <i>CsGTR2</i> (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 3. *Thlaspi arvense* NGT applications (yellow: 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	<i>FAD2</i> (fatty acid desaturase 2), <i>ROD1</i> (reduced oleate desaturation 1) and <i>FAE1</i> (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	<i>FAE1</i> (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

7. References

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