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Review

Camelina as a sustainable oilseed crop: Contributions of plant breeding and genetic engineering

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Camelina is an underutilized Brassicaceae oilseed plant with a considerable agronomic potential for biofuel and vegetable oil production in temperate regions. In contrast to most Brassicaceae, camelina is resistant to alternaria black spot and other diseases and pests. Sequencing of the camelina genome revealed an undifferentiated allohexaploid genome with a comparatively large number of genes and low percentage of repetitive DNA. As there is a close relationship between camelina and the genetic model plant *Arabidopsis*, this review aims at exploring the potential of translating basic *Arabidopsis* results into a camelina oilseed crop for food and non-food applications. Recently, *Arabidopsis* genes for drought resistance or increased photosynthesis and overall productivity have successfully been expressed in camelina. In addition, gene constructs affecting lipid metabolism pathways have been engineered into camelina for synthesizing either long-chain polyunsaturated fatty acids, hydroxy fatty acids or high-oleic oils in particular camelina strains, which is of great interest in human food, industrial or biofuel applications, respectively. These results confirm the potential of camelina to serve as a biotechnology platform in biorefinery applications thus justifying further investment in breeding and genetic research for combining agronomic potential, unique oil quality features and biosafety into an agricultural production system.

Keywords: Camelina · Genetic engineering · Genomics · Plant breeding

1 Introduction

Camelina (*Camelina sativa* [L.] Crtz.) is an oilseed crop of the Brassicaceae family (Fig. 1) adapted to temperate growing regions. Camelina is of East European/West Asian origin and has been utilized since the late Neolithic Era when it was domesticated in South-East Europe. During the Iron Age, cultivation of camelina was confirmed from West Asia throughout the European continent and northward to Scandinavia [1, 2]. In the course of time, camelina cultivation was continued on a lower level due to competition from different other crops (see [3] for more details of cultivation history).

Since the 1980s, the interest in camelina has been revived due to an increased need for both food and biofuel oils, and pilot-scale productions have been established in European countries as well as in North America. Moreover, camelina has been characterized as a low-input oilseed crop [4], and it also reveals remarkable resistance against common and wide-spread Brassicaceae diseases and pests (e.g. blackleg disease [5], flea beetle [6]). Therefore, camelina-derived biofuels have been considered as sustainable jet fuel or diesel alternatives reducing greenhouse gas emissions by 75–80% based on life cycle assessment as compared to petroleum-based products [7]. In particular market situations, camelina might also be

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Abbreviations: **AFLP**, amplified fragment length polymorphism; **C10:0**, capric acid; **C12:0**, lauric acid; **C16:0**, palmitic acid; **C18:0**, stearic acid; **C18:1**, oleic acid; **C18:2**, linoleic acid; **C18:3**, linolenic acid; **C20:1**, eicosenoic acid; **C20:1-OH**, lesquerolic acid; **C22:1**, erucic acid; **DHA**, docosahexaenoic acid; **EMS**, ethyl methanesulfonate; **EPA**, eicosapentaenoic acid; **FAD2**, fatty acid desaturase 2; **FAE1**, fatty acid elongase 1; **GTP**, guanosine-5'-triphosphate; **ITS**, internal transcribed spacer region; **QTL**, quantitative trait locus; **RAPD**, random amplified polymorphic DNA; **RNAi**, RNA interference; **SSR**, simple-sequence repeat

Figure 1. Camelina biology from flowering to seed harvest. (**A**) Flowering stage, (**B**) stem and pods infested by downy mildew (*Hyaloperonospora camelinae*), (**C**) pods at full maturity, and (**D**) camelina seeds. Scale bar: 1 mm (photos by C. Eynck and J. Vollmann).

economically profitable in on-farm biofuel production [8]. Apart from biofuel and other non-food applications, camelina oil is opening up another interesting perspective as a vegetable oil for food uses: camelina oil is a rare source of comparatively high amounts of alpha-linolenic acid [9] which is an essential omega-3 fatty acid with a number of health benefits [10]. As the levels of linolenic acid have been reduced in soybean and canola oils for minimizing the health risks associated with the formation of trans fatty acids during processing and for better shelf life, camelina oil might alternatively be utilized for supplying linolenic acid in healthy diets [11].

