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Enhancing seed oil content and fatty acid composition in camelina through overexpression of castor RcWRI1A and RcMYB306

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Abstract

Seed triacylglycerol (TAG), a major component of vegetable oil, consists of a glycerol esterified with three fatty acids. Vegetable oil has industrial applications and is widely used as edible oil. The increasing demand for plant oils, owing to population growth, it is crucial to enhance the oil content in seeds. We found castor WRINKLED1A (RcWRI1A) and R2R3-type MYB domain protein 306 (RcMYB306) which have homology with Arabidopsis WRI1 (AtWRI1) and AtMYB96 which regulate genes involved in fatty acid and TAG synthesis, respectively. These castor genes were separately and jointly overexpressed using seed-specific promoters in an oil crop, camelina (*Camelina sativa*). Overexpression of *RcWRI1A*, *RcMYB306*, or *RcWRI1A*+*RcMYB306* increased the total seed oil content in camelina. However, this increase was not significantly different from that observed during the overexpression of *RcWRI1A* or/and *RcMYB306*. *RcWRI1A* overexpression increased the fatty acid content, including 16:0, 18:2, 18:3. Contrastingly, *RcMYB306* overexpression increased the 18:1, 18:2, 18:3, 20:0 and 20:1 fatty acid. In the *RcWRI1A*+*RcMYB306* lines, changes in fatty acid composition demonstrated the combined effects of these transcription factors. These results suggest that RcWRI1A and RcMYB306 can be used to improve the productivity of oil crops.

Keywords Camelina, Castor, Fatty acid, MYB306, Triacylglycerol, WRI1

Introduction

Vegetable oil is an edible oil and is also used as a raw material in the industrial production of plastics, cosmetics, and lubricants [1]. It contains triacylglycerol (TAG), which comprises three fatty acids esterified with one glycerol [2]. The fatty acid composition of TAG in seeds differs depending on the plant species [3]. Olive oil has a high oleic acid (18:1^{Δ9}) content and is used for frying or salad dressing [4, 5]. Perilla, flaxseed, and walnut oil have health benefits and are high in α-linolenic acid (18:3^{Δ9,12,15}), an omega-3 fatty acid [6]. Castor (*Ricinus communis* L.) oil contains 80–90% ricinoleic acid (18:1^{Δ9}-OH) with a hydroxy group on 12th carbon. It is used as

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an industrial raw material in various manufacturing processes, such as lubricant and paint production [7]. The demand for vegetable oils is increasing worldwide [8]. However, crop productivity is decreasing because of climate change caused by global warming [9]. Therefore, technological advancements are required to increase the TAG content of seeds, subsequently increasing vegetable oil production per unit area. Camelina (*Camelina sativa*) is an oil crop belonging to the Brassicaceae family. It has a short life cycle, grows in barren areas, and is easily transformed through the floral dipping method, a genetic engineering platform for plant oil enhancement [10, 11].

The transcription factors and genes involved in fatty acid and TAG synthesis have been identified in *Arabidopsis* (*Arabidopsis thaliana*) [12]. Fatty acids synthesized in plastids are exported to the cytosol to form acyl-coenzyme A (CoA) pools [12]. In the Kennedy pathway, three acyl-CoA molecules are sequentially added to one molecule of glycerol-3-phosphate to synthesize TAG using glycerol-3-phosphate acyltransferase, lysophosphatidic acid acyltransferase, and diacylglycerol acyltransferase 1 (DGAT1) in the endoplasmic reticulum (ER) [13, 14]. DGAT is a rate-limiting enzyme that transfers acyl-CoA to the *sn*-3 position of diacylglycerol (DAG) to generate TAG [15, 16]. Unsaturated fatty acids from the *sn*-2 position of phosphatidylcholine (PC) are transferred to the *sn*-3 position of DAG by phospholipid: DAG acyltransferase (PDAT) to synthesize TAG [17].

Arabidopsis WRINKLED1 (AtWRI1) belongs to a family of APETALA2 (AP2) transcription factors that regulate genes involved in fatty acid synthesis [18]. Orthologs of AtWRI1 have also been found in *Brassica*, corn, camelina, soybean, and castor [19–23]. MYBs are the largest family of the transcription factor in plants. The MYB protein is divided into several classes, one of which is the R2R3-type MYB TF, divided into 23 subgroups [24]. R2R3-type MYB domain protein 96 (MYB96), which exhibits drought stress or disease resistance related to ABA signaling, regulates *PDAT1* and *DGAT1* expression [25]. Furthermore, seed-specific overexpression of *AtMYB96* enhances the TAG content in *Arabidopsis* seeds [25]. In contrast, *AtMYB89* is a negative regulator for oil accumulation [26].

Castor is grown in tropical or subtropical regions and contains 30–50% oil in seeds [7]. Even though castor has high oil content in seeds, not much research has been conducted on the transcription factor of castor. In this study, two transcription factors, *RcWRI1A* and *RcMYB306*, were isolated from castor. It remains to be revealed whether overexpression of *RcWRI1A* and *RcMYB306* effectively increases oil content in oil crops. Therefore, seed-specific overexpression of *RcWRI1A* and *RcMYB306* were conducted in camelina as well as co-expression of *RcWRI1A* and *RcMYB306*. We investigated

whether heterologous expression of *RcWRI1A* or/and *RcMYB306* enhances the metabolism of fatty acids and TAG in camelina seeds and ultimately improves oil content.

