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Full Length Research Paper

Decreasing erucic acid level by RNAi-mediated silencing of fatty acid elongase 1 (*BnFAE1.1*) in rapeseeds (*Brassica napus* L.)

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The β-ketoacyl CoA synthase encoded by fatty acid elongase 1 gene (*BnFAE1.1*) is a rate-limiting enzyme regulating biosynthesis of erucic acid in rapeseeds *(Brassica napus)*. To develop low level of erucic acid in rapeseeds by intron-spliced hairpin RNA, an inverted repeat unit of a partial *BnFAE1.1* gene interrupted by a spliceable intron was cloned into pCAMBIA3301, and a seed-specific (Napin) promoter was used to control the transcription of the transgene. Four transgenic plants harboring a single copy of transgene were generated. Expression of endogenous *BnFAE1.1* gene in developing T_3 seeds was significantly reduced. In mature T_3 seeds, erucic acid was decreased by 60.8 to 99.1% compared with wild type seeds, and accounted for 0.36 to 15.56% of total fatty acids. The level of eicosenoic acid was also greatly decreased. Furthermore, it resulted in a significant increase in the level of oleic acid, but total fatty acid content in T_3 seeds was the same with that in wild type seeds. In conclusion, the expression of endogenous *BnFAE1.1* was efficiently silenced by the designed RNAi silencer, causing a significant down-regulation in the level of erucic acid. Therefore, the RNAi-mediated post-transcriptional silencing of *FAE1* gene to reduce oleic acid in rapeseeds was an efficient method to breed some new *B. napus* lines.

Key words: *Brassica napus* L., fatty acid elongase, intron-spliced hairpin RNA, down-regulation, erucic acid.

INTRODUCTION

Erucic acid (C22:1), a type of very long chain fatty acid (VLCFA), is one of the major storage fatty acids in Cruciferae seeds. As for its biosynthesis, chain length of C18 fatty acids as precursors are elongated by an enzyme complex known as elongase complex (Harwood, 1988), leading to the synthesis of C22 fatty acids with β-ketoacyl CoA synthase (KCS) encoded by fatty acid elongase 1 (*FAE1*) gene acting as a rate-limiting enzyme (Lassner et al., 1996; Millar et al., 1997). Cloning and functional characterization of *FAE1* gene from several crops are well documented by numerous researchers (Lassner et al.,1996; Millar et al., 1997; Han et al., 2001; Katavic et al., 2001; Das et al., 2002; Mietkiewska et al., 2007). In transgenic mustard, *Brassica juncea*, an over-expression of *BjFAE1* gene caused a 36% increase in erucic acid and antisense gene-mediated down-regulation of this gene caused 86% decrease in erucic acid to as low as 5% in the seed oil of transgenic plants (Kanrar et al., 2006). Regulated by a seed-specific napin promoter, seed-specific expression of nasturtium *Tropaeolum majus FAE1* gene resulted up to 8-fold increase in erucic acid proportions in Arabidopsis seed oil (Barret et al., 1998). Seed-specific expression of *Crambe*

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Abbreviations: DAP, Days after pollination; **FAE1,** fatty acid elongase 1; **ihpRNA,** intron-spliced hairpin RNA; **LEA,** low erucic acid; **PPT,** phosphinothricin; **TFAs,** total fatty acids; **UFA,** unsaturated fatty acid; **VLCFA,** very long chain fatty acid; **WT,** wild type.

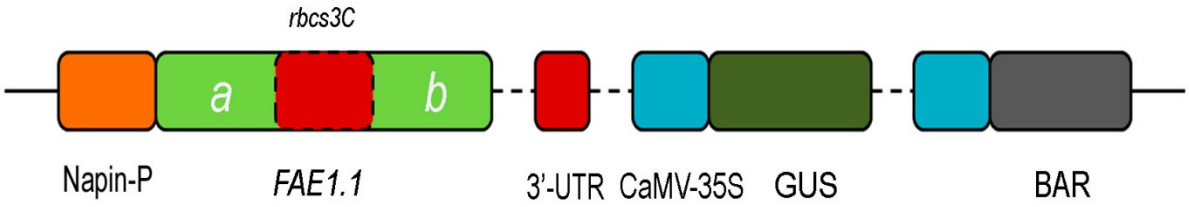

Figure 1. The partial construct of RNAi fragment.

abyssinica *FAE1* gene in *Arabidopsis thaliana* resulted up to a 12-fold increase in the proportion of erucic acid, and in transgenic high-erucic *Brassica carinata* plants, the proportion of erucic acid was as high as 51.9% in the best transgenic line, a net increase of 40% compared with WT lines (Mietkiewska et al., 2007). *BnFAE1.1* was isolated from a cDNA library of immature *B. napus* HEAR embryos and its expression was restricted to the embryo (Barret et al., 1998). Over-expression of the coding region of *B. napus FAE1.1* gene in developing seeds of transgenic *B. napus* resulted in a significant increase in the levels of eicosenoic acid and erucic acid (Han et al., 2001).