Camelina is a self-pollinating crop species with low outcrossing rates ranging between 0.01–0.28% in rows 20 to 60 cm apart [12]. In the 1980s, Seehuber [13] investigated the genetic variation present in agronomic and seed quality characters of camelina germplasm accessions and initiated the first camelina breeding program focusing on the formation of base populations for further yield improvement [14]. Subsequently, breeding activities were carried out in several European countries as well as in the United States and in Canada [3]. Meanwhile, genetic engineering approaches have been successful in modifying the fatty acid composition of camelina oil for the targeted production of individual fatty acids of particular interest such as fish oil-like long chain omega-3 fatty acids [15]. Camelina is closely related to the genetic model species *Arabidopsis thaliana* (L.) Heynh., which suggests good transferability of genetic and genomic tools developed in *Arabidopsis* so far [16]. Thus, camelina apparently has the potential to function as a biotechnology platform for various food, health, biofuel and other non-food oilseed applications. Therefore, the objective of this review is to synthesize plant breeding and biotechnology results in order to highlight potentials and further research needs towards the wider utilization of camelina as a sustainable oilseed crop.

2 Agronomic performance and camelina oil quality

2.1 Grain yield and oil content of camelina

Competitiveness in agronomic performance as compared to established oil crops is a key factor for the economically successful establishment of a new crop. Higher seed yields would also reduce the environmental impact of camelina oil and biofuel production [17]. Camelina has been listed among the most promising new crops for oil production in temperate regions due to its wide adaptability, low input requirement, short crop cycle and other advantages [18]. Although its modern breeding history is rather short, camelina experiments have revealed an acceptable yield performance and other favorable agronomic features as compared to other new crops which might be due to camelina's long history of adaptation. A summary of published research on variation of camelina seed yield and oil content in European and North American locations is presented in Table 1. Grain yields of over 3000 kg/ha have been achieved with genebank accessions, newly developed cultivars and breeding lines [14, 19, 20] in favorable environments, whereas low yields might indicate a weak crop establishment due to drought stress, non-optimum sowing time or other negative environmental influences. Considerable genetic differences in yield stability between genotypes across a larger number of experiments have been reported [19] which suggests that breeding either for adaptation to specific environments or for broader stability could improve yield performance. Similar to grain yield, a considerable variation has also been found in camelina oil content ranging from 30 to 49% (Table 1). For both grain yield and oil content, significant genetic variation as well as genotype by environment interaction have been described in particular experiments suggesting that selection for improved yield or oil content would be efficient. As reported for other oilseeds, significantly positive correlations between grain yield and oil content have been described [21, 22]. In contrast, Guy et al. [19] reported negative correlations between the two characters across a set of camelina genotypes in particular environments which underlines the need for monitoring of the grain yield/oil content relationship of individual populations in breeding for oil yield.

2.2 Fatty acid composition of camelina oil

In most of the established oil crops, a few major fatty acids such as palmitic (C16:0), oleic (C18:1) or linoleic acid (C18:2) are predominant. In a few novel oilseeds such as cuphea (*Cuphea* spp.), medium-chain fatty acids,

Table 1. Ranges of variation in camelina seed yield and oil content in experimental research from locations in Europe and North America

Location	Yield range (kg/ha)	Oil content range (%)	Major source of variation	Reference
Germany	1100–2650	32.0–49.0	Breeding lines	[21]
Austria	1574–2248	40.5–46.7	Breeding lines, seed size	[22]
Ireland	1630–3200	43.1–44.7	Sowing date, N rates	[93]
Denmark	1270–2360	40.4–46.7	Spring/fall sowing	[25]
Germany	1290–3230	34.3–42.4	Breeding lines	[14]
Germany	500–2620	32.1–42.3	Genebank accessions	[13]
West Canada	1000–3000	37.0–46.3	N fertilizer rates, environments	[32]
Minnesota, USA	800–1900	37.7–41.0	Cultivars, sowing date	[94]
Pacific northwest USA	127–3302	29.6–36.8	Cultivars, spring/fall planting	[19]
East Canada	1400–2050	35.5–37.8	N, S fertilization	[95]
Nebraska, USA	556–1456	29.8–34.3	Sowing date	[26]
East Canada	426–2568	35.5–40.1	Cultivars, N rate	[96]
West Canada	962–3320	35.8–43.2	Genebank accessions, environments	[20]
Minnesota, USA	1007–1218	34.3–37.5	Genebank accessions	[4]

