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Improved fatty acid profiles in seeds of *Camelina sativa* by artificial microRNA mediated *FATB* gene suppression

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ABSTRACT

The fatty acid profile of plant oils determines their quality and uses. Saturated fatty acids are often not desirable from the standpoints of nutrition and some industrial applications. *Camelina sativa* is a re-emerged oilseed crop, however its oil needs to be improved to meet different application requirements. In this study, saturated fatty acids were greatly reduced by down-regulating genes encoding the fatty acyl-ACP thioesterases (FATB). An artificial microRNA (*amiFATB*) was created by replacing a microRNA sequence in the camelina *Csa-miR159a* gene with a *FATB* gene specific sequence. Seed-specific expression of *amiFATB* caused a 45% reduction of palmitic acid (16:0) and a 38% reduction of stearic acid (18:0) compared to wildtype seeds. The total saturated fatty acid content was decreased by 35% from 14.6% to 9.4% of total fatty acids. When *amiFATB* was expressed in a high-oleic acid transgenic line, it caused further increased oleic acid content. This work demonstrates that the *FATB* genes in camelina can be effectively knocked down by an artificial microRNA targeting gene-specific sequences, thus provides an additional tool to improve seed oils for desired properties.

1. Introduction

Vegetable oils are an important source of food and feed, and increasingly industrial feedstocks such as biofuels [1]. To provide desired functionalities and nutritional attributes, genetic engineering is being conducted to improve fatty acid composition by manipulating key genes involved in lipid metabolism. In oilseeds, newly synthesized fatty acids are exported from plastids mainly in the form of the monounsaturated oleic acid (18:1) along with smaller amounts of the saturated 16:0 and 18:0. These fatty acids are released from acyl-acyl carrier protein (acyl-ACP) by two different classes of thioesterases (FAT). The FATA has a higher activity on 18:1 while the FATB prefers saturated substrates [2]. A large proportion of oleic acid is modified before being incorporated into triacylglycerol through either desaturation by the desaturases FAD2 and FAD3 acting on the phosphatidylcholine (PC) substrates to form the polyunsaturated linoleic (18:2) and linolenic (18:3) acids, or elongation to form 20:1 and 22:1 by the fatty acid elongase FAE1 [3]. Controlling these metabolic steps may effectively improve plant oils for required nutritional or industrial properties [4,5].

Camelina (*Camelina sativa*) is a promising oilseed crop that is under intensive development mainly for bioenergy production. An important limitation for camelina is the suboptimal quality of its oil [6], which contains high contents of undesirable polyunsaturated (50%–60% of the total fatty acid), saturated (~10%), and the very-long chain (~12%) fatty acids [7], which are known to be associated with low oxidative stability, poor cold flow and high melting point, respectively, of the biodiesel [8]. Previously, we have successfully decreased polyunsaturated and very-long-chain fatty acids by suppression or deletion of *FAD2* and *FAE1* genes in camelina using RNA interference (RNAi) or CRISPR/Cas9 technologies [7,9,10]. The objective of this study is to decrease saturated fatty acids and to increase oleic acid. Mutations or knockdown of *FATB* genes have been shown to be associated with low saturates in seed oils such as canola and soybean [11,12]. Here, we used a microRNA mediated approach to downregulate the *FATB* genes in camelina. MicroRNAs are short 20–24 nucleotide non-coding RNA sequences. They control gene expression by binding to specific complementary mRNA targets in the untranslated regions or the coding sequence,