Although, erucic acid has wide industrial uses for lubricants and plastics (Ohlrogge, 1994), it is nutritionally unhealthy and undesirable for human consumption, resulting in development of new cultivars with low erucic acid (LEA) as a source of oils. Gene engineering provides efficient ways for generation of LEA *B. napus* germplasms. As a key enzyme responsible for biosynthesis of erucic acid, *FAE1* gene is an important target for down-regulation of erucic acid accumulation. Antisense gene of *BjFAE1* was used to decrease the level of erucic acid in *B. juncea* (Kanrar et al., 2006). Although, anti-sense *FAE1* gene has been applied to decrease the level of erucic acid in HEA rapeseed, it reduced the level of erucic acid from 48 to 33% in T_1 seeds (Zebarjad et al., 2006), which was much higher than that defined by LEA cultivars such as “canola”. Therefore, it is necessary to develop transgenic LEA rapeseed cultivars via much more efficient technology.

Intron containing constructs (ihpRNA) generally give 90 to 100% of independent transgenic plants showing silencing, and the degree of silencing with these constructs is much greater than that obtained by using either co-suppression or anti-sense constructs (Wesley et al., 2001). The ihpRNA-mediated post-transcriptional silencing of *FAE1* gene to efficiently decrease erucic acid biosynthesis was less reported. *BnFAE1.1* was a seed specifically expressed gene, and the highest transcript level was detected in immature embryos at about 24 to 30 days after pollination (DAP) concomitant with the accumulation of erucic acid in rapeseed oil (Han et al., 2001).

In the present study, *BnFAE1.1* gene was used as the target for ihpRNA-mediated post-transcriptional silencing under the control of a seed-specific napin promoter. In transgenic *Brassica* lines, expression of endogenous *BnFAE1.1* gene was effectively down-regulated in immature seeds, and it resulted in a significantly decreased level of erucic acid and eicosenoic acid as well, in addition to a highly increased percent of oleic acid in rapeseed oil.

MATERIALS AND METHODS

Vector construction for RNAi

The vector construct containing an *rbcs3C* gene (GenBank accession: X04334) intron of 83 bp-length from pea *Pisum sativum*, flanked by the specific inverted repeats (designated as FAE1.1-A, and FAE1.1-B) of *B. napus FAE1.1* gene (GenBank accession: AX473306, nt 330-827), a rapeseed napin gene promoter (GenBank accession: AF420598, 1142bp), and 3'-UTR of the CaMV 35S (GenBank accession: AF234297, 209 bp) as terminator, were cloned into pUC19 (Figure 1). Using rapeseed genomic DNA as templates, a seed-specific napin promoter sequence was amplified with primers NapinF and NapinR; two inverted sequences of *BnFAE1.1* (FAE1.1-A, and FAE1.1-B) were amplified with the following primers: FAE1i-1F and FAE1i-1R, FAE1i-2F and FAE1i-2R, respectively. Primers rbcs F and rbcs R were used for cloning *rbcs3C* intron with pea genomic DNA as template. Primers 35SUTRF and 35SUTRR were used for amplification of *CaMV* 35S 3'-UTR from pCAMBIA3301. A list of the primers used is given in Table 1. Also, according to a CTAB protocol (Ausubel et al., 1995; Doyle and Doyle, 1987), genomic DNA was isolated from young leaves of rapeseed and pea, respectively. Subsequently, the RNAi cassette was digested by restriction enzymes *Sal*I and *Sac*I, and then ligated into pCAMBIA3301, followed by characterization of restriction enzyme digestion coupled with sequencing (performed by TaKaRa Biotechnology Co. Ltd, Dalian, China). The construct was then designated as pBERi and subsequently transferred into *Agrobacterium tumefaciens* LBA4404 by freeze-thaw method.

