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Full Length Research Paper

Fatty acid elongase 1 (FAE1) promoter as a candidate for genetic engineering of fatty acids to improve seed oil composition

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As an important *cis*-regulatory element, a promoter plays a key role in plant gene expression and regulation, and has been widely used in plant genetic engineering. The fatty acid elongase 1 (FAE1) promoter was isolated from *Arabidopsis thaliana*. Sequence analysis showed that the FAE1 promoter contains two Skn-1 motifs, one O_2-site, and one G-box, which play important roles in controlling seed-preferred expression. Stearoyl-acyl carrier protein desaturase (SAD) catalyzes the conversion of stearic to oleic acid. 23 FAE1pro:GUS and 31 FAE1pro:SAD transgenic lines were obtained and confirmed by polymerase chain reaction (PCR). In the FAE1pro:GUS transgenic lines, β-glucuronidase (GUS) staining was observed in immature leaves, young stems, flowers, and pods, indicating that FAE1 promoter is highly active in these tissues. Real-time PCR revealed that the FAE1 promoter-driven maize *SAD* gene was highly expressed in the seeds, followed by the pods and flowers; however, its expression level was low in the roots, stems, and leaves. Stearic acid and long-chain saturated fatty acid contents of seeds were significantly decreased; further, the ratio of total saturated fatty acids to unsaturated fatty acids was also reduced. These results indicate that FAE1 promoter is ideal for genetic engineering to improve seed oil composition.

Key words: *Arabidopsis thaliana*, FAE1 promoter, stearoyl-acyl carrier protein desaturase (*SAD*), seed fatty acid composition.

INTRODUCTION

In most economically valuable crops, such as soybean and canola, seeds are natural storage organs that accumulate high levels of lipids. Many genes are involved in the biosynthesis and accumulation of storage lipids. Among these, the stearoyl-acyl carrier protein desaturase (*SAD*) gene plays a key role in determining the content of stearate acid and long-chain saturated fatty acids, as well as the ratio of total saturated fatty acids to unsaturated fatty acids in plants (Knutzon et al., 1992; Lindqvist et al., 1996). *SAD* has been used in genetic engineering to improve the fatty acid composition in seeds (Knutzon et al., 1992; Liu et al., 2002; Zarhloul et al., 2006). However, previous studies have shown that seeds with constitutive expression of the *SAD* gene probably did not germinate (Knutzon et al. 1992); therefore, a seed-preferred promoter with an appropriate developmental timing is as a key strategy for genetic engineering.

Many seed-preferred promoters have been isolated or used in the genetic engineering of fatty acids, including the β-ketoacyl-CoA synthase (KCS) gene promoter (Han et al., 2001), the unknown seed protein (USP) promoter (Bäumlein et al., 1991b), the FatB4 promoter of the acyl-thioesterase gene (Töpfer and Martini, 1994), the Dc3 promoter from *Daucus carota* (Seffens et al., 1990),

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Abbreviations: KCS, β-Ketoacyl-CoA synthase; **USP,** unknown seed protein; **SAD,** stearoyl-acyl carrier protein desaturase; **PCR,** polymerase chain reaction; **cDNA,** complementary deoxyribonucleic acid; **FAE1,** fatty acid elongase 1; **GUS,** β-glucuronidase.

Table 1. Sequences of oligonucleotide primers used in this study.

Primer	Sequence (5′-3′)	Usage
FAE1 forward primer	AAGCTTCTAGTAGATTGGTTGGTTGGTTTCC	FAE1 promoter cloning
FAE1 reverse primer	TCTAGATGCTCTGTTTGTGTCGGAAAATAATGG	FAE1 promoter cloning
ACTIN1 forward primer	CATCAGGAAGGACTTGTACGG	Real-time PCR
ACTIN1 reserves primer	GATGGACCTGACTCGTCATAC	Real-time PCR
uidA forward primer	TCCTGTAGAAACCCCAACCCG	Amplifying *uidA* gene by PCR
uidA reverse primer	GGCCCTTCACTGCCACTGACC	Amplifying *uidA* gene by PCR
SAD forward primer	GGATATGAGGCAGATTGAGAAGACA	Amplifying *SAD* gene by PCR
SAD reverse primer	TGAAGCAAGGGTGCAAAGGTAG	Amplifying *SAD* gene by PCR

the legumin protein gene promoter (LeB4) (Bäumlein et al., 1991a), the conlinin 1 promoter, and napin promoter (Cheng et al., 2010). Some characteristics of fatty acids may require expression of more than one transgene (Liu et al., 2002). Use of the same promoter for multiple transgenes is not advisable, because promoter homology can lead to transgene silencing (Brusslan and Tobin, 1995; Park et al., 1996). Therefore, it is necessary to evaluate potential seed-preferred promoters for the genetic engineering of fatty acids.