i.e. capric (C10:0) or lauric acid (C12:0) are synthesized, whereas hydroxy fatty acids such as lesquerolic acid (C20:1-OH) can be found in lesquerella (*Physaria* spp.). In addition, in most Brassicaceae oilseeds such as non-canola-quality rapeseed cultivars or crambe (*Crambe abyssinica* Hochst. Ex. R.E. Fries) erucic acid (C22:1) is synthesized. The interest in such unusual fatty acids is due to their unique physicochemical properties and a wider range of oleochemical reactions for various different applications as compared to common vegetable oils [23]. Camelina seed oil is unique in two respects: (i) the polyunsaturated alpha-linolenic acid (C18:3) is the major fatty acid; and (ii) the concentration of erucic acid (C22:1) is comparatively low for a Brassicaceae species whilst eicosenoic acid (C20:1) is synthesized instead as a long-chain fatty acid (Table 2). The variation in camelina fatty acid concentration from different research is summarized in Table 2. Linolenic acid is commonly found between 30 and 43% depending on genotype, agronomic treatment or environmental conditions [e.g. 20, 22, 24]. Linolenic acid concentration was higher in a winter-sown cultivar as compared to a spring-sown one [25], after earlier planting [26], at a higher N-fertilizer rate [27], and in seeds of larger 1000-seed weight [22]. As described for other oilseeds, the linolenic acid content is apparently lower in warmer climates such as Spain [28] than in cooler ones such as northern Germany [13]. Linseed (*Linum usitatissimum* L.) oil is the only vegetable oil higher in linolenic acid (range: 50–70% C18:3) than camelina [23]. Erucic acid content of camelina oil is below 3% in most cases, whereas eicosenoic acid levels are in the range of 11–19%, and saturated fatty acids are commonly present in concentrations below 10%. While many of the experiments listed in Table 2 reported genetic differences in particular fatty acid concentrations, these differences appear rather small and are of a quantitative nature only. A slightly larger variation in fatty acid composition has been described in mutant populations [29, 30]. Similar to other oil crops, particular mutant genotypes could be utilized in crosses within selection programs to modify the concentration of

Table 2. Variation in concentration of major fatty acids in camelina seed oil as reported from different field experiments

Concentration range (%) of major fatty acids							Comment	Reference
C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	C22:1		
6.0–7.6	2.3–3.5	14.3–18.9	18.6–26.3	25.1–32.4	12.4–15.3	2.2–3.4	Genebank accessions	[28]
5.1–5.4	2.4–2.5	12.5–13.9	15.3–16.5	36.4–38.6	14.8–15.1	2.6–3.0	N rates, seasons	[27]
5.5–6.3	2.2–3.0	13.3–18.1	16.1–18.6	33.3–37.7	11.2–12.9	2.2–3.5	Environments	[97]
5.8–6.0	2.4–2.5	14.9–16.2	18.9–21.1	32.0–37.1	11.8–12.8	2.5–3.1	Sowing date	[26]
5.2–6.8	2.3–3.2	14.5–18.7	14.7–20.4	29.2–35.4	14.4–17.6	2.4–4.0	Genotypes	[22]
–	–	11.2–16.5	14.3–19.3	34.1–41.2	12.3–16.8	1.9–3.8	Genebank accessions	[20]
5.3–5.6	2.3–2.7	14.2–16.9	13.5–16.5	34.9–39.7	15.1–15.8	2.6–3.0	Environments	[24]
3.7–9.0	1.3–9.6	14.2–38.7	13.4–26.5	19.1–37.7	5.9–17.9	0.8–2.1	Mutant population	[29]
4.1–8.5	1.7–3.7	8.1–20.9	11.0–27.6	22.5–40.8	11.8–18.1	1.8–5.7	Selected mutants	[30]
5.3–5.6	2.2–2.7	14.7–16.5	12.9–16.3	33.7–40.3	14.7–15.1	2.5–3.0	Fall/spring cultivars	[25]
5.2–7.1	1.0–1.8	13.2–17.7	14.7–20.4	36.9–43.1	13.5–18.9	2.1–3.3	Genebank accessions	[13]

individual fatty acids: linolenic acid content could be increased for technical applications, while for camelina biodiesel applications it should be lowered because of a number of drawbacks associated with the chemical properties of linolenic acid methyl esters [31]. Moreover, in food applications the level of erucic acid might be reduced for food safety reasons, as higher intake of erucic acid was associated with cardiac lipidosis in animal experiments [27].

In summary, the unique fatty acid composition of camelina oil points to a considerable fatty acid desaturase (i.e. biosynthesis of polyunsaturated fatty acids) and fatty acid elongase potential (i.e. carbon chains longer than C18), both of which could be enhanced in genetic engineering approaches for the production of tailored fatty acid profiles (see Section 4.4).

In addition to the oil component, camelina bears a seed protein (content 20–29%, [22, 32]) which appears as a valuable by-product, but will not be covered in this review.

Thus, based on agronomic and oil quality characteristics described above, camelina can be grown as a winter- or spring-sown crop in semi-arid, temperate or even short-season environments such as Canada and the northern United States [3, 4, 19, 20] as well as in Central [21, 22] and Northern [25] Europe and Asia for biofuel and food oil production. Camelina is comparable to other oil crops in its reaction to inputs such as nitrogen fertilizers [32], while its present yield level (Table 1) makes it competitive to other oil crops [3] and for on-farm biofuel production [8].