Generation of transgenic rapeseed plants

Seeds of *B. napus* cv. Y015 (a cultivar with about 40% erucic acid in rapeseed oil, developed by Bioengineering Department of Zhengzhou University, China, were sterilized and plated on germination medium (1/2 MS medium, 30 g/L sucrose, 7 g/L agar, pH5.8) at 25°C with a 16 : 8 h light/dark cycles. After 5 to 6 days, the cytoledonary petioles were excised, pre-cultured for 2 days and used as explants for transformation mediated by *A. tumefaciens* strain LBA4404 containing pBERi (Moloney et al., 1989), followed

Table 1. List of primers in the experimental procedures

Primer name	Sequence
Napin F	5'-CGCTGCAGTCTTCATCGGTGATTGATTCCTT-3'
Napin R	5'-CGCCATGGATCCTGTATGTTTTTAATCTTGT-3'
FAE1i-1F	5'-GAGGATCCATGGATGGCCACCAACACGGAACAAG-3'
FAE1i-1R	5'-CAACTAGTCCTTCTCGGAACGGCACGTGCGATG-3'
FAE1i-2F	5'-GTCCTAGGTCCTTCTCGGAACGGCACGTG-3'
FAE1i-2R	5'-GTGGTACCCCACCAACACGGAACAAGCAAT-3'.
rbcs F	5'-CTGGATCCACTAGTTGCAGGTGACAGAAACAT-3'
rbcs R	5'-AGGGTACCCCTAGGACACCTAAATATTCAACA-3'
35SUTRF	5'-GGTGGTACCACGCCGAATTAATTCGGGGGAT-3'
35SUTRR	5'-TGAGCTCAGTAGATGCCGACCGGATCTGTC-3'
FAE1iF1	5'-CCACCAACACGGAACAAGCAAT-3'
rbcsR1	5'-TCAACATAAACGGTTAACAAACTTG-3'
Bar F	5'-CGAGTCTACCATGAGCCCAGAACGACGCC-3'
Bar R	5'-CAAACTCGAGTCAAATCTCGGTGACGGGCA-3'
ACTF	5'-CGCCGCTTAACCCTAAGGCTAACAG-3'
ACTR	5'-TTCTCTTTAATGTCACGGACGATTT-3'
FAE1F	5'-TGGTGAACTCAAGCATGTTTAATCC-3'
FAE1R	5'-CGGCATGTATACAAAAATGGTCGAT-3'

by co-cultivation on regeneration medium (30 g/L sucrose, 2mg/L 6-benzyladenine, 0.15 mg/L α-naphthalene acetic acid, 5 mg/L $AgNO_3$, 7 g/L agar) in the dark at 22°C for 48 h. Subsequently, the explants were transferred to regeneration medium supplemented with 250 mg/L cefotaxime and 12 mg/L phosphinothricin (PPT) for 3 to 6 weeks selection. The regenerated green shoots resistant to PPT were transferred to a rooting medium (30 g/L sucrose, 2 mg/L 3-indole butyric acid, 0.05 mg/L α-naphthalene acetic acid, 250 mg/L cefotaxime, 12 mg/L PPT and 7 g/L agar). After the roots were well established, the candidate transgenic plantlets were transferred to soil and grown in a chamber (16 h light at 19°C/8 h dark at 14°C).

Polymerase chain reaction screening and segregation

Genomic DNA of young leaves from T_0 plants and wild type (WT) control plants was isolated (Ausubel et al., 1995). Two primers FAE1iF1 and rbcsR1 (paired with sense sequence of *BnFAE1.1*, and *rbcs3C* intron sequence, respectively) were used to confirm integration of transgene into genomic DNA of transgenic plants following normal PCR procedure (94°C for 5 min; 94°C for 1 min, 57°C for 1 min, 72°C for 70 s, 35 cycles; 72°C for 10 min). Genomic DNA isolated from WT control plants, was used as the negative control. Subsequently, T_0 plants were self-pollinated and progeny seeds of each plant were harvested separately.

To analyze segregation of transgene in T_0 plants, one hundred T_1 seeds harvested from each T_0 plants were germinated and detected by PCR according to previously described protocol. T_1 plants that harbored transgene with segregation ratio of about 3:1 were grown and selfed to generate T_2 seeds, and collected separately followed by PCR analysis. Subsequently, T_2 plants with homozygous transgene were self-pollinated to generate T_3 seeds, and the T_3 seeds derived from each homozygous T_2 plants were pooled together for fatty acid composition analysis.

