Chapter 8
Camelina: An Emerging Oilseed Platform for Advanced Biofuels and Bio-Based Materials

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Abstract *Camelina* (*Camelina sativa* (L.) Crantz) is a Brassicaceae oilseed crop with valuable agronomic and biotechnological attributes that make it an attractive renewable feedstock for biofuels and bio-based materials. *Camelina* seeds contain 30–40 % oil and can achieve oil yields per hectare that surpass established oilseed crops such as soybean. *Camelina* is also productive under conditions of limited rainfall and low soil fertility. As a short season, frost tolerant oilseed, *Camelina* is amenable to double cropping systems and fallow year production. Simple, non-labor intensive *Agrobacterium*-based transformation methods have recently been described for *Camelina* that can be used in combination with breeding to rapidly improve seed quality and agronomic traits to advance *Camelina* as a production platform for biofuels and industrial feedstocks in geographical regions such as the North American Great Plains that currently have little oilseed production for edible vegetable oils.

Keywords *Camelina* • Oilseed crop • Biofuels • Bio-based materials • Biodiesel • Fatty acids

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8.1 Introduction

Camelina sativa or *Camelina*, known also as false flax or gold of pleasure, is an annual oilseed species of the Brassicaceae family. *Camelina* has received growing interest as biofuel crop for the production of vegetable oils for biodiesel and aviation fuel because of its productivity in geographic regions that are not currently used for large-scale oilseed production and the possibility of growing *Camelina* in farming systems (e.g., double cropping) that do not compete with crops for food production. *Camelina* currently has a number of seed quality and agronomic issues that limit its wider use for biofuel and possibly other industrial feedstock production. These limitations can now be addressed by advances in biotechnology and the increasing availability of genomic resources to facilitate breeding.

Camelina is native to Eastern Europe and Central Asia (Putnam et al. 1993). It has been cultivated in Europe since the Bronze age, as early as 1500–400 BC (Bouby 1998; Zubr 2010). It was introduced to North America from Europe most likely as a weed along with flax and is well adapted to Southern Canada, Northern Great Plains and Pacific Northwest of the U.S. *Camelina* grows 0.3–1 m high, stems are smooth and branched with arrow shaped leaves that are 5–8 cm long (Fig. 8.1). Flowers are small and consist of four pale or greenish yellow petals and give rise to pear-shaped pods (5–10 mm) containing as many as 16 seeds. Seeds are small

Fig. 8.1 *Camelina sativa* (*Camelina*). **a** Flowering *Camelina* plant. **b** Mature pods. **c** Comparison of *Arabidopsis* (*Arabidopsis thaliana*), *Camelina*, and rapeseed (*Brassica napus*) seed sizes

yellow–brown or brown, oblong, rough with rigid surface (Putnam et al. 1993). Seeds contain 30–40 % oil on dry weight basis of which 64 % is polyunsaturated, including 30–45 % of the omega-3 fatty acid α-linolenic acid (Vollmann et al. 1996).

Historically *Camelina* has been valued for the vegetable oil extracted from its seeds (Moloney et al. 1998; Jaskiewicz and Matyka 2003; Flachowsky et al. 2011). The ancient Romans used *Camelina* oil as massage oil, lamp fuel, as well as cooking oil. In modern times, *Camelina* oil has gained a niche market, particularly in Europe, for its nutraceutical value because of its high content of α-linolenic acid (Hurtaud and Peyraud 2007; Ehrensing and Guy 2008). *Camelina* meal obtained after oil extraction, contains 10–11 % fiber, 10–14 % oil, and about 40 % protein making it a valuable product for use (Korsrud et al. 1978; Onyilagha et al. 2012) in cattle, chicken and hog feed (Ehrensing and Guy 2008). However, due to the presence of glucosinolates (19–23 μmol/g) in *Camelina* meal regulations require limited daily use to avoid negative impacts on livestock productivity although the amount is not higher than that of the widely used canola meal (Matthäus and Angelini 2005; Moser 2010). U.S. Food and Drug Administration regulations currently restrict the use of *Camelina* meal to ≤10 % of the total diet of beef cattle, broiler chickens, and laying hens and ≤2 % of the diet of growing swine for United States livestock production.

8.2 Agronomic Properties

Camelina has recently gained renewed interest as a low input biofuel feedstock because of its minimal agronomic input requirements for productivity on marginal lands with limited fertility and water resources. *Camelina* requires low to moderate amounts of fertilizers and is productive with nitrogen and phosphorus levels of as low as 90–100 and 67 kg/ha, respectively (McVay and Lamb 2007; Ehrensing and Guy 2008; Sipalova et al. 2011; Solis et al. 2013). Field trials with cambic chernozem soil in Timisoara, Romania showed that only addition of the fertilizers nitrogen (100 kg/ha) and phosphorus (60 kg/ha) increased seed yield from 932 to 1,813 kg/ha and oil up to 584 kg/ha increasing the oil yield by 25 % when plants were sowed at 25 cm row distance which was found to be optimal (Imbrea et al. 2011). Similar doses of nitrogen fertilizer were recommended for *Camelina* growth to achieve optimal yield based on the results of the laboratory experiments (Sipalova et al. 2011).