The FAE1 promoter isolated from *Arabidopsis thaliana* is highly active and embryo specific (Rossak et al., 2001). The promoter has been used to increase the amount and composition of carotenoids in canola seeds by genetic engineering (Fujisawa et al., 2009). Rossak et al. (2001) speculated that the FAE1 promoter is superior to the napin promoter and may be ideal for genetic engineering of fatty acids to improve seed oil composition. However, the FAE1 promoter has not been tested, and little is known about its effectiveness in the genetic engineering of fatty acids. The purpose of this study was to clone the FAE1 promoter, in combination with the maize SAD gene, and to study its potential application for genetic improvement of fatty acid content in plants.

Plant materials and growth condition

The *A. thaliana* ecotype Columbia (Col-0) was used in this study. Seeds were sown on Murashige-Skoog (MS) medium and were subjected to cold treatment at 4°C for 2 days. Seedling of *Arabidiosis* were typically planted 12 per 64 cm^2 pot in moistened potting soil (Sunshin Mix # 1; Sun Gro Horticulture Inc., Bellevue, W, USA), and grown in a greenhouse under darkness for 8 h at 20°C and under light for 16 h at 23°C, with a light intensity of 60-150 µmol m^{-2} s^{-1} (Chung et al., 2000). Plants at the flowering stage were transformed by the floral dip method (Clough and Bent, 1998). Seeds harvested from the transformed plants were treated with 70% ethanol for 60 s, then with 1% Sodium hypochlorite for 15 min, followed by three rinses with sterile water. Sterilized seeds grown on (MS) medium containing 100 mg·L^{-1} kanamycin, 50 mg·L^{-1} ampicillin, and the resistant plants were obtained.

Bacterial strain, plasmid, and *Agrobacterium* infection

The specific primers for FAE1 promoter are listed in Table 1. The FAE1 promoter was isolated from *A. thaliana* by polymerase chain reaction (PCR), and PCR conditions used were: 2 min of initial denaturation at 94°C, 30 cycles of 94°C for 15 s, 65°C for 30 s, 72°C for 45 s, followed by a final extension at 72°C for 7 min (Rossak et al., 2001). The *cis*-regulatory elements in FAE1 promoter were predicted using PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html). The FAE1 promoter fragment was subcloned into the pBI121 plasmid at the *Hind*III and *Xba*I cloning sites by replacing the CaMV35s sequence in order to develop FAE1pro:GUS (Figure 1a). A full-length cDNA fragment of the maize SAD gene was isolated by Liu et al. (2009), then was subcloned into pBI121FAE1 at the *Xba*I and *Sst*I cloning sites by replacing the *uidA* sequence in order to develop FAE1pro:SAD (Figure 1b). The above 2 constructs were introduced into *Agrobacterium tumefaciens* GV3101 by a freeze and thaw method (An et al., 1988), and the integrity of the 2 constructs in *Agrobacterium* cells was confirmed by performing restriction enzyme analysis. The bacterial cultures were grown in 50 mL of Luria-Bertani (LB) liquid medium supplemented with 50 mg·L^{-1} rifampicin and 100 mg·L^{-1} kanamycin at 28°C and 150 rpm. Cells were harvested by centrifugation and re-suspended in an infiltration medium to an OD_{600} of 0.8 (Clough and Bent, 1998). The *Agrobacterium*-mediated transformation of *Arabidopsis* was conducted as described by Clough and Bent (1998).

Histochemical GUS assays

GUS assays (Jefferson et al., 1987) were conducted on 23 transgenic FAE1pro:GUS lines. Three T_2 seedlings, roots, stems, leaves, flowers, and pods were randomly chosen for histochemical GUS assays, with 3 biological replications. Control samples were obtained from non-transformed plants. Histochemical reactions were performed at 37°C for several hours. After staining, these samples were rinsed in 70% ethanol for 5 min, and were soaked in Federal Aviation Administration (FAA) liquid.