3 Disease and pest resistance of camelina

As a member of the Brassicaceae family, camelina is affected by similar pests and diseases that pose a threat to other cruciferous crops. For a comprehensive review of the most important diseases of *C. sativa*, the reader is referred to Séguin-Swartz et al. [33].

Interestingly, two fungal diseases of great importance for Brassica crop production world-wide are completely missing from the disease reports: alternaria black spot caused by *Alternaria brassicae* (Berk.) Sacc. and *A. brassicicola* (Schw.) Wiltsh., and blackleg or stem canker, caused by *Leptosphaeria maculans* (Desm.) Ces. & de Not. (anamorph *Phoma lingam* Tode ex Fr.).

While no source of resistance is known within the *Brassica* species [34], resistance of camelina to *Alternaria* spp. was reported by numerous authors [35–37]. The production of two indole phytoalexins, camalexin and methoxy-camalexin, has been implicated in the containment of *Alternaria* spp. in *C. sativa* [38] and *A. thaliana* [39–41]. Camalexin is structurally similar to the synthetic systemic fungicide thiabendazole and is not found in any other cultivated crucifer species [38, 42]. It exerts direct antimicrobial activity to *Alternaria* spp. in vitro [39, 43, 44]; however, it cannot be excluded that camalexin also contributes to *Alternaria* resistance indirectly through inhibition of the production of the host-specific *Alternaria* toxin destruxin B [45]. Further, it was shown that *C. sativa* is able to detoxify destruxin B [46].

The economic importance of black spot disease for Brassica crop production world-wide and the complete resistance of *C. sativa* have made the latter an obvious choice for intraspecific hybridizations with other Brassicas. But strong cross-incompatibility [47] and the differences in ploidy between *C. sativa* and cultivated Brassica species render the transfer of *Alternaria* resistance difficult [48]. Attempts to introduce camelina-derived *Alternaria* resistance to *B. carinata* [49] and *B. oleracea* [50, 51] through protoplast fusions were hampered by insufficient rhizogenesis [49, 50] and sterility [51] of the hybrids. Moreover, transfer of resistance based on the elicitation of camalexin may not be as straightforward as one might think: the detoxification of the phytoalexin brassinin in cultures of *L. maculans* increased substantially in the presence of camalexin [52]. This suggests that introducing the camalexin pathway into plants that produce brassinin, such as *B. napus*, despite increasing *Alternaria* resistance, might at the same time increase susceptibility to *L. maculans*. In conclusion, the consequences of expressing a phytoalexin in a new plant species need to be investigated carefully.

Leptosphaeria maculans, causal agent of blackleg or stem canker, is the most ubiquitous pathogen of Brassica crops [53, 54]. It is generally accepted that camelina is virtually immune to this pathogen [5, 33], making it an attractive source of resistance for the improvement of susceptible Brassica crops; despite this, very little research has been done to elucidate the mechanisms underlying blackleg resistance in camelina. Again, phytoalexin production may be one of the contributing factors [45].

In addition to complete resistance to black spot and black leg disease, camelina exhibits variation for resistance to damping-off and root-rot (*Rhizoctonia solani* Kühn) [33, 55], sclerotinia stem rot (*Sclerotinia sclerotiorum* [Lib.] de Bary) [56] and downy mildew (*Hyaloperonospora camelinae* [Gäum.] Göker, Voglmayr, Riethmüller, Weiß et Oberwinkler, comb. nov.) [57], indicating that the development of resistant cultivars is feasible. Camelina is, however, susceptible to clubroot (*Plasmodiophora brassicae* Woronin), white rust (*Albugo candida* [Pers.] [O.] Kunze) and aster yellows (*Candidatus* Phytoplasma asteris Lee et al.), diseases that may pose a threat with increasing camelina production unless resistant cultivars or effective management practices are developed [33].

Flea beetles (*Phyllotreta cruciferae* Goeze and *P. striolata* F. [Coleoptera: Chrysomelidae]) are insect pests of great importance for canola (*B. napus*, *B. rapa*) and mustard (*B. juncea*) production in the northern Great Plains of the USA and Canada [58]. While flea beetles can also be

found on camelina plants in the field, they do not feed on this species [59, 60]. Rather, feeding on camelina is not initiated unless the beetles are confined on plants for several days [59]. However, once initiated, feeding tended to continue, indicating that resistance of *C. sativa* to *Phyllotreta* may be a result of the absence of feeding cues rather than the existence of feeding deterrents. This conclusion is corroborated by findings of Henderson et al. [6].