Despite being a cool season crop, *Camelina* is well suited for dryland cropping systems in which soil moisture and rainfall can be maximized by planting in early months of spring depending on location. For example, as high as 1,912 kg/ha of grain and 506 kg/ha oil yield were achieved in dryland conditions near Havre, Montana USA, while grain yield of 3,250 kg/ha was obtained in Austria if water was not limiting throughout vegetation period (Vollmann et al. 1996; McVay and Lamb 2007; Ehrensing and Guy 2008). The 2 year trials in arid zones of Southwestern USA showed that *Camelina* can be a low water use crop. *Camelina* grown from January to May in Maricopa, Arizona yielded in over 1,500 kg/ha seed

with maximum seasonal water use of just 47–49 cm, furthermore the seed yield loss was not significant unless the soil water depletion before irrigation reached 70 % and higher (Hunsaker et al. 2011).

Camelina has traditionally been grown without pesticides and has allelopathic effects on weed species (Ehrensing and Guy 2008; Gesch and Cermak 2011). Herbicide research on *Camelina* is currently ongoing. Being a minor weed, *Camelina* is usually not a problem in other crops and does not have seed dormancy. Research showed that by planting *Camelina* seeds in winter or early spring, herbicide use can be avoided since *Camelina* seeds can germinate at low temperatures and seedlings are frost tolerant (Robinson 1987) and suppress many weed species, except perennial weeds, especially if seeded at high density (Ehrensing and Guy 2008; Gesch and Cermak 2011). *Camelina* was shown to be a viable winter crop for the Northern Corn Belt of the U.S., where seed yields as high as 1,317 kg/ha and oil yields as high as 420 g/kg were obtained from fall sown crop promoting good weed suppression (Gesch and Cermak 2011). Thus sowing does not require pre-emergence weed control, greatly reducing both production costs and environmental damage. Susceptibility of *Camelina* to herbicides inhibiting acetolactate synthase (ALS), that are commonly used for wheat cultivation in the Pacific Northwest of the U.S., has limited cultivation of *Camelina* as an oilseed crop in the area (Hanson et al. 2004; Pavlista et al. 2011).

Unlike other Brassicaceae crops such as rapeseed, *Camelina* does not require insecticide application. *Camelina* is resistant to crucifer insect pests due to large concentrations of the insect deterrent quercetin glycosides in its tissues (Onyilagha et al. 2012; Naranjo and Stefanek 2012). *Camelina* is also highly resistant to blackleg disease caused by the fungus *Leptosphaeria maculans*, which is a major pathognen of many Brassicaceae crops, such as canola. *Camelina* is susceptible to downy *Peronospora Camelinae* (downy mildew), *Alternaria brassicae* and the saprotrophic fungus *Rhizoctonia*, but none of these pathogens has been reported to cause major yield losses in *Camelina* (Robinson 1987; Salisbury et al. 1995; Ehrensing and Guy 2008).

Because it has a relatively short growth cycle (80–100 days) and frost tolerance, *Camelina* can be used in double cropping systems during cool periods of the year (Gesch and Archer 2012). Field trials showed that short season cultivars of soybean could be double cropped after winter *Camelina* in the upper U.S. Midwest (Gesch and Archer 2012). The net return from the *Camelina*-soybean double crop was higher than that of mono-cropped soybean in the period analyzed (Gesch and Archer 2012). *Camelina* can also be grown following wheat, barley, peas and lentils, but should not be planted following Brassicaceae crops to avoid increased risks by pests and diseases (Fleenor 2011). In addition, *Camelina* has been considered as a tertiary crop for rotations in northeastern Colorado where wheat is rotated with crops such as corn (Brandess 2012). In this region, *Camelina* could be planted in October after the first crop in year one and harvested before July of year two. This could then be followed by land recuperation period of July to mid-September when winter wheat could be planted and harvested in July of year three. This is followed by a fallow period until April or May of the year four when

the rotation restarts (Brandess 2012). This rotation would allow farmers to grow three crops versus two in three years (Brandess 2012). Since it is best adapted to cooler climates, *Camelina* could also be grown in winter in areas with mild winters (Gesch and Cermak 2011).

