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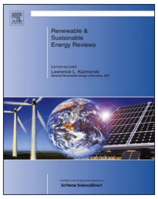

Advances in genetic improvement of *Camelina sativa* for biofuel and industrial bio-products

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ABSTRACT

Ever-increasing global energy demand, diminishing fossil fuel reserves and environmental concerns have forced to look for renewable and sustainable alternative energy sources preferentially from non-food crops. Camelina being a short-duration, low-cost, non-food oilseed crop with high content of oil (45%) rich in unsaturated fatty acids and capable of growing in marginal lands has emerged as a potential alternative for biofuel (with low carbon emission) and industrial bio-products. However, the fatty acid profile needs to be refined to make it more efficient for biodiesel and bio-products. Attempts to improve crop yield, oil content and composition through conventional and mutation breeding have been limited due to inadequate genetic diversity and availability of mutants. Simple and easy transformation and recent upsurge in 'omics' data (trancriptomics and genomics) has resulted in better understanding of lipid biosynthesis and its regulation, and thus has made it possible to produce unusual lipids with modified fatty acids for new functionalities. However, further improvement is still awaited for carbon assimilation efficiency, resistance to various abiotic and biotic stresses, seed yield, oil content and composition. This review extensively analyses the recent advances and challenges in using molecular markers, genomics, transcriptomics, miRNAs and transgenesis for improvement in biotic and abiotic stresses, carbon assimilation capabilities, seed yield, oil content and composition in camelina for biodiesel fuel properties, nutrition and high value-added industrial products like bioplastics, wax esters and terpenoids.

1. Introduction

Depleting non-renewable fossil-fuel resources, rising emissions of greenhouse gases, fuel demand, and escalating prices are forcing towards sustainable alternative fuels. Biofuels have been identified as an important component of our future energy supply because they are renewable, efficient and clean burning fuels. Various edible vegetable oil crops like soybean, palm, rape seed, groundnut, sunflower and flax, and non-edible oils like cotton and jatropha are used for biodiesel production [1]. Currently, palm, soybean, rape seed and sunflower are the major biodiesel producing plants accounting 79% of the total world production of biodiesel. However, these feed stocks compete with food crop for high quality arable land and water. Therefore, oilseed crops used for biofuel production should be from non-human food sources to sustain food production [2]. Crambe (*Crambe abyssinica*), *Camelina sativa*, *Jatropha curcas, Pongamia pinnata,* Carinata (*Brassica carinata*) and Pennycress (*Thlaspi arvense*) are the few important alternative oilseed plants [3,4]. Thermal efficiency, emissions and fuel consumptions have shown that camelina, pennycress and carinata had very similar engine performance to the traditional oils [5]. Yield of jatropha and others diminishes substantially on marginal land, *Camelina sativa,* however, grows well on marginal land with low inputs like less water, fertilizers and pesticides and has recently emerged as a promising low cost renewable source for biodiesel and industrial bio-products [6]. Use of camelina diesel reduces green house gases [GHG] emission up to 40–60% in comparison to petroleum diesel [7]. Therefore, it is essential to increase oil content and modify fatty acids to optimum composition specific for biodiesel fuel properties, nutrition and high value-added industrial products [8,9]. Due to the limited germplasm of pre-commercial lines of *C. sativa,* conventional breeding is not a very promising approach; therefore, studies should focus on genetic engineering which can be achieved with

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relative ease and in a shorter time compared to other oilseed crops [9–12]. It has been estimated that the fossil fuel reserves will be consumed by the year 2050. Hence, developing genetically superior plants for biofuel production is an efficient alternative and sustainable key to improve the competency and viability of biofuels [13].

Camelina improvement has been largely remained unexploited due to the limited availability of genetic and genomic tools [14–16]. However, the current interest in research on camelina agronomic potential, molecular markers, genetic resources including 'omics', genetic/metabolic engineering etc, has provided major concerned advances. The objective of this paper is to present available literature for this low cost non-food sustainable oil seed crop in an integrated form to specifically discuss the recent biotechnological advances made in genetic improvement to show up its potential for efficient utilization for biofuel and industrial applications. The review also highlights several challenges hindering genetic improvement of camelina and the strategies to overcome them which needs to be addressed in future researches to ensure wider application and economic viability of camelina as a biofuel and industrial crop.

2. Camelina: beneficial attributes

Camelina [*Camelina sativa* (L.) Cranz], (false flax, gold-of-pleasure) is a primeval but underexploited oilseed crop of the family *Brassicaceae* with a genome size of 750 Mbp with 2n=40 chromosomes [15]. Traditionally, it has been used for both human consumption and several non-food applications such as the production of soap, cosmetics, oil for lamp and safe paints [17]. Camelina is a better crop for bio-diesel production because it is a fast growing, self pollinating, hexaploid plant with short life cycle (85–100 days) and high yield potential (seed yield, 1500–3000 kg ha^{-1} $year^{-1}$ with oil yield 540–1410 kg ha^{-1} $year^{-1}$), requires minimal agronomic inputs and hence is well suitable for temperate climates and poor soils and yields economically useful by-products [7]. It has been suggested as a low cost feed stock for biodiesel production [18]. Camelina oil contained the same properties as that of rapeseed with an added advantage of having high iodine content, which protects against more rapid deterioration of the lubricating oil. Camelina seeds contain 36–47% oil which is twice that of soybean (18–22%) [19] and is rich in polyunsaturated fatty acids (90%) such as α-linolenic acid (18:3, 30–43%), linoleic acid (18:2, 20–25%), oleic acid (18:1, 15–20%) and low levels of gondoic (20:1) and erucic acids (22:1) (less than 10%) [20]. Higher levels of unsaturated fatty acids blend with oleic acid make camelina oil suitable for jet fuel, biodiesel and industrial lubricants.