Materials and methods

Plant materials and transformation

Camelina sativa cultivar Suneson was used for transformation. Wild-type and transgenic plant seeds were germinated on moist filter paper in a culture chamber at 16 °C under a 16 h light/8 h dark photoperiod. Plants were grown at 20 °C in a growth chamber under a 16 h light/8 h dark photoperiod. The *Agrobacterium* strain GV3101 was used to transform the pBinGlyRed3 vector. *Agrobacterium* cells were inoculated into 500 mL LB medium and incubated at 28 °C. Cultured cells were centrifuged at 3000 × *g* for 10 min at 4 °C. They were subsequently dissolved in a solution containing 5% sucrose and 0.05% (v/v) Silwet-L77. Eighteen plants were transformed using the floral dipping method [10]. After floral dipping, the plants were wrapped in black plastic for a day to maintain the humidity. This process was performed three times at intervals of 5–7 d. The fluorescent seeds were obtained using a green flash and a red filter to identify transgenic plants.

Gene cloning and vector construction

Total RNA was extracted from developing castor seeds, and cDNA was synthesized using a cDNA synthesis kit (Takara, Kusatsu, Japan). We designed primers covering the full-length open reading frame (ORF) to amplify the coding sequence (CDS) of *RcWRI1A* and *RcMYB306* (Table S1). The PCR products were eluted using a PCR purification kit (Cosmo Genetech, Seoul, Korea) and cloned into the pGEM-T vector (Promega, Madison, WI, USA). The final vector, pBinGlyRed3, included the glycine promoter for seed-specific expression and *DsRed3* as a selection marker [27]. There is an *EcoRI* site on both sides of the CDS in the pGEM-T vector and a unique *EcoRI* site in pBinGlyRed3. Therefore, *RcWRI1A* and *RcMYB306* were cloned using *EcoRI*. To construct a co-expression vector containing both genes, *RcMYB306* was cloned into a pKMS2 vector containing an oleosin promoter using *NotI* enzymes on both sides of the pGEM-T vector. Then, the oleosin promoter:*RcMYB306*:terminator cassette was cut out using the *AscI* restriction enzyme. This cassette was inserted into the *AscI* site of the pBinGlyRed3-*RcWRI1A* vector to complete the cloning of the co-expression vector.

Phylogenetic tree and protein sequence alignment

All protein sequences used for phylogenetic tree and sequence alignment were obtained from the National Center for Biotechnology Information (NCBI) and The

Arabidopsis Information Resource (TAIR). The phylogenetic tree was generated by the Neighbor-Joining method with 1000 bootstrap replications in the MEGA7 program. Protein sequence alignment was conducted in DNAMAN program using Clustal W method.

Fatty acid analysis

The fatty acid composition and total oil content were analyzed through gas chromatography (GC). Seven seeds were reacted with 500 μ L toluene and 1 mL of 5% H_2SO_4 including pentadecanoic acid (15:0) standard (100 μ g/mL) in an 85 $^\circ\text{C}$ water bath for 2 h. Next, 1 mL of 0.9% NaCl and 1 mL hexane were added. The solution was mixed and centrifuged at $330 \times g$ for 2 min to extract fatty acid methyl esters (FAME). The addition of 1 mL hexane, followed by centrifugation, was repeated three times. Then, the solution was evaporated using nitrogen gas. The FAME was diluted with 200 μ L hexane and transferred into a GC vial for analysis using a GC-2030 (Shimadzu, Kyoto, Japan) machine and a DB-23 column (30 m $\times$ 0.25 mm, 0.25 μ m film; Agilent, Santa Clara, CA, USA). The oven temperature range increased from 190 $^\circ\text{C}$ to 230 $^\circ\text{C}$ by 5 $^\circ\text{C}$ per min.

RT-PCR and RT-qPCR analysis

Total RNA was isolated from developing seeds in camellina transgenic plants using the method described in reference [28]. 2 μ g of cDNA was synthesized using the PrimeScript 1st Strand cDNA Synthesis Kit (Takara, Kusatsu, Japan). Developing seeds were obtained during the oil accumulation period (18–24 DAF) based on reference [29]. Reverse transcription real-time PCR (RT-qPCR) was performed using TB Green Premix Ex Taq™ II (Takara, Kusatsu, Japan) reagent in a StepOne-Plus Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). By subtracting the C_t values of the target gene and the endogenous control, the C_t value was determined. *ACTIN2* was used as a control. To measure the relative expression level, the two C_t values were subtracted and obtained the value of $2(-\Delta\Delta C_t)$. Primers used in this study are listed in Table S1.