Although the oil content of seeds is lower than that of *Brassica napus* on dry weight basis, the oil yield per hectare can reach as high as that of *B. napus* if good agronomic management is practiced (Putnam et al. 1993; Imbrea et al. 2011). *Camelina* has a low seeding rate (as low as 3–5 kg/ha) compared to other agronomic crops, including canola to establish dense stands (McVay and Lamb 2007; Pilgeram et al. 2007). In addition, existing equipment that is used for harvesting and processing of other crops can be adapted to *Camelina* (Brandess 2012). These agricultural attributes of *Camelina* give it compelling properties and make it a favorable oilseed crop to be grown in agronomically demanding lands with low-inputs.

8.3 Genetic Improvement: Variety Selection, Breeding, Genomic Resources, Biotechnology

Limiting the full potential of *Camelina* as a biofuel oilseed crop is the need to improve a number of agronomic, yield, and oil quality traits. In contrast to other Brassicaceae oilseeds such as canola and rapeseed, *Camelina* has not undergone extensive breeding and only a relatively small number of cultivars are available for commercial production. From an agronomic production standpoint, improvement in traits such as heat tolerance, downy mildew resistance, water and nitrogen use efficiencies, and herbicide resistance are desirable. For biodiesel and industrial uses such as bio-based lubricants, enhancement in seed oil content from the current 30–40 % of seed weight to levels of 40–50 % of seed weight, as is currently found in elite rapeseed germplasm, is a major target for *Camelina* crop improvement. An additional target for biodiesel and bio-lubricants is the reduction of the high polyunsaturated fatty acid content of the seed oil [35–39 % linolenic acid (18:3) and 20–25 % linoleic acid (18:2)] and replacement with high content of the more oxidatively stable monounsaturated fatty acid oleic acid (18:1). Furthermore, reductions in seed glucosinolate levels would allow for increased use of *Camelina* meal for livestock production.

Varietal selection and screening of germplasm following mutagenesis are among the approaches used to date for *Camelina* crop improvement. Several *Camelina* varieties have been selected for higher oil content and improved fatty acid composition. For example, the cultivar Blaine Creek is richer in ω-3 fatty acids, while Suneson has 2–3 % higher oil content, and is rich in α-linolenic acid (Ehrensing and Guy 2008). Lines with resistance to acetolactate synthase (ALS)-targeting herbicides imazethapyr and sulfosulfuron and altered seed fatty acid composition, including increased oleic acid content, have also been identified screening of mutagenized populations (Vollamnn et al. 1997; Buchsenschutz-Nothdurft et al. 1998; Kang et al. 2011; Walsh et al. 2012).

Recent advances in *Camelina* genomics are also providing avenues for *Camelina* improvement through marker-assisted breeding. Molecular genetic maps have been assembled for *Camelina* using random amplified polymorphic DNA (RAPD) markers and amplified fragment length polymorphisms (Vollmann et al. 2005; Gehringer et al. 2006). These maps have been used to localize QTLs for agronomic characteristics such as seed yield, oil content, 1,000-seed weight, and plant height (Gehringer et al. 2006). More recent AFLP fingerprinting data using 53 accessions from different origins showed a high genetic diversity in the species which could offer opportunities for breeding (Ghamkhar et al. 2010). The chromosome number of *Camelina* is 2n = 40, which was confirmed by linkage map using 157 AFLP markers and 3 *Brassica* SSR markers (Gehringer et al. 2006). Genetic mapping based on AFLP, SSR and ILP makers indicated that *Camelina* is a hexaploid (Hutcheon et al. 2010). This was supported by isolation of three copies of *FATTY ACID DESATURASE 2* (*FAD2*) and *FATTY ACID ELONGASE 1* (*FAE1*) genes, both of which are single copy in *Arabidopsis* (Gehringer et al. 2006; Galasso et al. 2011). Like other important crops, polyploidy of *Camelina* will likely complicate efforts to develop molecular markers and assemble whole genome sequence. Advanced technologies for molecular genetics and genomics, including RNA-seq and next-generation genome and transcriptome sequencing, will likely provide unprecedented opportunities to accelerate improvement of agronomic and seed quality traits for *Camelina* (Varshney et al. 2009; Edwards et al. 2012).