Transgene analysis

Total genomic deoxyribonucleic acid (DNA) was extracted from young leaves of transgenic *Arabidiosis* plants in accordance with the method of Weigel and Glazebrook (2002). PCR primers for the *uidA* and *SAD* (Liu et al., 2009) genes were designed by primer 5.0

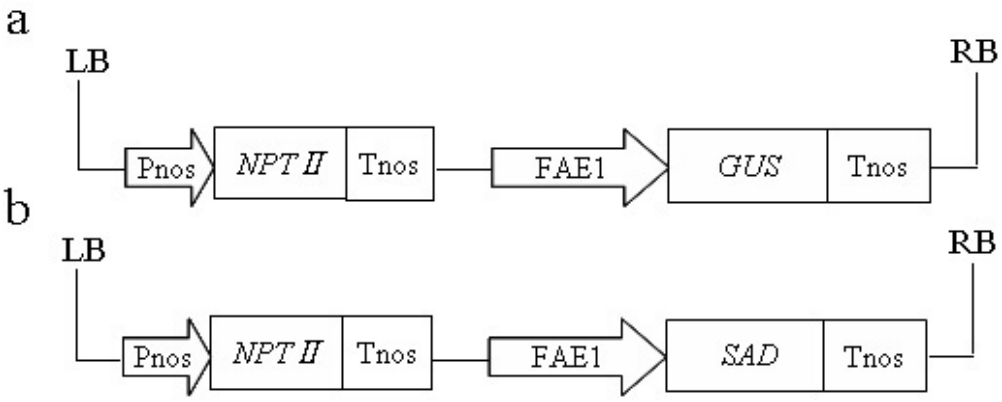

Figure 1. Schematic representation of the FAE1-GUS (a) and FAE1-SAD (b) constructs (not to scale). LB and RB, T-DNA left and right borders, respectively; Pnos, nopline synthase gene promoter; Tnos: nopline synthase gene terminator; ***NPTII***: neomycin phosphor-transferase II; **GUS**, β-glucuronidase gene; **FAE1**, fatty acid elongation 1 condensing enzyme promoter; **SAD**, stearoyl-acyl carrier protein desaturase.

software and are listed in Table 1. The PCR parameters for *gusA* were the same as those for *SAD*, including 1 cycle of 95°C for 3 min; 35 cycles of 95°C for 1 min, 58°C for 1 min, and 72°C for 1 min; and 1 cycle of 72°C for 10 min. Samples were stored at 4°C. The PCR product for *gusA* was 826-bp long and that for *SAD* was 517-bp long, and these products were separated on 1% (w/v) agarose gels with EtBr.

Real-time polymerase chain reaction (PCR) analysis of SAD gene expression

The roots, stems, leaves, flowers, pods, and seeds were collected from mature FAE1pro:SAD transgenic plants for ribonucleic acid (RNA) extraction using the RNAiso reagent (Takara, Japan). Complementary deoxyribonucleic acid (cDNA) was synthesized using the oligo(dT) primers following a first-strand cDNA synthesis kit (Takara, Japan). The *actin1* gene (NM179953) was used as an endogenous control to normalize the expression data (Du et al., 2010). SAD and *actin1* primers are listed in Table 1. Real-time PCR was conducted using the SYBR real-time PCR kit (Takara, Japan) with IQ™ SYBR® Green Supermix according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA). The reaction conditions were as follows: 94°C for 1 min, followed by 40 cycles of 95°C for 10 s, and then 60°C for 20 s. Maize SAD gene expression level in the root of the SAD-9 transgenic line was used as the control. The resulting melting curves were visually inspected to ensure specificity of product detection, and gene expression was quantified using the comparative CT (Cycle Threshold) method. Experiments were performed three times, and all results are presented as the mean ± standard deviation (SD) (Zhou et al., 2009).