Results

Isolation of castor WRI1A and MYB306 cDNA for increasing seed oil in camellina

AtWRI1 functions as a master regulator by regulating transcription level of fatty acid and TAG biosynthesis genes [30]. The heterologous expression of various *WRI1* genes increases the TAG content of seeds in several plants [18, 19, 31, 32]. In addition, the overexpression of *AtMYB96* using a seed-specific promoter increased the TAG content in Arabidopsis [25]. In this study, we expressed *RcWRI1A* and *RcMYB306* individually or simultaneously to increase TAG levels in camellina seeds.

We attempted to compare the efficiency of these transcription factors in increasing oil content. Furthermore, we investigated whether the co-expression of two transcription factors could increase oil content compared to a single transcription factor.

The castor genes with the highest amino acid sequence homology to AtWRI1 and AtMYB96 were selected from the castor genomic database (<http://www.plantgdb.org>) using the BLASTP program. The highest homology with AtWRI1 and AtMYB96 in the protein database translated using the entire castor transcript was 30,069.m000440 and 30,138.m004082, with values of 6.0e^{-102} and 6.0e^{-91} , respectively. Analysis of BLAST and phylogenetic tree was conducted on the isolated transcript in the castor genomic database. As a result, 30,069.m000440 and 30,138.m004082 were estimated to be *RcWRI1A* and *RcMYB306*, respectively (Fig. 1). It was confirmed that castor WRI1 has a 14-3-3 binding motif, a conserved VYL motif, and two AP2 domains such as other WRI1 (Fig. S1). The amino acid sequence of *RcWRI1A* showed 61% identity with AtWRI1. *RcWRI1A* has the same sequence as a spliced transcript of castor WRI1 [23]. The phylogenetic tree between *RcMYB306* and AtMYBs revealed that the *RcMYB306* belongs to subgroup 1 and is close to AtMYB30 (Fig. S2). However, since AtMYB306 does not exist in Arabidopsis, the phylogenetic tree was generated between *RcMYB306*, several plant MYB306, and AtMYBs belonging to subgroup 1 (Fig. 1b). As a result, it was confirmed that *RcMYB306* was closer to MYB306s than to AtMYB30. In addition, the R2 and R3 domains of *RcMYB306* are conserved similar to other MYB306s (Fig. S3). According to the alignment result and phylogenetic tree, two genes were designated as *RcWRI1A* and *RcMYB306* in this study.

Selection of camellina transformants introduced with *RcWRI1A*, *RcMYB306*, and *RcWRI1A* + *RcMYB306* vectors

To investigate whether *RcWRI1A* and *RcMYB306* enhance TAG biosynthesis in seeds, these two genes were cloned into seed-specific expression vectors. Two vectors were constructed to express *RcWRI1A* and *RcMYB306* using a seed-specific glycinin promoter (Fig. 2a). To co-express *RcWRI1A* and *RcMYB306*, the two genes were cloned into a single vector containing glycinin and oleosin promoters (Fig. 2a).

Transgenic plants transformed with *GlyP: RcWRI1A*, *GlyP: RcMYB306*, or *GlyP: RcWRI1A + OleP: RcMYB306* vectors were selected from T_1 seeds. T_1 plants overexpressing (OE) *RcWRI1A*, *RcMYB306*, or *RcWRI1A + RcMYB306* were designated as W1–W10, M1–M10, and WM1–WM10, respectively. To detect only the transgene and not the endogenous genes, a forward primer was designed to target the rear of the transgene, and a reverse primer was designed to target the