Expression analysis of endogenous BnFAE1.1 gene in immature T_3 seeds

Developing T_3 seeds and WT control seeds were separately harvested 30 DAP, frozen immediately in liquid nitrogen, and subsequently stored at -86°C until homogenized. Seeds (approximately 20) were ground in a cold mortar followed by total RNA isolation according to the protocol described in RNA isolation kit (Watson Biotechnologies, Shanghai, China). *B. napus* actin gene (designated as *BnACT*, GenBank Acc. No. AF111812) was used as an internal control, and expression of endogenous *BnFAE1.1* gene in developing seeds was investigated by semi-quantitative one step RT-PCR analysis (One Step RT-PCR kit, TaKaRa, Dalian, China). Primers ACTF and ACTR were used to detect transcript of *BnACT*, primers FAE1F and FAE1R were applied to analyze expression of *BnFAE1.1* gene. With 1 µg / sample used as template, the reaction was performed by reverse transcription at 50°C for 30 min, and then denatured at 95°C for 2 min followed by 25 cycles of amplification (95°C for 1 min, 57°C for 1min, 72°C for 1min). Subsequently, the PCR products were analyzed on a 1% agarose gel supplemented with ethidium bromide. All the experiments were repeated for three times.

Analysis of fatty acid composition in mature T_3 seeds

T3 seeds (approximately 100 per sample) were homogenized and used to extract oil, followed by transmethylation (Kaushik and Agnihotri, 1997). The fatty acid methyl esters (FAMEs) were extracted into heptane, and 0.3 µL supernatant (containing FAMEs) was analyzed by GC-2001 gas chromatography (Jinpu Analytical Instrument Co. Ltd, Shangdong, China) according to its manual. Briefly, the samples were injected in free fatty acid phrase (FFAP) column (0.3 mm × 30 m); operating conditions: injector, 260°C, detector, 300°C, oven, 310°C; FID H2: 40.0 ml/min; carrier gas

makeup nitrogen: 30.0 ml/min. The WT seeds from cv. Y015 were used as controls. Seeds derived from the same homozygous T_1 plants were analyzed in three replications.

RESULTS

Silencing target sequences and plasmid construction

FAE1 has been considered to be a crucial target for modifying the nature and amount of VLCFA (Puyaubert et al., 2005). The 498 bp-length (nt 330-827) sequence of *BnFAE1.1* gene was chosen to be the target for RNA silencing. To enhance efficiency of RNA silencing, strong seed-specific storage protein (napin) promoter was applied to control expression of transgene and the plasmid was constructed to specifically suppress expression of FAE1.1. The target sequence was incorporated into pCAMBIA3301 in inverted-repeat orientations interrupted by a pea rbcS3C intron (83bp). In addition, the vector carried GUS gene and a phosphinothricin (PPT)-resistant *Bar* gene, both driven by CaMV 35S promoter, respectively. The constructed vector was designated as pBERi.

Transformation and regeneration of transgenic plants harboring a single copy of transgene

With cytoledonary petioles as the explants, plasmid pBERi was transformed into *B. napus* mediated by *A. tumefaciens* LBA4404. Subsequently, the PPT-resistant plantlets were regenerated, and the insertion of silencing fragments was confirmed by genomic DNA PCR (data not shown). In total, 25 transgenic plants were grown and self-pollinated to generate T_1 seeds, followed by separate harvest. One to several copies of transgene was possibly inserted into rapeseed genome. To achieve transgenic lines with expected stable traits, transgenic lines with a single copy of ihpRNA gene were confirmed. Based on the Mendelian law, T_0 plants with segregation ratio of 3 : 1 were probably predicted to harbor single copy transgene. 100 T_1 seeds generated by each T_0 plants were germinated, and then existence of ihpRNA gene was analyzed by genomic DNA PCR. As a result, segregation ratio of transgene from four lines (TR1 - 4) was statistically 3 : 1, which revealed they had a single copy of the transgene (Table 2). T_1 plants with a single transgene were independently grown and self-pollinated to generate homozygous T_2 seeds. As aforementioned, T_2 homogenous plants of transgene were screened by PCR and separately grown to generate T_3 seeds.