Determination of fatty acid composition and content

Seeds were harvested from each transgenic (T_2) line. Three transgenic controls (FAE-2, FAE-8, and FAE-11) and 7 transgenic lines (SAD-9, SAD-11, SAD-12, SAD-15, SAD-21, SAD-22, and SAD-27) were chosen for analyzing the composition of fatty acids. Approximately, 0.1-0.15 g of seeds was used for each assay, and 3 replicates were conducted. Total lipid was extracted from seeds as described by Sukhija and Palmquist (1988) with some modifications: 0.1-0.15 g of each sample was weighed and transferred to 15 ml culture tubes. To each tube, 4 ml methyl alcohol:acetyl chloride (10:1) were added, followed by 5 ml of internal reference (methyl nonadecanoate, Sigma, USA), with 1 mg/ml in hexane, and after general vortexing, heated for 2 h in a water bath at 80°C. After which the contents were cooled to room temperature, 5 ml of 7% K_2CO_3 were added to neutralize extracted fatty acids, and the upper phase was transferred to sample bottles. Samples at 250°C were automatically injected (1 µl) and separated in the GC system equipped with a HP-INNOWAX polyethylene glycol capillary column (30 m× 320 µm×0.5 µm, Agi;emt Tecjmp;pgoes) (Yang et al., 2010). The fatty acid composition was analyzed using gas chromatography (Hewlett-Packard, Palo Alto, CA). The using of gas chromatographe has been described in previous studies (Yang et al., 2010). Fatty acids were identified by comparison of their retention times with that of the internal reference. Fatty acid data were acquired on ChemStation software (Agilent Technologies), and imported into Excel, and the ratio of total saturated to unsaturated fatty acids was analyzed. Means data for the individual transgenic lines were compared using the *t* test.

RESULTS

Isolation of the FAE1 promoter from *Arabidopsis* and bioinformatics analysis

A 934 bp fragment was obtained. Sequence fidelity of the cloned fragment to reported one (Rossak et al., 2001) was confirmed by sequencing. The main *cis*-elements that are characteristic of a promoter sequence were revealed by PlantCARE prediction, including the TATA (-25 to -28 bp) and CAAT (-52 to -55 bp) boxes, which are the basic transcriptional *cis*-regulatory elements. Many *cis*-elements related to endosperm-specific expression, such as two Skn-1 motifs, one O2-site, and one G-box, were found in the FAE1 promoter (Figure 2). The two Skn-1 motifs were on the negative strand at positions -660 to -664 bp and -714 to -718 bp; the O2-site was on the plus strand at -656 to -665 bp; and the G-box was on the plus strand at -726 to -735 bp (Figure 2). The G-box, O2-site, and Skn-1 motif are important *cis*-regulatory

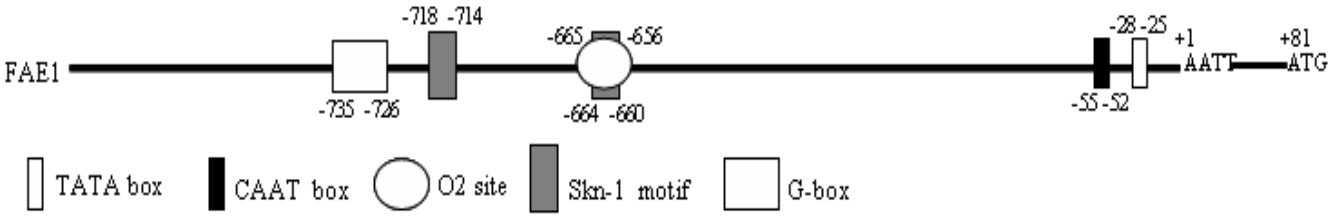

Figure 2. Analysis of key cis-regulatory elements in the FAE1 promoter. The transcriptional start site AATT is given, and the cis-regulatory elements are presented schematically with the orientation and position in base pairs relative to this site. One TATA box, one CAAT box, one O2-site, one G-box, and two Skn-1 motifs were present in the FAE1 promoter.