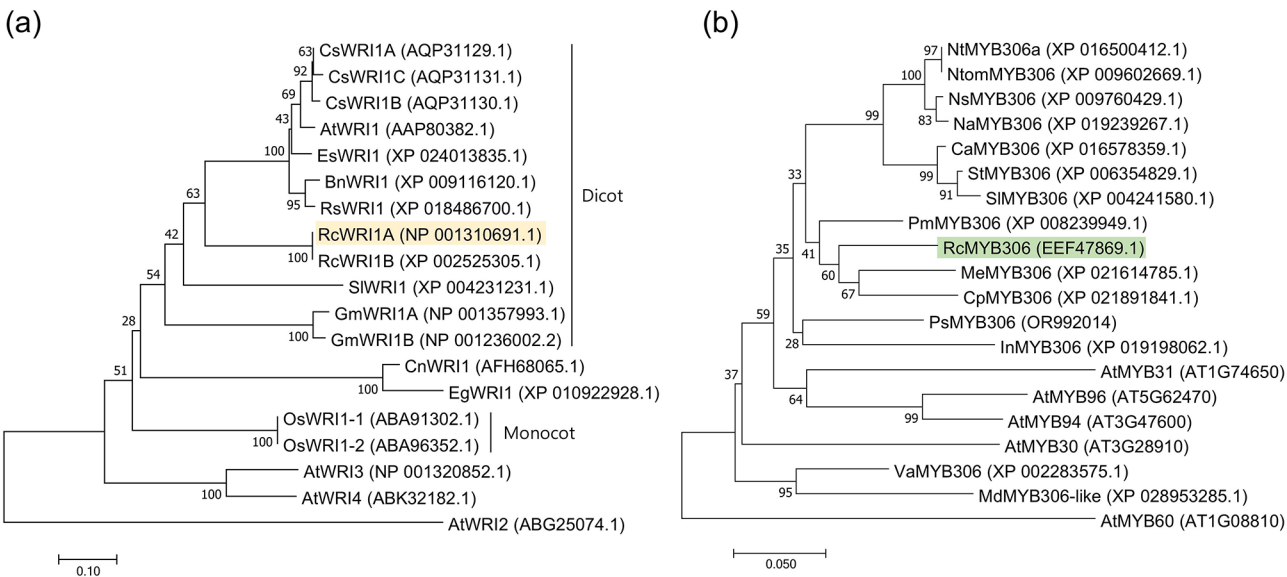


Fig. 1 Evolutionary relationships of **(a)** RcWRI1A and **(b)** RcMYB306 with various plants WRI1 and MYB306. The phylogenetic tree was generated by the Neighbor-Joining method with a bootstrap test (1000 replicates) in the MEGA7 program (<https://www.megasoftware.net>)

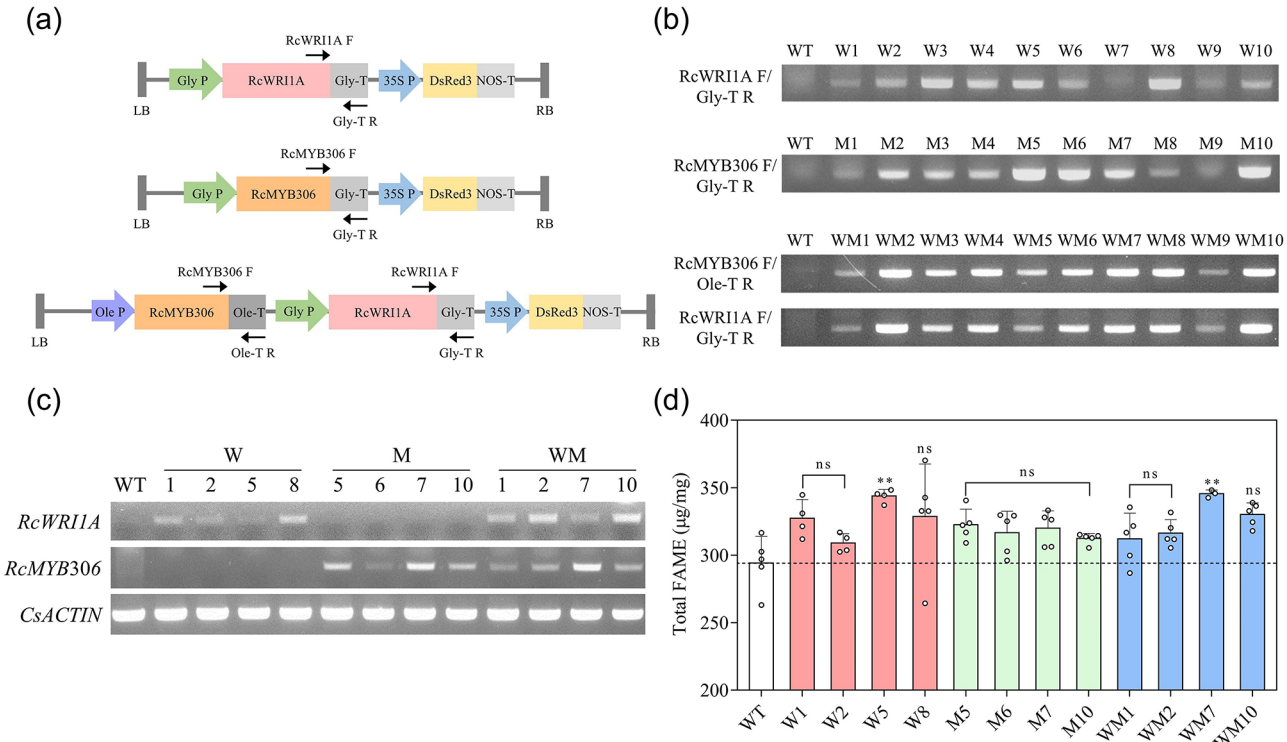


Fig. 2 T-DNA vector map and selection of transgenic plants. **(a)** A vector map for seed-specific expression of *RcWRI1A* or/and *RcMYB306*. GlyP, Glycinin promoter (701 bp); *RcWRI1A*, *RcWRI1A* cDNA (1365 bp); Gly-T, Glycinin terminator (458 bp); NOS-T, Nos terminator; LB, left border; RB, right border; *RcMYB306*, *RcMYB306* cDNA (1035 bp); OleP, oleosin promoter (580 bp); Gly-T R, Glycinin terminator reverse primer; 35 S P, 35 S promoter. **(b)** Transgene PCR using genomic DNA of T₁ plants. WT, wild type; W, *RcWRI1A* OE line; M, *RcMYB306* OE line; WM, *RcWRI1A* + *RcMYB306* OE line. **(c)** RT-PCR analysis of transgenes from RNA isolated from developing seeds of T₁ plant. **(d)** Total FAME (μg/mg) of T₂ seeds harvested from T₁ transformants. Error bars are SD of the mean. Statistical significance is indicated by an asterisk and was evaluated using one-way ANOVA with Tukey's multiple comparison test (**p < 0.01)

front of the terminator (Fig. 2a). PCR of the genomic DNA confirmed the expected 477 bp (*RcWRI1A*) and 456 bp (*RcMYB306*) PCR bands in transgenic plants but revealed no PCR bands in wild type (WT) (Fig. 2b).