3. Genetic improvement of camelina

The genus *Camelina* belongs to the tribe *Camelineae* of the family *Brassicaceae*. The other members of this tribe are the model plant, *Arabidopsis thaliana* and the weedy species,*Capsella bursa pastoris*. The genus *Camelina* has 11 species [21], of which only five species; *C. sativa*, *C. microcarpa*, *C. rumelica*, *C. alyssum,* and *C. hispida* are present in Europe and USA, and three species, C. *sativa*, *C. microcarpa*, and *C. alyssum* belongs to Canada. Among them, only C. *sativa* and *C. microcarpa* are cultivated. Based on archeological evidence, camelina is a native of Northern Europe [22], while based on genetic diversity, Russia or the Ukraine have also been suggested as its centre of origin as well [23]. Limited numbers of camelina genetic resources are maintained at several collecting centers or institutions, e. g. a total of 793, 137 and 44 accessions are listed in the European Catalogue of Plant Germplasm Collection (http://eurisco.ecpgr.org), the Plant Gene Resources of Canada database (http://pgr3.agr.gc.ca) and USDA-National Plant Germplasm System (http://www.ars.grin.gov.npgs/). All the accessions have not been screened for agronomic and biochemical traits [20]. *C. sativa* is very close to Arabidopsis and hence translating findings of this model plant can be explored for developing genetic and genomic tools in *C. sativa* for applied research to a great extent [15].

3.1. Conventional breeding

Camelina is mainly a self-pollinated crop [17] but cross-pollination also occurs through bees (insects) [24]. Till now, there has been very little interest in camelina breeding. However, observed variability can be exploited for improvement of camelina through hybridization breeding. The breeding approaches and procedures for camelina improvement are excellently presented elsewhere [20]. *C. sativa* can be crossed with other camelina species, *C. alyssum*, *C. microcarpa* and *C. rumelica* [25,26] while attempts to cross *C. sativa* with the distantly related crop species *Brassica napus*, *B. rapa*, and *B. juncea* were unsuccessful [25,27]. Even, it is not cross-fertile with a close relative of tribe *Camelineae*, Arabidopsis [26]. However, it is crossable with another member of the same tribe, *C. bursa-pastoris*, and produced a few hybrid plants which could not develop seeds [26,28]. Thus, the pollen mediated gene flow from camelina to other camelina species is possible but to the related species is limited. Plant breeding efforts are required to develop camelina with high-seed yield and high oil content with optimum composition of fatty acids for new functionalities.

The followings are the research goals for camelina improvement.

1. Increase in camelina seed size and total oil content
2. Alteration of the fatty acid composition of camelina seeds to reduce unsaturation and fatty acid chain length [C12-C16], introduction of unusual hydroxyl fatty acids, omega-7 unsaturated fatty acids, acetyl–triacylglycerol (TAG) and increase in the levels of Omega-3 LC-PUFA
3. Increase in photosynthetic CO2 fixation capabilities
4. Enhanced resistance to drought and heat
5. Development of novel molecular markers for camelina breeding programs
6. Development of camelina genetic resources (omics-platforms)
7. Production of terpenes, wax esters and polyhydroxybutyrate (PHB) in Camelina

3.2. Mutation breeding

Mutation breeding is important for developing novel camelina varieties with altered and desirable fatty acid profiles for biodiesel use. Seeds of *C. sativa* were irradiated with gamma-rays [29] or treated with a chemical mutagen, ethyl methane sulfonate (EMS) [30] to identify mutants with modified seed oil fatty acid composition and decreased or increased linolenic acid (C18:3) content. A large population of mutants was developed by EMS mutagenesis [15]. They identified individuals mutated in each of the three copies of both *Fatty Acid Desaturase 2* (*FAD2*) and *Fatty Acid Elongase 1* (*FAE1*) genes present in camelina genome by Targeting Induced Local Lesions In Genomes (TILLING). Mutations knocking out *FAD2* or *FAE1* may result in reduction of the polyunsaturated fatty acids or long chain fatty acids thereby creating a broad spectrum of *C. sativa* varieties possessing useful biodiesel properties. Mutants of camelina resistant to acetolactate synthase (ALS) inhibitor herbicides, imazethapyr, sulfonylureas, and flucarbazone were developed by exposing seeds to ethyl methane sulfonate and screening the plants at M2 generation for increased resistance to these herbicides [31]. The high levels of resistance will provide resistance to soil residual herbicides and thus will increase adoption of camelina as a rotation crop. Further, it has increased the option of herbicides for weed control in growing camelina.