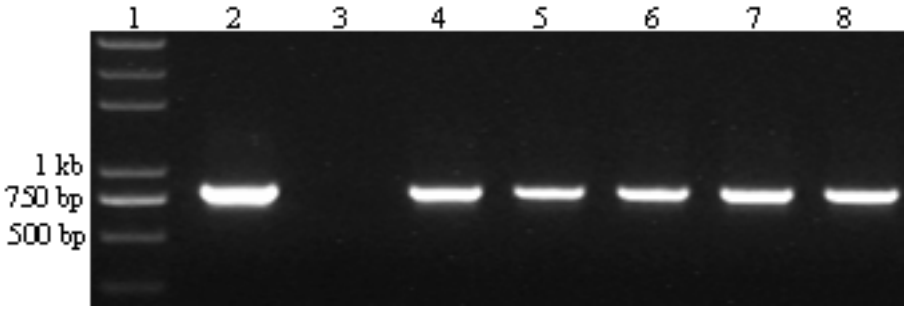

Figure 3. PCR analysis of transgenic plants using *gusA* primers. Total genomic DNA was extracted from 23 transgenic lines, respectively and was amplified with GUS primers. The products were separated on 1% (w/v) agarose gels. The results show the expected 826 bp DNA fragment, indicating that the lines were transgenic and the FAE1-GUS construct was integrated in the genome. Lane 1, DNA maker DL2000; lane 2, pBI121 FAE1::GUS plasmid; lane 3, WT *Arabidopsis thaliana*; lanes 4-8, transgenic FAE1-GUS lines.

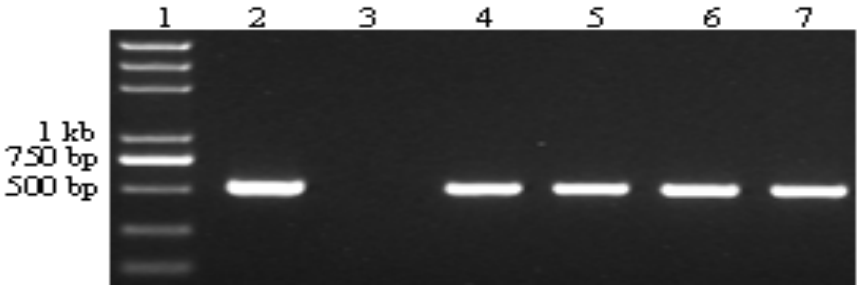

Figure 4. PCR analysis of transgenic plants using SAD primers. 517-bp DNA fragments were amplified from 31 transgenic FAE1-SAD lines, demonstrating that FAE1-SAD constructs have been integrated into *Arabidopsis* genome. Lane 1, DNA maker DL2000; lane 2, pBI121 FAE1::GUS plasmid; lane 3, Wild-type *Arabidopsis thaliana*; lanes 4-7, transgenic FAE1-SAD lines.

elements required for endosperm expression, indicating that the FAE1 promoter may be active in the endosperm.

Molecular characterization of transgenic plants

Resistant plants emerged from the MS medium after approximately 2 weeks of kanamycin selection. Twenty-three FAE1pro:GUS and 31 FAE1pro:SAD putative transgenic plants were obtained, and they did not show any morphological alterations when compared to non-transgenic plants. Total genomic DNA was extracted from the young leaves of each plant and was used for PCR analysis. Primer pairs specific for *uidA* and *SAD* gene detection were employed, and PCR products that were 826 and 517 bp in length were obtained from FAE1pro:GUS and FAE1pro:SAD lines, respectively confirming that all tested plants were transgenic (Figures 3 and 4).

Histochemical analysis of GUS gene activity was performed in these 23 transgenic FAE1pro:GUS lines (T_2). The rosette and cauline leaves, roots, flowers, pods and