Four independent plants were randomly selected among the 10 T_1 plants in each transgenic plant and RNA was extracted from the developing seeds of the T_1 transgenic plants. Then, RT-PCR was performed to assess *RcWRI1A* and *RcMYB306* expression. This analysis confirmed that the two genes were stably expressed in each of the four transformed lines (Fig. 2c). The total FAME content in the seeds was analyzed in transgenic plants. The total FAME per mg in T_2 seeds was increased in the three kinds of transgenic plants compared to the WT but was not significantly increased in some of the over-expressed lines (Fig. 2d). Because the T_1 generation was not a homozygous plant, W5, W8, M5, M7, WM7, and WM10 lines, which had high transgene expression in developing seeds, were selected and transmitted to the T_2 generation plants. The PCR was conducted using gDNA to confirm whether the transgene was inserted in the T_2 generation (Fig. S4). Furthermore, two independent lines were selected for the T_3 generation.

Fatty acid analysis of *RcWRI1A*, *RcMYB306*, and *RcWRI1A*+*RcMYB306* transgenic plants

Fatty acid analysis was performed on T_4 seeds derived from T_3 plants to investigate whether the *RcWRI1A*- and *RcMYB306*-induced oil content increase was maintained in the T_3 generation (Fig. 3a, b). Except for *RcWRI1A* OE #8-2-3 and #8-2-4 lines, the total FAME per mg increased by 14% in the *RcWRI1A* OE #5-5-1 and #5-5-6 lines compared to that in the WT (Fig. 3a). All transgenic plants in *RcMYB306* and *RcWRI1A*+*RcMYB306* OE lines had higher total FAME per mg of seeds (Fig. 3a). *RcMYB306* OE lines increased the total FAME per mg by 7–10% compared to the WT (Fig. 3a). In addition, the co-expression of *RcWRI1A* and *RcMYB306* caused 13–17% increase in total FAME per mg in seeds (Fig. 3a). The *RcWRI1A*, *RcMYB306*, and *RcWRI1A*+*RcMYB306* OE lines increased the total FAME per seed by 14–21%, 13–19%, and 15–26%, respectively (Fig. 3b).

Since the total fatty acid content of the three types of overexpression plants increased, the change in fatty acid content per seed was investigated compared to WT (Fig. 3c). The *RcWRI1A* OE lines significantly increased the 16:0, 18:2, and 18:3 contents in seed oil. In the *RcMYB306* OE lines, 18:1, 18:3 contents significantly increased, and 18:2 content slightly