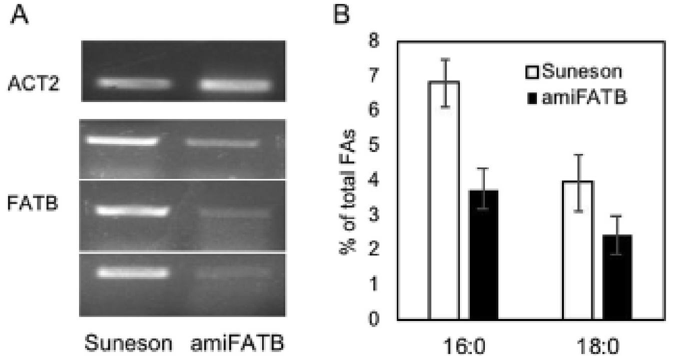

Fig. 2. The effect of amiFATB on *FATB* expression and saturated fatty acids accumulation. (A) RT-PCR detection of *FATB* in amiFATB transgenics and Suneson. The actin (*ACT2*) was used as a control gene. (B) The levels of 16:0 and 18:0 in Suneson and amiFATB seeds.

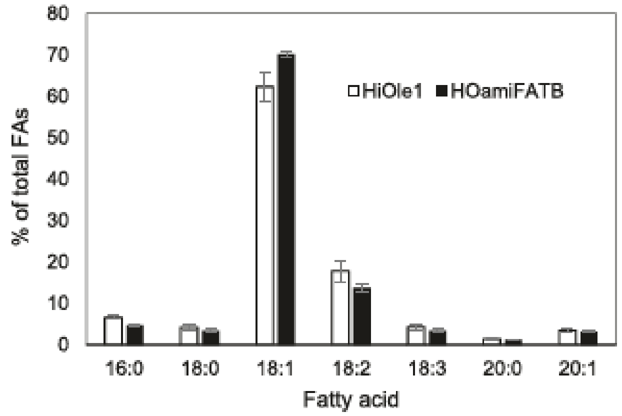

Fig. 3. A comparison of seed fatty acid profiles between a high-oleic line with its *FATB* knockdowns.

amplified all three homologous *FATB* genes (Table S1). The results showed that *FATB* transcript levels were significantly lower in transgenic lines than in the wildtype (Fig. 2A). This indicated that the *amiFATB* transgene has effectively knocked down *FATB* genes in camelina developing seed.

To assess the effect of the *amiFATB* transgene on fatty acid metabolism in camelina, fatty acid composition was determined in mature T3 seeds by gas chromatography. The results showed that levels of saturated fatty acids were decreased in the amiFATB seeds compared to those in the non-transgenic Suneson. Especially, a 45% reduction of 16:0 and a 38% reduction of 18:0 were observed in amiFATB compared to Suneson (Fig. 2B). This result was consistent with the enzyme substrate specificity of FATB on saturated (16:0 and 18:0) acyl-ACPs in plastids [2]. Other saturated fatty acids (20:0 and 22:0) were also slightly decreased, consequently the total saturated fatty acids were decreased from 14.6% in Suneson to 9.4% in amiFATB seeds, about 35% reduction compared with non-transgenic seeds (Table 1).

3.3. *The effect of FATB-knockdown in a high-oleic line*

Corresponding to decreased saturated fatty acids, the levels of oleic acid and other unsaturated fatty acids were slightly increased in the amiFATB seeds (Table 1). Previously, we created transgenic camelina lines that accumulated high levels of oleic acid by antisense or hairpin mediated RNAi knockdown on *FAD2*, *FAD3* and *FAE1* genes [7,9]. To explore whether down-regulating *FATB* expression may further increase the oleic acid content, we transformed the amiFATB construct into a high-oleic line (HiOle1) containing the FAD2-FAD3-FAE1 RNAi construct and a herbicide resistance marker [9]. Twenty transgenic lines were obtained that contained both herbicide and DsRed selection markers. Consequently, six T3 HOamiFATB lines that showed clearly decreased *FATB* transcripts determined by semi-qRT-PCR were analyzed for their seed fatty acid composition. The results showed that saturated fatty acids (16:0, 18:0 and 20:0) were decreased as expected. The composition of unsaturated fatty acids was also changed. Significantly, the oleic acid was increased from about 62% to over 70% (Fig. 3).