Silencing of FAE1 gene decreased biosynthesis of erucic acid in seeds

As previously documented, the highest transcript levels of *BnFAE1.1* gene were found in immature embryos at about 24-30 DAP concomitant with the accumulation of erucic acid in rapeseed oil (Han et al., 2001). Moreover, the concentration of napin protein appears at 20 DAP and increases continuously until reaching a maximum between 30 and 35 DAP (Hoglund et al., 1992). In immature T_3 seeds of 30 DAP, transcript level of endogenous *FAE1.1* was investigated by semi-quantitative one step RT-PCR. Data indicate that for the expression of the internal control actin gene, there was no difference between WT control and transgenic seeds. However, *FAE1.1* gene was greatly suppressed compared with that in WT control seeds. Furthermore, the transcripts of *FAE1.1* gene in developing seeds of TR4-3-1 lines were down-regulated over 96% and represented the lowest level. In other three transgenic lines, transcripts of *FAE1.1* gene accounted for less than 35% of that in WT seeds (Figure 2A). In addition, relative intensity of transcripts (ratio: *FAE1.1* / Actin) decreased from 33.7% in WT control to approx. 11% (in TR1-8-3, TR2-5-2 and TR3-2-4), and 1.3% in TR4-3-1, respectively (Figure 2B). It indicated that expression of endogenous *FAE1.1* gene was efficiently suppressed in immature seeds of all the analyzed transgenic lines.

To explore alteration caused by RNA silencing of *FAE1.1* gene, the level of erucic acid in mature T_3 seeds were determined by GC analysis. It revealed that erucic acid level was much lower in T_3 seeds from all the analyzed transgenic lines than that in WT control seeds with a significance (p = 0.05) (Figure 3). Erucic acid content in TR4-3-1 seeds was decreased by 99.1% compared with WT control seeds, and it accounted for only 0.36% of total fatty acids (TFAs). And in T_3 seeds from the other three lines (TR1-8-3, TR2-5-2, and TR3-2-4) it was down-regulated more than 60% (Figure 4). Corresponding to silencing of *FAE1.1* gene in immature T_3 seeds, erucic acid level in mature T_3 seeds indicated a remarkable down-regulation.

Silencing of FAE1 gene has distinct impact on levels of eicosenoic acid and oleic acid

Silencing of *FAE1* gene and down-regulation of erucic acid content may affect levels of other fatty acids. Eicosenoic acid in VLCFA family and oleic acid are the precursors for biosynthesis of erucic acid that was catalyzed by elonga````se complex including *FAE1*. Data show that eicosenoic acid content was also significantly down-regulated (p = 0.05) in T_3 seeds similar to *FAE1.1* transcripts and the erucic acid level. In contrast, oleic acid content was greatly enhanced (Figure 3). Oleic acid in WT seeds accounted for 22.5% of TFAs, but in T_3 seeds it accounted for more than 50%. In TR4-3-1 seeds, it represented the highest level (60%) among the transgenic lines. There was a significantly negative correlation between oleic acid content and the levels of eicosenoic and erucic acid (p = 0.01).

Table 2. Segregation ratio analysis of ihpRNA transgene by PCR screening in T_1 seeds.

Line	Number of positive seed	Number of negative seed	Segregation ratio
TR1	71	29	2.448
TR2	69	31	2.226
TR3	77	23	3.348
TR4	73	27	2.704