Finding and acceptance of host plants by insects are processes that are determined by their perception of the chemical composition of the plant's surface and volatile compounds [6]. Glucosinolates are one group of compounds found within and on the surface of cruciferous plants that can act as deterrents or attractants of insect pests and that have been shown to play a part in the recognition of host plants by insects such as the flea beetle [61]. *C. sativa* has comparatively low levels of glucosinolates [62, 63] which may not present sufficient chemosensory stimuli to initiate feeding. Secondly, *C. sativa* possesses three glucosinolates [62] that are not found in any of the cultivated Brassica species. Thus, next to quantitative variation qualitative variation in glucosinolate content may also explain host selection [58, 64].

The camelina/flea beetle system is the best-studied interaction between this plant species and insects, and only isolated reports exist that mention the resistance of camelina to other insect pests. Thus, *C. sativa* was shown to have a deterring effect on diamondback moth (*Plutella xylostella*) [65] and mustard sawfly (*Athalia proxima*) [66]. Similarly, female cabbage root flies (*Delia brassicae* syn. *D. radicum*) did not oviposit on *C. sativa* plants [67]. Camelina also appeared to be resistant to the cabbage seedpod weevil (*Ceutorhynchus obstrictus*) [68]. Although the test results of the latter study were inconclusive due to poor plant development, they are corroborated by the finding of a dramatic reduction in leaf area consumption by *Ceutorhynchus pallidactylus* (cabbage stem weevil) in *Camelina alyssum* compared to that in *B. napus* [69]. The unique characteristics of resistance to diverse insect pests in camelina may help in the manipulation of feeding stimuli in crops like *B. napus* and consequently reduce the impact of insect pests on Brassica crops [70].

4 Breeding approaches for camelina

4.1 Evolutionary characteristic: Allohexaploid genome status

The cultivated *Camelina sativa* has a chromosome number of 2n = 40 in most counts, which was also reported for some other species of the genus *Camelina* [71]. Several important Brassicaceae crops with a similarly high chromosome number such as oilseed rape (2n = 38) are of polyploid origin. In oilseed rape (*Brassica napus* L.), this evolutionary status is extensively utilized in resynthesis breeding, by which the wide gene pools of the two ancestor species turnip rape (*Brassica rapa* L.) and cabbage (*Brassica oleracea* L.) contribute to increasing genetic variation in rapeseed [72]. The genomic status of *B. napus* or other Brassicaceae species and the high chromosome number of camelina strongly suggest a polyploid nature of camelina as well.

Hutcheon et al. [16] were the first to present clear evidence of a hexaploid genome status of camelina by analyzing two genes belonging to the fatty acid biosynthesis pathway. They characterized a fatty acid desaturase (*FAD2*) and a fatty acid elongase (*FAE1*) gene in different camelina species. Using Southern blot analysis, they found three copies of both *FAD2* and *FAE1* in *C. sativa*, while only one copy of each is present in *Arabidopsis*. Sequencing of the *FAD2* and *FAE1* genes revealed high levels of identity between the three gene copies and between each of them and their putative *Arabidopsis* ortholog gene both on the nucleotide sequence and the encoding protein level; furthermore, expression studies using real time quantitative PCR confirmed that all three copies of both genes could be functional and expressed in developing seeds rather than being silenced. Finally, Hutcheon et al. [16] studied the duplication history of *FAD2* and *FAE1* gene copies in *C. sativa*, *C. microcarpa*, *C. rumelica*, *C. laxa* and *C. hispida*; for both genes, two copies were most similar between *C. sativa* and *C. microcarpa*, while for the third copy *C. sativa* and *C. microcarpa* were differently associated with other species. These results, chromosome counts and genome size data all suggest a hexaploid genome status of *C. sativa*, *C. microcarpa* and probably *C. alyssum*, a tetraploid status of *C. rumelica* and a diploid one for *C. laxa* and *C. hispida*. Similarly, Kwak et al. [73] were sequencing glycine-rich RNA-binding proteins of camelina which play a role in stress tolerance; three highly similar cDNAs were found supporting the allohexaploid origin of the species. Moreover, hybridization experiments between *C. sativa* and *C. alyssum* revealed a high level of interfertility, a lower one for *C. sativa* and *C. microcarpa* crosses, and the lowest one with mainly sterile F_1 plants between *C. sativa* and *C. rumelica* [71]; this corroborates the findings regarding the ploidy levels suggested above.