4. Discussion

The fatty acyl-ACP thioester FATB is a major determinant for saturated fatty acid accumulation in oilseeds. Low palmitate (16:0) in soybean was associated with the mutation of a *FATB* gene [11], and the Arabidopsis *fatb* knockout mutant accumulated only 50% saturated fatty acids of the wild type level [21]. In this study, we demonstrated that seed-specific expression of an artificial microRNA (*amiFATB*) significantly decreased *FATB* gene transcripts in developing seeds and caused about 45% and 38% reduction of 16:0 and 18:0, respectively, in camelina seed oil. The reduction of the saturated fatty acid content in amiFATB was at a similar level found in the Arabidopsis *fatb* mutant [21]. In tissues of the *fatb* plant, the remaining about 50% saturated fatty acids were presumably synthesized by other genes including the plastidial glycerol-3-phosphate:acyl-ACP acyltransferase (ACT1). Significantly, plant growth of the *fatb* and *fatb*/*act1* mutants were severely retarded, indicating that saturated fatty acids are essential for normal plant growth [21]. It is therefore infeasible to generate the *FATB* knockout camelina plants, which may affect plant growth while not be able to further reduce saturated fatty acids in seed. We did not observe abnormal seed germination and plant growth of the amiFATB lines.

A major goal of oilseed breeding is to increase the desired oleic acid content. Our results indicated that *FATB* knockdown not only decreased saturated fatty acids, but also caused changes in unsaturated fatty acids especially enhanced oleic acid accumulation. Simultaneous downregulation of *FATB* and *FAD2* in soybean has been shown to increase oleic acid and decrease palmitic acid [22]. Similarly, expression of *amiFATB* in a *FAD2-FAD3-FAE1* RNAi line also increased oleic acid in camelina. Besides decreased saturates, the increased oleic acid in the HOamiFATB seeds seemed to be also at the expenses of linoleic acid (18:2). It is not clear whether reduced

Table 1
Fatty acid composition in Suneson and amiFATB seeds.

		16:0	18:0	18:1	18:2	18:3	20:0	20:1	20:2	20:3	22:0	22:1
Suneson	Ave	6.8	3.9	11.5	18.8	36.4	3.3	13.0	1.7	1.3	0.5	2.8
	SD	0.7	0.8	0.7	2.1	2.6	0.7	1.1	0.2	0.2	0.1	0.4
amiFATB[a]	Ave	3.8	2.4	13.4	18.9	37.5	2.8	13.9	1.8	1.3	0.4	3.5
	SD	0.6	0.5	1.6	1.6	4.0	0.4	0.6	0.1	0.2	0.1	0.5

[a] Data represent average and standard deviation from 4 independent lines.

flux of 16:0 and 18:0 into the cytosolic triacylglycerol biosynthesis pathway or the *FATB* gene knockdown affected FAD2 activity in the RNAi line. Nevertheless, our results and previous studies [22] suggest that simultaneous knockdown of genes encoding key fatty acid modification enzymes including FATB, FAD2, FAD3 and FAE1 may enhance accumulation of oleic acid in oilseeds. Further increasing oleic acid in HOamiFATB seeds may be achieved by eliminating the very-long-chain fatty acids (20:0 and 20:1) by deleting *FAE1* genes. It was possible to knockout *FAE1* genes in camelina by the CRISPR-Cas9 technology, which blocked fatty acyl flux into the elongation pathway and resulted in enhanced accumulation of 18:1 and its derived polyunsaturated fatty acids (18:2 and 18:3) [10]. Unlike *FAE1* which can be deleted without causing abnormal plant growth, *FATB* and *FAD2* are essential for normal plant growth [21,23,24]. Our results provide an additional tool to effectively downregulate gene expression by artificial microRNAs. Combining this with other biotechnological tools for gene silencing will allow for the development of high-oleic camelina for improved oil qualities.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2018.06.051.

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