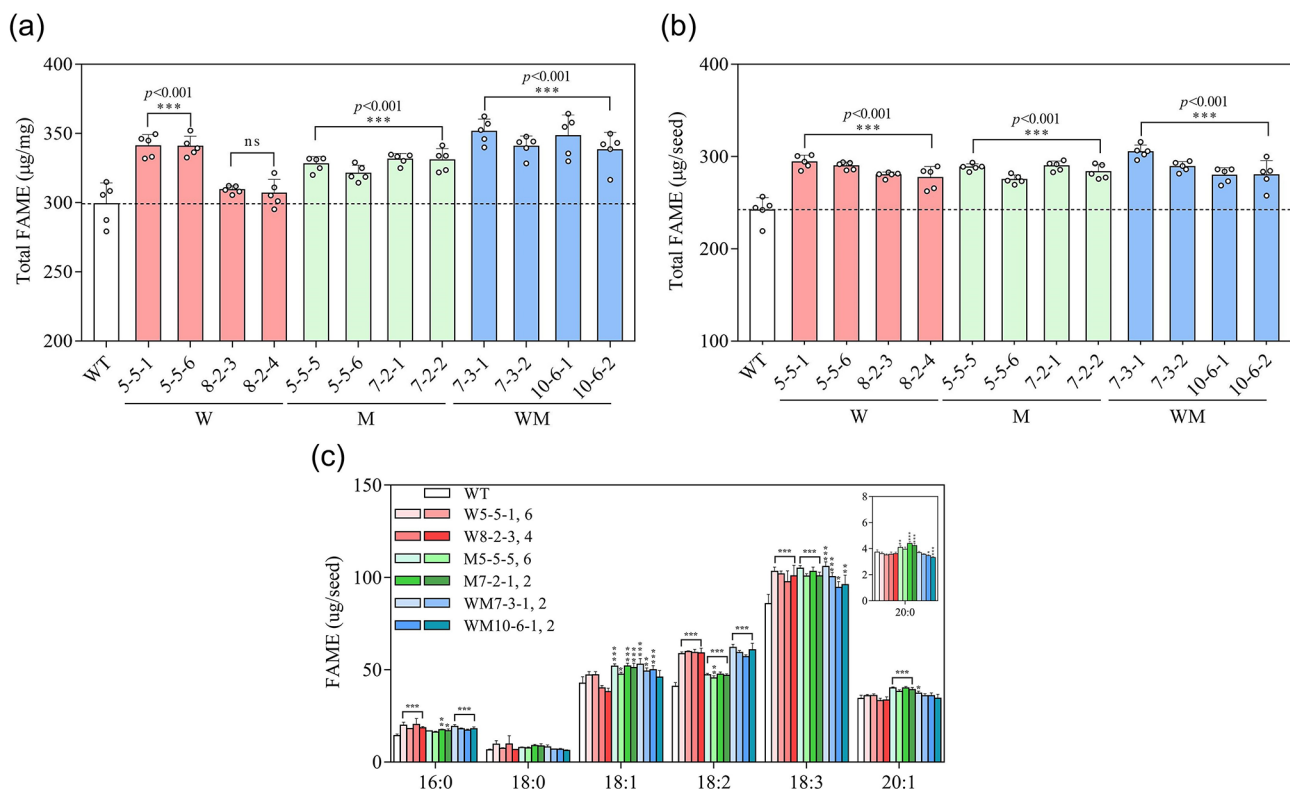


Fig. 3 Fatty acid analysis of T_3 transgenic plants. **(a)** Analysis of total FAME content ($\mu\text{g/mg}$) in T_4 seeds and **(b)** total FAME content ($\mu\text{g/seed}$) in T_4 seeds. **(c)** The FAME per seed in T_4 seeds compared to the WT. Error bars are SD of the mean. Statistical significance is indicated by an asterisk and was evaluated using one-way ANOVA with Tukey's multiple comparison test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

increased. In addition, 20:0 and 20:1, which did not change in *RcWRI1A* OE lines, increased in *RcMYB306* OE lines. The *RcWRI1A*+*RcMYB306* OE lines showed changes in fatty acid increase that were somewhat different from those of the *RcWRI1A* and *RcMYB306* OE lines, respectively. The increases in 16:0, 18:2, and 18:3 were similar to *RcWRI1A* OE lines, but the increases in 18:1 which showed increases such as *RcMYB306* OE lines (Fig. 3c). In addition, 20:0 and 20:1 was decreased in *RcWRI1A*+*RcMYB306* OE lines compared that of *RcMYB306* OE lines (Fig. 3c). This result showed that seed fatty acid content was increased in the *RcWRI1A* and *RcMYB306* OE lines. There was no significant difference in total FAME but composition of FAME per seed in the *RcWRI1A*+*RcMYB306* OE lines was different compared to the *RcWRI1A* or *RcMYB306* OE lines (Fig. 3). Even though total FAME was increased in *RcWRI1A*, *RcMYB306*, and *RcWRI1A*+*RcMYB306* OE lines, seed size was not affected in all lines (Fig. S5).

Regulation of fatty acid and target gene expression by *RcWRI1A* and *RcMYB306* in camelina

The expression of fatty acid biosynthesis genes targeted by each of transcription factors was analyzed between *RcWRI1A*, *RcMYB306*, and *RcWRI1A*+*RcMYB306* OE lines (Fig. 4). A comparative analysis was performed on one line of each OE lines. The transgenes (*RcWRI1A* and *RcMYB306*) were overexpressed in a seed-specific manner for each OE line (Fig. 4a). In the developing seeds of the *RcWRI1A* OE line, the expression of camelina *BCCP2*, *KASI*, *KASII*, *MAT*, *PDH* and *PKP2*, which were reported as targets of AtWRI1, was upregulated as expected. In addition, expression of *FAD2* which is a desaturase enzyme, was also increased. However, the expression of *FAE1*, *DGAT1*, and *PDAT1* was downregulated (Fig. 4b, c). In the *RcMYB306* OE line, expression of *KASI*, *KASII*, *MAT*, *PDH* and *PKP2* were upregulated similarly to the *RcWRI1A* OE line, and expression of *FATA* and *FAD3*, which were unchanged in the *RcWRI1A* OE line, were increased. Expression of *FAE1* and *DGAT1* was also upregulated (Fig. 4b, c). The *RcWRI1A*+*RcMYB306* OE