Recently, Kagale et al. [74] used a next-generation sequencing approach for characterizing the *C. sativa* genome in more detail: based on the relatively large genome size of 785 Mb over 89 400 genes were predicted, which is about three times the gene number of *Arabidopsis* species suggesting a whole-genome triplication of a diploid ancestor species. Remarkably, repetitive elements were present at 28% which is similar to *Arabidopsis* species and much lower compared to other crop plants of similar genome size. High levels of synteny between *C. sativa* chromosomes and chromosome collinearity between *C. sativa* and both *A. thaliana* and *A. lyrata* were found with each *Arabidopsis* chromosome being repre-

sented in three independent chromosomes in *C. sativa*; based on these data, Kagale et al. [74] assume that the genome structure of *C. sativa* (1n = 20) consists of one sub-genome with six chromosomes and two sub-genomes with seven chromosomes each. Moreover, 80% of 736 genes involved in various steps of the lipid biosynthesis pathway were present in three copies confirming earlier findings [16], while only 15% of the triplicate lipid metabolism genes revealed functional diversification [74]. As loss of function or gene function diversification are commonly observed in diploidized paleopolyploids, the high number of protein-coding genes, a high level of gene expression and low differentiation between genomes within *C. sativa* suggest that the hybridization of the ancestor sub-genomes has occurred relatively recently compared to other polyploid crop species.

The evolutionary status of *C. sativa* has a number of consequences for camelina breeding: an allohexaploid genome with a high number of triplicately expressed and non-silenced genes suggests a complex inheritance and various regulatory interactions between different loci of most traits, which makes traditional selection procedures more complicated in segregating populations. This also coincides with the observation that single-gene based Mendelian segregation has not been described in segregating camelina populations on the phenotypic level. On the other hand, the close relationship between *C. sativa*, *C. alyssum* and *C. microcarpa* and their hexaploid genome structure could be utilized for gene introgression and enhancement of genetic variation in camelina breeding. As an example, resistance to acetolactate synthase inhibition based herbicides could be introgressed into camelina from *C. microcarpa*, as a resistant biotype has been found [75]. This might broaden the weed control options in camelina production. Variation in disease resistance, seed composition or other traits of interest is largely unknown at present for wild and weedy *Camelina* species [76], but useful variation might be introgressed from related hexaploid species. Moreover, sterile camelina hybrids have been described in interspecific hybridizations [71]; similar to oilseed rape, nuclear genetic or cytoplasmic male sterility systems could be developed from such populations for utilizing heterosis in future hybrid camelina breeding.

4.2 Breeding procedures in camelina

Flowers of camelina are small and rarely visited by insects. As mentioned above, camelina outcrossing rates are very low and similar to the level of soybean, and outcrossing occurs over short distances only [12]. Flower visits by honey bees, wild bees and other insects were observed, but seed set parameters were not significantly different between open pollination and self-pollination [77]. Thus, camelina can be considered as a predominantly autogamous species.

Based on the flowering biology of camelina, pure line breeding appears as the method of choice for cultivar development. Following artificial hybridization, segregating generations can be handled using pedigree or bulk breeding procedures. For rapid generation advance to reach homozygosity, the single-seed descent method has been utilized in camelina breeding [14] as well as in mapping experiments [21]; as spring-types of camelina need no vernalization, three to four generations of a segregating population could be grown per year in a greenhouse similar to rapid cycling brassicas.

Apart from hybridization, mutation induction approaches have also been used to generate novel genetic variation in camelina. Seed treatment with EMS or seed irradiation with gamma rays from a ^{60}Co source have been effective to modify fatty acid composition [29, 30]. EMS seed treatment was also successfully used to reduce the sensitivity of camelina to acetolactate synthase inhibitor herbicides conferring resistance to imazethapyr, sulfosulfuron and flucarbazone [78].

The production of doubled-haploids derived from anther cultures or isolated microspores is another important technique for accelerating breeding programs. In a protocol for microspore embryogenesis in camelina [79], the best microspore embryogenesis rates were reported when small flower buds of <1 mm bud size were used which resembles a late-uninucleate stage of microspore development; about 70% of the regenerated plantlets had a spontaneous chromosome doubling followed by a normal seed set, but the efficiency of the doubled-haploid production system is low needing further improvement.

4.3 Genetic resources and genomics applications

Genebank accessions of camelina are maintained by several collecting institutions, but the number of accessions available is comparatively low in most collections reflecting the insignificant role of camelina as a field crop in the past. As an example, a total of 793 accessions of *C. sativa* are listed in EURISCO (http://eurisco.ecpgr.org), the European catalogue of plant germplasm collections as of June 2014. According to the EURISCO catalogue, most camelina accessions are held in Germany, Poland, the Czech Republic, Bulgaria and Austria, but many accessions appear to be duplicated in national inventories. The Plant Gene Resources of Canada database (http://pgrc3.agr.gc.ca/) is listing 137 *C. sativa* accessions, and the USDA National Plant Germplasm System (http://www.ars-grin.gov/npgs/) is holding 44 accessions according to taxonomic searches as of June 2014.