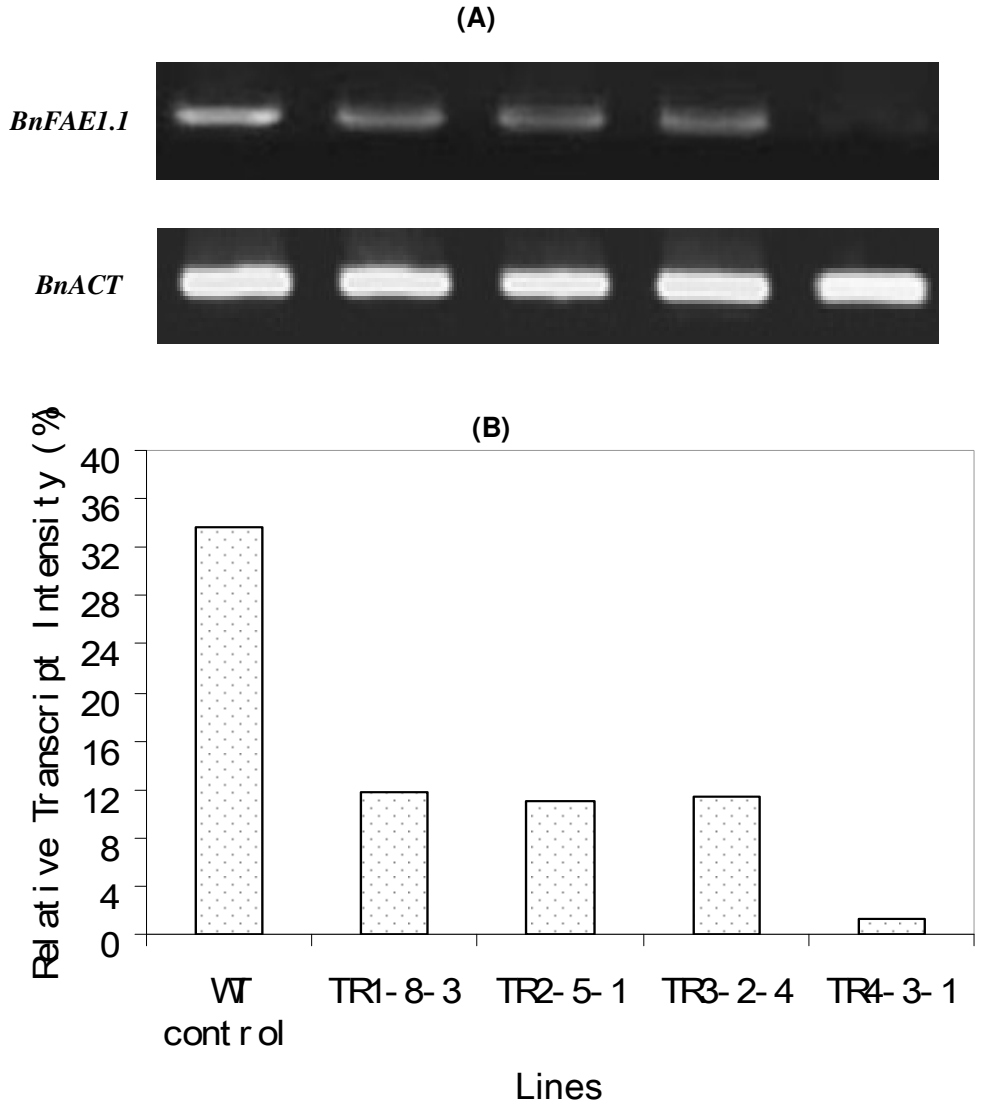

Figure 2. Semi-quantitative RT-PCR analysis of *FAE1.1* gene transcripts in T_3 developing seeds. The total RNA used as the templates (1 µg / sample) for RT-PCR were isolated from the developing seeds harvested about 30 DAP from WT plant (WT control) and transgenic lines (TR1-8-3, TR2-5-1, TR3-2-4 and TR4-3-1). Expression of actin gene in *B. napus* (*BnACT*) was used as an internal control, and the experiment was repeated for three times. (A), the amplified fragments of *BnFAE1.1* and *BnACT* are 641 bp and 322 bp, respectively; (B), Relative transcript intensity is the ratio of transcripts (*BnFAE1.1* / *BnACT*).

Furthermore, levels of the other four fatty acids (palmitic, stearic, linoleic and linolenic acid) indicated no remarkable difference between WT seeds and T3 seeds (Figure 3), and the level of total C18 unsaturated fatty acids (UFAs) in T3 seeds was significantly higher than that in WT seeds ($p = 0.05$). However, the level of TFAs