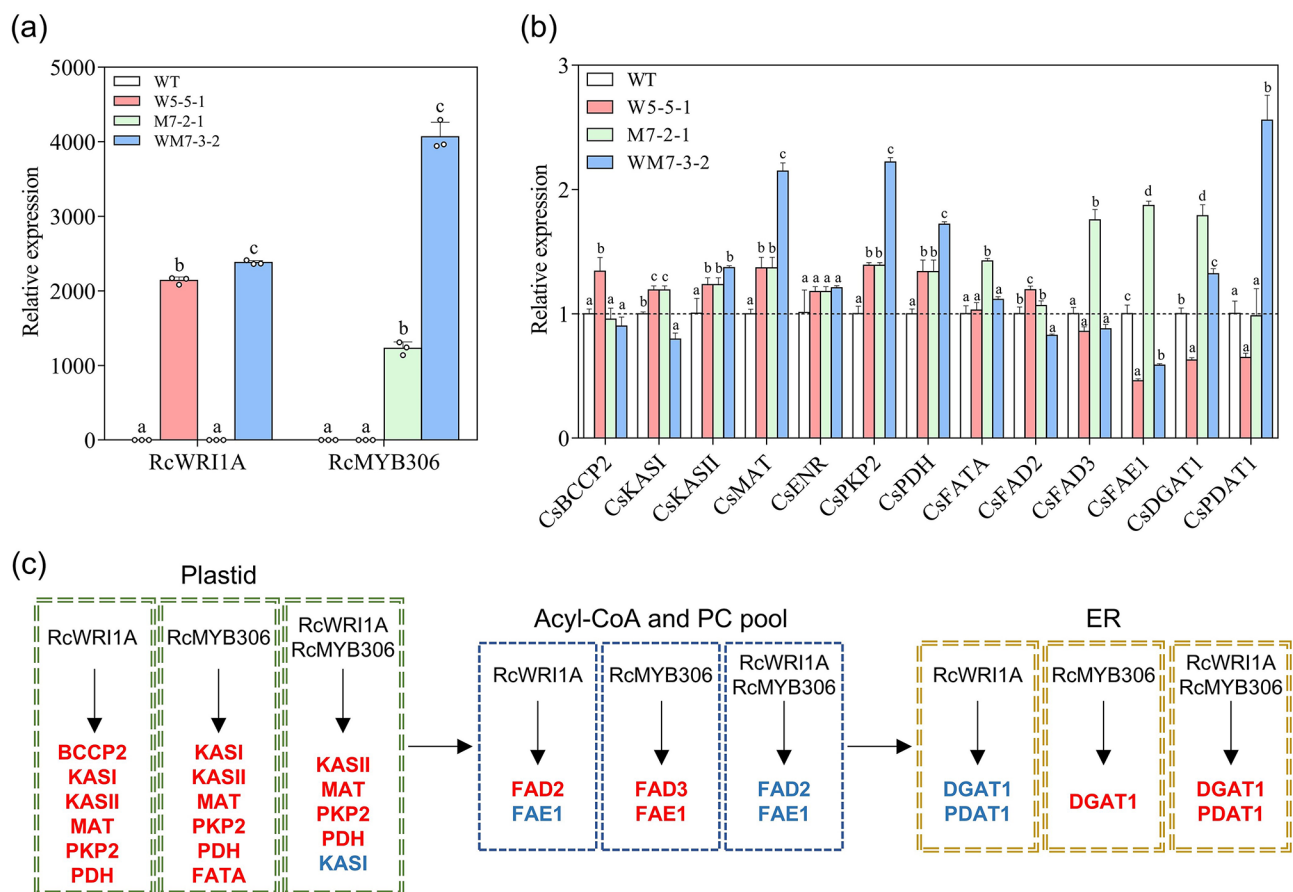


Fig. 4 RT-qPCR analysis in developing seeds of transgenic plants compared to the wild type. **(a)** Expression pattern of *RcWRI1A* and *RcMYB306* in transgenic plants. **(b)** Expression pattern of fatty acid biosynthesis genes and acyltransferase genes. **(c)** Upregulated and downregulated genes are indicated by red and blue color in *RcWRI1A* OE, *RcMYB306* OE lines, and *RcWRI1A*+*RcMYB306* lines. Error bars are SD of the mean. Statistical significance is indicated by different letters and was evaluated using one-way ANOVA with Tukey's multiple comparison test ($p < 0.05$)

lines showed the expression pattern of mixed genes of *RcWRI1A* and *RcMYB306* OE lines. *MAT*, *PKP2*, *PDH*, and *PDAT1* genes showed a greater increase than in the single OE line. However, the expression of *KASI*, *FAD2*, and *FAE1* was reduced more than that of overexpression of single gene (Fig. 4b, c).

Discussion

Much research has been performed to improve the oil content in oil crops [33, 34]. One of the strategies for increasing TAG in leaves promote the increase of fatty acid content by transcription factors (Push), transfer fatty acids to TAG through acyltransferases (Pull), and maintain the oil body structure (Protect); this is referred to as the 3P strategy [35, 36]. In this study, push and pull were induced by seed-specific overexpression of *RcWRI1A* and *RcMYB306* in camelina seeds (Fig. 2). Seed-specific overexpression of *RcWRI1A* and *RcMYB306* increased the fatty acid content by 14% and by 7–10% respectively in camelina T₄ seeds, with positive effects observed for *RcWRI1A* and *RcMYB306* individual overexpression (Fig. 3). The simultaneous expression of *RcWRI1A* and *RcMYB306* did not more increase seed oil content but resulted in different fatty acid composition compared with that of *RcWRI1A* or *RcMYB306* OE lines (Fig. 3).