Seehuber [13] described phenotypic diversity of agronomic and seed quality characters of a set of camelina genebank accessions emphasizing a large variation and high heritability for time to flowering, plant height and 1000-seed weight with less variation for fatty acid con-

Table 3. Camelina genetic engineering and traits expressed in genetically transformed plants

Gene/Construct	Traits expressed	Reference
Discosoma sp. *DsRed* marker	Red seed fluorescence	[86]
Castor fatty acid hydroxylase	Hydroxy fatty acid synthesis	[86]
Acetolactate synthase marker, *REVOLUTA* gene constructs	Chlorsulfuron herbicide marker, marker-free constructs for PCR-based selection	[87]
Beta-glucuronidase and *hpt* genes	Gus reporter gene, hygromycin resistance selection marker	[98]
Microalgal and yeast fatty acid elongase and desaturase genes	Long-chain omega-3 fatty acid production (EPA[a)], DHA[b)]	[99, 100]
Five- and seven-gene cassettes with fatty acid desaturase and elongase genes	High-level omega-3 fatty acid accumulation (EPA[a], DHA[b])	[15]
Suppression or overexpression of different lipid synthesis genes	High-palmitic, high-oleic, high-linoleic acid oil	[88]
RNAi suppression constructs targeted to storage protein and oil biosynthesis expression	Seed storage protein and fatty acid content variations	[84]
Bar and *nptII* selection marker genes	Basta and kanamycin resistance	[84]
Arabidopsis *MYB96*	Drought resistance through cuticular wax	[90]
Arabidopsis *AGG3* signalling protein gene	Rapid plant development, larger seed size and seed number, drought tolerance	[89]

a) eicosapentaenoic acid (C20:5-omega-3)
b) docosahexaenoic acid (C22:6-omega-3)

centrations. Vollmann et al. [80] were grouping 130 accessions according to seed size, seed oil and protein content into four groups; for a subset of genotypes, they identified 15 polymorphic RAPD markers, but the correspondence between genetic and phenotypic estimates of diversity was rather weak. Manca et al. [81] developed SSR markers from a genomic DNA library of camelina. While most of their SSR primer pairs amplified multiple fragments, a number of informative polymorphic primers were extracted for clustering 40 genebank accessions into distinct groups.

For discriminating between *Camelina* species and for detection of interspecific hybrids, Séguin-Swartz et al. [71] used sequence analysis of the ITS region as a marker system. In a QTL-mapping approach, Gehringer et al. [21] utilized AFLP markers for constructing a genetic linkage map; analyzing a bi-parental population, they identified QTL regions for seed yield, 1000-seed weight, plant height, oil content and individual fatty acid concentrations demonstrating the feasibility of marker-assisted selection. However, due to the current progress in sequencing and bioinformatics, future camelina breeding might greatly benefit from high-density genetic maps and genomic information derived from recent genome and transcriptome sequencing approaches [74, 82–84]. For instance, transcriptome analysis of developing camelina seeds revealed various genes related to seed storage protein and lipid biosynthesis metabolism; this could be utilized in gene suppression or transgene expression approaches for targeting camelina seed quality towards specific protein or lipid properties [84].

4.4 Camelina as a genetic engineering platform

Camelina has been attributed as a platform crop for bio-industrial oil production [85]. Apart from acceptable yield and processing potential, fully developed tools are available for breeding and trait improvement through genetic engineering due to a high sequence identity of genes between *Arabidopsis* and camelina. Genetic transformation protocols based on *Agrobacterium*-mediated floral dip transformation have been developed [86, 87] which has led to a number of successful transformation reports over the last years (Table 3). Apart from selection markers, genes for altering fatty acid composition, improved seed and oil yield or drought resistance have been expressed in camelina.

RNAi suppression of fatty acid desaturase (*FAD2*) and elongase (*FAE1*) genes resulted in reduced concentrations of linoleic, linolenic and eicosaenoic acids, whereas oleic acid was accumulated at a level of 66% [88]; high-oleic strains of camelina are of huge interest for biofuel production and other technical uses, as high-linolenic camelina biodiesel is bearing disadvantages such as reduced oxidative stability and low cetane number [31]. Compared to natural genetic or environmental variation in fatty acid composition (Table 2), suppression lines have a huge impact in tailoring oil composition traits. Other

strains of camelina engineered with fatty acid desaturase and elongase gene cassettes produced up to 31% of eicosapentaenoic acid (EPA, C20:5-omega-3) or up to 14% docosahexaenoic acid (DHA, C22:6-omega-3) [15], both of which have unique health and dietary importance and are provided primarily by marine fish oils at present. Camelina lines overexpressing the *AGG3* signalling GTP-binding protein showed increased photosynthesis efficiency and were associated with higher fruit number, 1000-seed weight, seed yield and oil content compared to controls [89]; this demonstrates the feasibility to engineer quantitative agronomic traits through the regulation of plant organ size. Another important application with particular significance for biofuel production on marginal land is an improved tolerance to drought which was achieved by overexpressing an *Arabidopsis* gene for cuticular wax biosynthesis on camelina leaf surfaces [90].