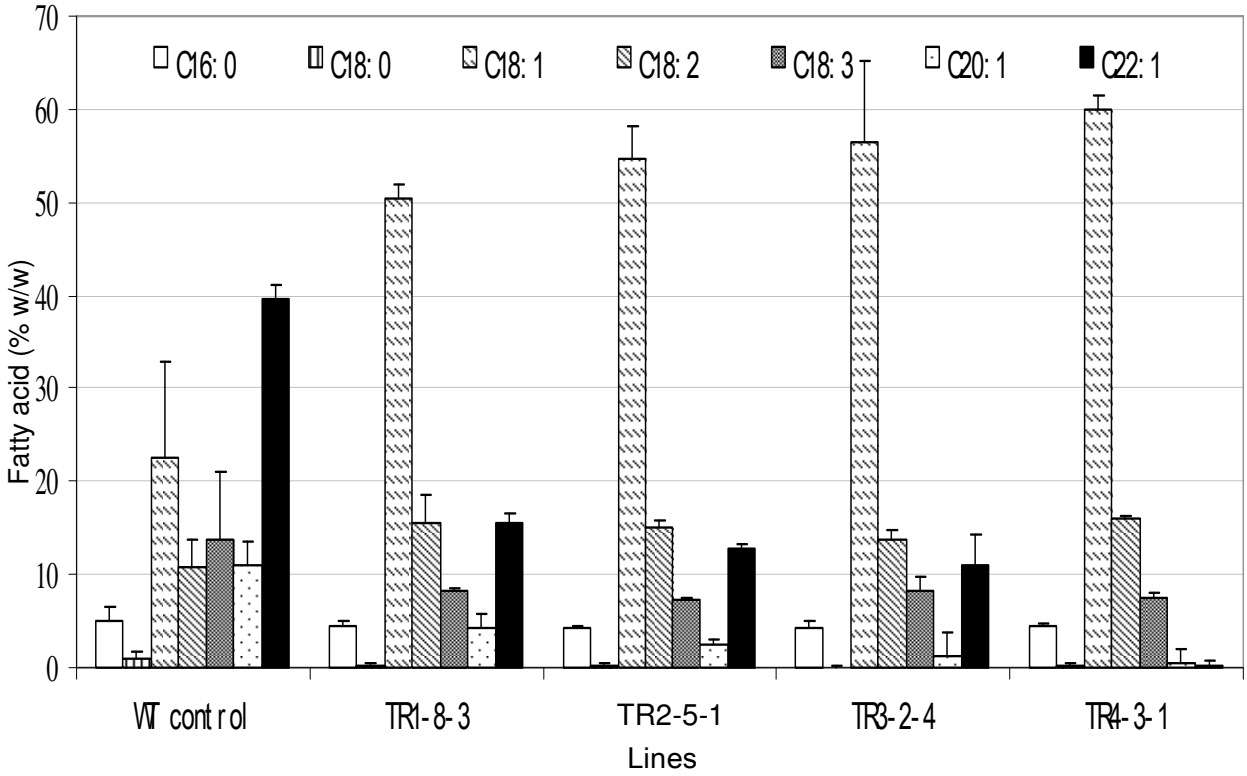

Figure 3. Fatty acid composition in T3 seeds. The fatty acid composition in T3 mature seeds was measured by GC analysis with WT seeds as control. And each bar represents the average of three measurements ± SD of the pooled seed samples from five individual plants.

in T3 seeds was the same as that in WT seeds.

DISCUSSION

Post-transcriptional gene silencing (PTGS) occurs via a mechanism responsible for the production of double-stranded or self-complementary hpRNA, and the constructs designed to express such transcripts were an efficient way of inducing targeted gene silencing (Chuang and Meyerowitz, 2000; Smith et al., 2000; Levin et al., 2001; Matzke et al., 2001; Vance and Vaucheret, 2001; Wesley et al., 2001). In the present study, intron-spliced hairpin gene silencing construct (ihpRNA) was efficient in suppressing expression of *FAE1.1* gene in developing T_3 seeds, and it resulted in significant down-regulation in the levels of erucic acid and eicosenoic acid in addition to a remarkable increase in the level of oleic acid in rapeseed oil. In the analyzed four lines, the level of erucic acid was reduced by 60 to 99% in seeds (Figure 4). This indicated efficient silencing of target gene (*FAE1.1*) in transgenic rapeseed. Application of a functional intron sequence (pea rbcS3C intron) coupled with a seed-specific napin promoter probably led to such a high efficiency and proportion of independent silenced lines.

PCR analysis showed a segregation of the single copy transgene in four T_0 plants (TR1-8-3, TR2-5-2, TR3-2-4 and TR4-3-1) accorded with Mendelian law. However, the other T_0 plants are predicted to harbor several copies of transgene based on PCR screening of their T_1 plants. In this report, we did not confirm integration of ihpRNA transgene by genomic DNA blotting analysis, considering that the silencing target fragment existed synchronously both in rapeseed genome and in transgene. Napin is an abundant seed-specific storage protein, and its promoter has been widely used to modulate fatty acid composition in *Brassica* species (Brough et al., 1996; Katavic et al., 2000, 2001; Stoutjesdijk et al., 2002; Jadhav et al., 2005). The level of erucic acid was successfully increased and/or decreased in oilseeds under the control of the seed-specific napin promoter (Katavic et al., 2000, 2001; Mietkiewska et al., 2004, 2007; Jadhav et al., 2005). Furthermore, the transcripts of both napin and *FAE1.1* gene peaked at approx. 30 DAP concomitant with the accumulation of erucic acid in developing *B. napus* seeds (Hoglund et al., 1992; Han et al., 2001).