Gene expression analysis explains the differences between fatty acid content and fatty acid composition in the *RcWRI1A*, *RcMYB306*, and *RcWRI1A*+*RcMYB306* OE lines (Figs. 3 and 4). Expression of *PKP2*, *MAT*, *KASI*, *KASIII*, *ENR*, and *FATA* was significantly downregulated in *AtWRI1* mutant [18]. In addition, *PKP-β1*, *BCCP2*, *ACP1*, and *KASI* are directly regulated by *AtWRI1* [37, 38]. In previous report, transient expression of *RcWRI1A* in *Nicotiana benthamiana* leaf increases the expression of *ACP1*, *PDH-E1α*, *KASI*, *BCCP2*, *PKP-α*, *PKP-β1* [23]. In our study, *RcWRI1A* overexpression of camelina led to upregulation expression of *PKP2*, *BCCP2*, *KASI*, which are direct targets of *AtWRI1*, in addition to increasing the expression of *KASII*, *MAT*, *PDH* (Fig. 4b). When expression of *RcWRI1A* in *N. benthamiana*, increase the 18:1, 18:3, 22:0 fatty acids and decrease 18:0 and 20:0 fatty acids in leaves [23]. It showed a different pattern in the seed fatty acid composition of the *RcWRI1A* OE lines, which has no decrease in 18:0 and 20:0 fatty acids and an increase in 16:0, 18:2, and 18:3 content (Fig. 3c). The 18:2 content increase in *RcWRI1A* OE lines may be due to the increased expression of *FAD2* and the increase in 16:0 is expected to be due to the higher expression of *KASI*. Unexpectedly, the expression of *FAD3* was not upregulated but the 18:3 content was increased (Fig. 4b).

The increase of 20:1 content in *RcMYB306* OE lines is presumed to be an increase in *FAE1* expression. This result is consistent with the increased expression of *FAE1* and 20:1 content in the *AtMYB96* overexpression line

[39]. In addition, *AtMYB96* has been shown to upregulate the expression of *DGAT1* and *PDAT1* [25]. Expression of *DGAT1* was also elevated in *RcMYB306* OE lines, but *PDAT1* expression was not significantly different from WT (Fig. 4b). When the seed fatty acid composition was examined in *AtMYB96* OE lines, the 16:0, 18:0, 18:1, 18:2 and 18:3 contents all increased [25]. This is consistent with the increase in most fatty acid levels in *RcMYB306* OE lines (Fig. 3c). In particular, the 18:1 content did not increase in the *RcWRI1A* OE line but increased in the *RcMYB306* OE line. The reason is the increase of *DGAT1* expression in the *RcMYB306* OE line, which results in an increase of 18:1 content, which is a *DGAT1* substrate [40]. In addition, the increase in 18:3 content may be due to increased expression of *FAD3*. However, the *RcMYB306* OE line also showed increased expression of *KASI*, *KASII*, *MAT*, *PKP2*, *PDH*, and *FATA* unlike *AtMYB96* OE lines (Fig. 4b).

MYB306 research was conducted in several plants [41–43]. *Paeonia suffruticosa* MYB306 (PsMYB306) negatively regulate bud dormancy by directly binding to the promoter of PsNCED3 and activating it [41]. *NtMYB306a* is highly expressed in trichomes and is involved in wax alkane biosynthesis in leaves [42]. Apple MdMYB306-like interacts with MdMYB17 and MdbHLH33 and inhibits anthocyanin synthesis [43]. Since MYB306 shows various functions in various crops, additional research in other fields as well as controlling the oil content of *RcMYB306* appears to be necessary.

The *RcWRI1A*+*RcMYB306* OE line did not differ significantly from the increase in total fatty acid content of *RcWRI1A* and *RcMYB306* OE lines (Fig. 3). This means that among the fatty acid biosynthesis genes targeted by each transcription factor, the expression of some genes increased more than that of the single OE lines, on the other hand, the expression of some genes decreased more than that of the single OE lines (Fig. 4). This suggests that co-overexpression of the exogenous transcription factors *RcWRI1A* and *RcMYB306* causes the interaction between them, leading to upregulation of some target genes and downregulation of some genes in fatty acid metabolism in camelina (Fig. 4).

In conclusion, we demonstrated the potential of *RcWRI1A* and *RcMYB306* to enhance fatty acid content in camelina seeds when expressed individually. The simultaneous expression of both transcription factors changes the fatty acid composition but not lead to an increase in seed oil content compared to that of *RcWRI1A* or *RcMYB306* OE lines. Complex genetic interactions, metabolic imbalances, and regulatory interference may have contributed to this outcome. Therefore, further investigations are required to address these challenges and optimize strategies for increasing camelina seed oil production.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13765-024-00927-1>.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

Not applicable.

Author contributions

H.U.K supervised the project. H.U.K and M.-E.P designed the research. M.-E.P performed fatty acid analysis, vector construction, camelina transformation. M.-E.P, I.K, H.J.L performed RT-qPCR analysis. H.U.K and M.-E.P wrote paper. H.U.K, M.-E.P, M.C.S, K.-R.L analyzed the data. All authors read and approved the final manuscript.

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Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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