The examples given illustrate the potential of camelina in translating basic genetic and biotechnology results into different biorefinery implementations for agricultural production of biofuels and other vegetable oils. In addition, genetically engineered camelina has a low-risk biosafety status due to its low outcrossing rate [12, 77], no intercrossing with common Brassicaceae species [91] and weak weed potential [92] compared to other Brassicaceae crops, thus making it particularly suitable as a platform crop.

5 Further research needs for camelina

Although camelina has a significant agronomic potential and a unique fatty acid composition, grain yield and oil content should be major breeding targets for making the crop more competitive to other well-established oilseeds. Due to phytoalexin production, camelina is resistant to major Brassicaceae diseases, in which respect it could also serve as a resistance model for other important species. As the genome of camelina is allohexaploid with a large number of non-silenced and interacting gene loci, genomic selection might accelerate the breeding progress over phenotypic selection because it could handle complex epistatic effects more efficiently. The sequence collinearity to *Arabidopsis* can be utilized in various genomic and transgenic approaches such as metabolic engineering of lipid biosynthesis. More research is also needed for maximizing the concentration of target fatty acids in transgenic camelina strains while minimizing undesirable metabolomic by-products, for engineering the seed protein, and on biosafety issues.

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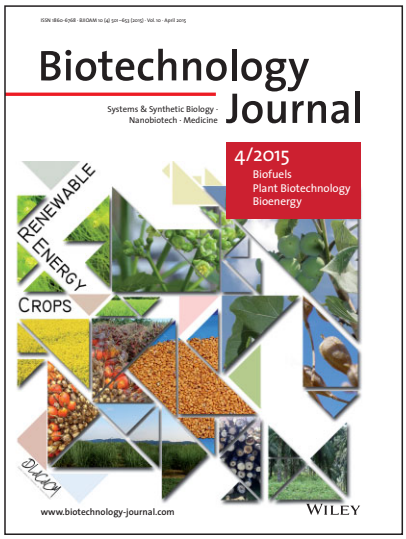

Cover illustration

Special issue: Renewable Energy Crops, edited by Margit Laimer, Fatemeh Maghuly, Johann Vollmann and Nicolas Carels. Given the growing importance of environmental advantages of biofuel plants reducing greenhouse gas emissions as compared to fossil fuel consumption, second and third generation biofuels today are produced from both annual and perennial non-food biofuel crops, e.g. switchgrass, poplar and *Miscanthus*, or from new and non-food oil crops, like *Jatropha*, *Camelina* and oil palm. To achieve an economic production of biofuels for our future need of sustainable energy, genetic improvement of the plant material must be obtained by a range of different biotechnologies.

Biotechnology Journal – list of articles published in the April 2015 issue.

Research Article

Production of curcuminoids from tyrosine by a metabolically engineered *Escherichia coli* using caffeic acid as an intermediate

Joana L. Rodrigues, Rafael G. Araújo, Kristala L. J. Prather, Leon D. Kluskens, and Ligia R. Rodrigues

http://dx.doi.org/10.1002/biot.201400637

Research Article

Buffer-free therapeutic antibody preparations provide a viable alternative to conventionally buffered solutions: From protein buffer capacity prediction to bioprocess applications

Sven Bahrenburg, Anne R. Karow, Patrick Garidel

http://dx.doi.org/10.1002/biot.201400531

Research Article

Over-expression of *ICE2* stabilizes cytochrome P450 reductase in *Saccharomyces cerevisiae* and *Pichia pastoris*

Anita Emmerstorfer, Miriam Wimmer-Teubenbacher, Tamara Wriessnegger, Erich Leitner, Monika Müller, Iwona Kaluzna, Martin Schürmann, Daniel Mink, Günther Zellnig, Helmut Schwab and Harald Pichler

http://dx.doi.org/10.1002/biot.201400780

Research Article

Indole generates quiescent and metabolically active *Escherichia coli* cultures

Chih-Chin Chen, Rupali Walia, Krishna J. Mukherjee, Subhashree Mahalik and David K. Summers

http://dx.doi.org/10.1002/biot.201400381

Biotech Method

Optimized Sleeping Beauty transposons rapidly generate stable transgenic cell lines

Eric Kowarz, Denise Löscher, Rolf Marschalek

http://dx.doi.org/10.1002/biot.201400821