In the present work, under the control of rapeseed napin promoter, ihpRNA transgene was efficiently transcribed in immature seeds of 30 DAP, which was evidenced by remarkable silencing of endogenous *FAE1.1* gene. Based on previous study, although, a minimum of two *FAE1* isoforms were found in rapeseed

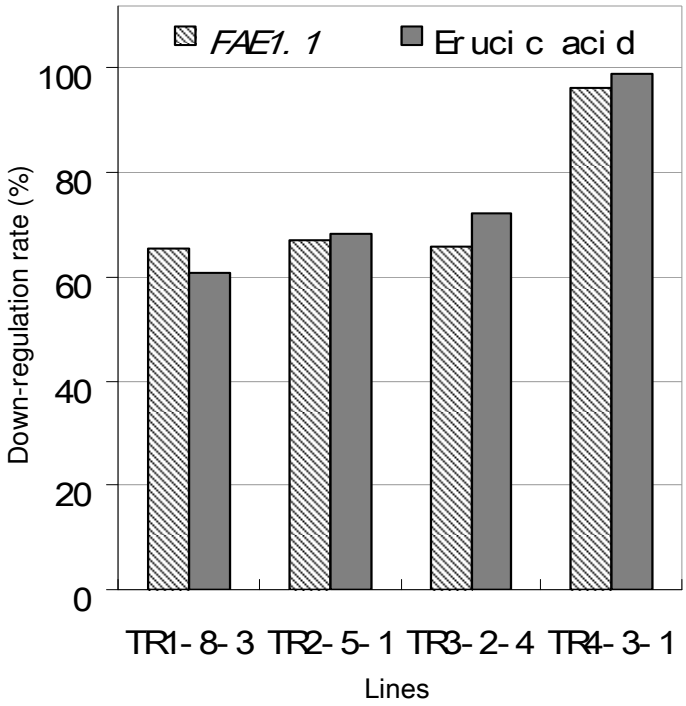

Figure 4. Down-regulation of *FAE1.1* gene and erucic acid composition in T_3 seeds. Transcript of *FAE1.1* gene in T_3 immature seeds (30 DPA) was analyzed by RT-PCR, and erucic acid composition in T_3 mature seeds was detected by GC analysis. Wild type seeds (WT control) were used as control.

genome, *BnFAE1.1* gene tightly linked with E1 locus was associated with the broad variation in erucic acid content in rapeseed and expressed at a high level (Barret et al.,1998; Puyaubert et al., 2001). In immature T_3 seeds from different homozygous lines with a single copy transgene, transcripts of endogenous *BnFAE1.1* gene were remarkably down-regulated at a different level (from 65 to 96%) (Figure 4). Different transcript intensity of transgene, which probably resulted from different insertion loci of ihpRNA in rapeseed genome, presumably caused such a distinct difference in silencing. Nevertheless, the effection of different insertion loci on transcript levels of transgene remain to be identified. In addition, the target sequence for ihpRNA silencing was homologous to those of its isoforms (example, *FAE1.2* linking with E2 locus) (Barret et al., 1998; Fourmann et al., 1998; Han et al., 2001; Puyaubert et al., 2001; Wu et al., 2008) and they were possibly silenced along with *FAE1.1* silencing in case expressed in developing seeds.

FAE1 gene was the rate-limiting enzyme for biosynthesis of VLCFAs, and efficient silencing of *FAE1* gene results in decreased levels of erucic and eicosenoic acid in rapeseed oil. Corresponding to down-regulation of *FAE1.1* gene, biosynthesis of both fatty acids was greatly suppressed in mature T_3 seeds. Erucic and eicosenoic acid accounted for not more than 15.6 and 4.3%, respectively. And in TR4-3-1 seeds, the level of erucic acid (0.36%) is much lower than 2% defined by LEA cultivars such as “canola” (Downey, 1990). Thereafter, reduction of VLCFAs content directly increased accumulation of oleic acid, which caused no significant fluctuation of the other C18 UFAs. This suggests that the transcript levels of other genes closely related to biosynthesis of C18 UFAs (example SAD, FAD2 and FAD3) were constant, although, their expression patterns were not investigated. However, it has been demonstrated that the decrease in the level of erucic acid increased accumulation of upstream fatty acids such as oleic acid and linoleic acid, but decreased the level of linolenic acid in transgenic rapeseed with antisense construct of the *FAE* gene (Zebarjad et al., 2006). In addition, Spearman correlation analysis indicated that transcript intensity of *FAE1.1* shared a significant negative correlation with erucic and eicosenoic acid content, and a significant positive correlation with the levels of oleic acid and the total C18 UFAs (p = 0.01) (data not shown). Therefore, the analyzed transgenic lines, especially TR4-3-1, probably indicate potential applications in the future breeding program.

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