Complementing the impact of breeding on crop improvement, *Camelina* is highly amenable to biotechnological enhancement through the use of *Agrobacterium tumefaciens*-mediated transformation. *Camelina* can be easily transformed using protocols similar those routinely used for *Arabidopsis thaliana* transformation. These methods include vacuum infiltration of flowers with a solution of *Agrobacterium* harboring a binary vector that contains the desired transgene (Lu and Kang 2008), or by simple floral dip with the *Agrobacterium* solution (Liu et al. 2012). Plants with *Agrobacterium*-infiltrated or -dipped flowers are grown to maturity. Seeds obtained from these plants are then screened to identify those containing the transgene. For this process, genes for resistance to antibiotics (e.g., kamamycin and hygromycin) or herbicides (e.g., glufosinate) can be used as selection markers for obtaining transgenic plants by screening of seeds on media containing the selective agent or by spraying seedlings with the selective agent in the case of herbicides (Lu and Kang 2008; Liu et al. 2012). Fluorescent protein selection markers such as DsRed under control of a seed-specific or constitutive promoter can also be used to identify transgenic seeds based on fluorescence of seeds with equipment as low tech as a green LED flashlight and red camera filter (Lu and Kang 2008). A wide variety of seed specific promoter/3'UTR cassettes can easily be inserted into binary vectors to express several candidate genes to modify seed oil traits. With these transformation methods and metabolic engineering toolbox, transgenic seeds can be obtained in as little as 6–8 weeks following *Agrobacterium* infiltration or dipping of flowers. Unlike transformation protocols for most crops, *Camelina* transformation can be done with minimal labor input and without the need for specialized technical skills. As such, *Agrobacterium*-based transformation offers a relatively simple and rapid, cost-effective approach for improvement of agronomic and seed quality traits.

Recent biotechnological efforts to improve the agronomic properties of *Camelina* have included transgenic expression of *Arabidopsis* purple acid phosphatase 2 that resulted in increased seed size and yield and faster growing plants relative to non-transformants. In addition, a recent report described the enhancement of the oleic acid content, a desirable biofuel trait, in *Camelina* seeds by anti-sense suppression using an inverted portion of the *Camelina CsFAD*2-1 gene under the control of the seed-specific promoter for the phaseolin gene (Kang et al. 2011). The resulting transgenic seeds contained 38–51 % oleic acid compared to 13–18 % oleic acid in seeds from non-transformed plants (Kang et al. 2011). In this study, seeds from a mutant of the *CsFAD*2-2 locus obtained from random mutagenesis that contained a premature stop at the Trp288 codon had ~27 % oleic acid (Kang et al. 2011). An interpretation of this result is that two or more of the three known *FAD*2 loci in *Camelina* contribute to the desaturation of oleic acid in seeds. Because of the high identity of these genes, it is possible to use antisense or RNA interference suppression with sequence from only one of these genes to get a mid- to high-oleic acid trait in *Camelina* seeds. It can be envisioned that additional enhancements in oleic acid content can be achieved through transgenic suppression of the *FAE*1 genes that are responsible for the elongation of oleic acid (18:1) to gondoic acid (20:1). Moreover, genes from other species could be transferred to *Camelina* to obtain additional biofuel-type traits, such as short- and medium-chain fatty acid-specific FatB-type thioesterases to achieve an oil functionality mimicking Jet A1 fuel. For industrial uses of *Camelina* oil, the castor bean (*Ricinus communis*) fatty acid hydroxylase has been successfully transferred to *Camelina* to produce ricinoleic acid and other hydroxy fatty acid in the seed oil of transformants (Lu and Kang 2008). As *Camelina* crop improvement progresses, it is likely that these efforts may involve a combination of varietal selection, mutagenic breeding, and biotechnological approaches. Indeed, the simple transformation protocols for *Camelina* hold considerable promise for rapid genetic improvement of this crop.

8.4 Current and Future Prospects for Commercial Production

Despite its considerable potential as a low input oilseed for biofuel production, *Camelina* has yet to see extensive commercial production. In the United States, large-scale production of *Camelina* has largely been restricted to the state of Montana where 8,400 ha (20,800 acres) were grown in 2009 (Anonymous 2011a). Spurring the recent interest in *Camelina* has been successful tests that have used *Camelina* oil as an ingredient of aviation fuel for commercial airliners and military jets. To stimulate increased commercial planting of *Camelina*, the U.S. Department of Agriculture Farm Service Agency announced in July 2011 a program targeted for the states of California, Washington, and Montana under the Biomass Crop Assistance Program to provide 5 year contracts for the production of up to 20,200 ha (50,000 acres) of *Camelina* for aviation fuel or other biomass conversion (Anonymous 2011b).

Table 8.1 Fatty acid composition of seed oils of *Camelina*, soybean, sunflower, and rapeseed

Oil source	Fatty acid composition (% of total fatty acids)							
	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:1
Camelina	7.5	4.0	14.9	21.4	31.7	5.1	11.8	3.3
Soybean	11.6	4.6	23.9	52.0	6.8	0.3	0.2	0.4
Sunflower	6.1	6.4	24.8	61.7	0.1	0.4	0.2	0.3
Rapeseed	3.3	1.7	18.1	14.1	7.7	2.8	8.2	44.2

More widespread production of *Camelina* in the North American Great Plains and the Pacific Northwest of the United States will undoubtedly require ready markets for *Camelina* oil and meal with cost-competitive pricing as well as the development of more extensive infrastructure for crushing of *Camelina* seeds and conversion of *Camelina* oil to biodiesel, aviation, or other fuel (Table 8.1).

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