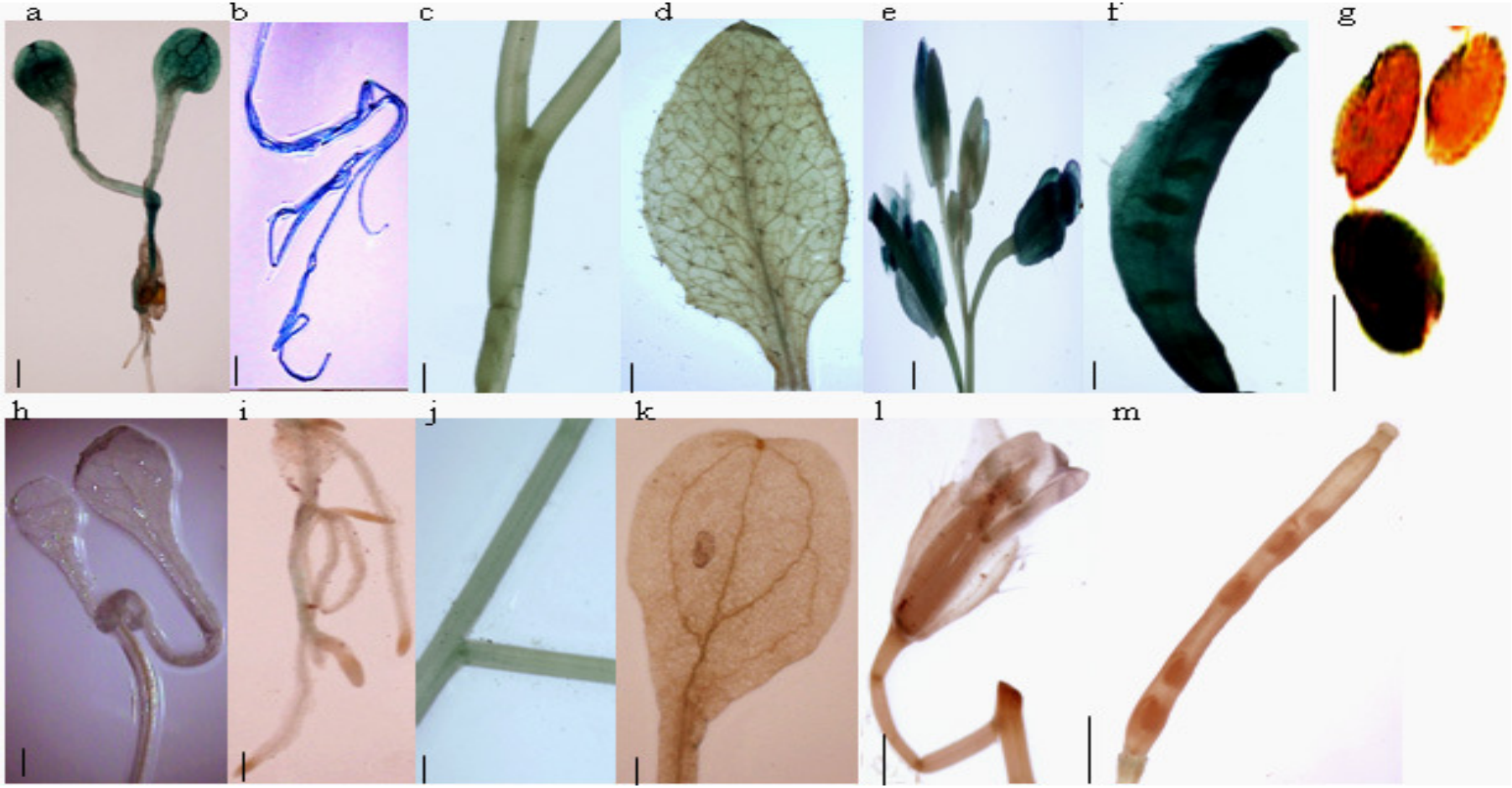

Figure 5. Histochemical analysis of GUS expression in T_2 transgenic *Arabidopsis* plants harboring the FAE1-GUS construct. In transgenic FAE1-GUS lines, GUS staining was not observed in the young roots (a), mature roots (b), mature stems (c), or mature leaves (d); young leaves and stems (a) displayed blue GUS staining; Strong GUS staining was exhibited in the flowers (e), pods (f) and seeds (g). h-m was WT *Arabidopsis* plants, and were used as the control. The scale bar indicates 1 mm.

immature seeds from 3 individual plants of each line were stained. At the rosette stage, all young leaves and stems showed blue GUS staining, but the roots did not (Figure 5a). However, after bolting, GUS activity was observed only in flowers, pods (Figure 5e and f), as well as seeds (Figure 5g), and not in the roots, stems, or leaves (Figure 5b, c and d). Taken together, these results suggest that the FAE1 promoter is active in rosette leaves and stems in the rosette stage and in flowers, pods, and seeds after bolting, confirming that the FAE1 promoter is not tissue specific.

Analysis of Stearoyl-acyl carrier protein desaturase (SAD) gene expression and fatty acid composition

Specific primer pairs for the maize SAD gene were designed, and the level of SAD gene expression

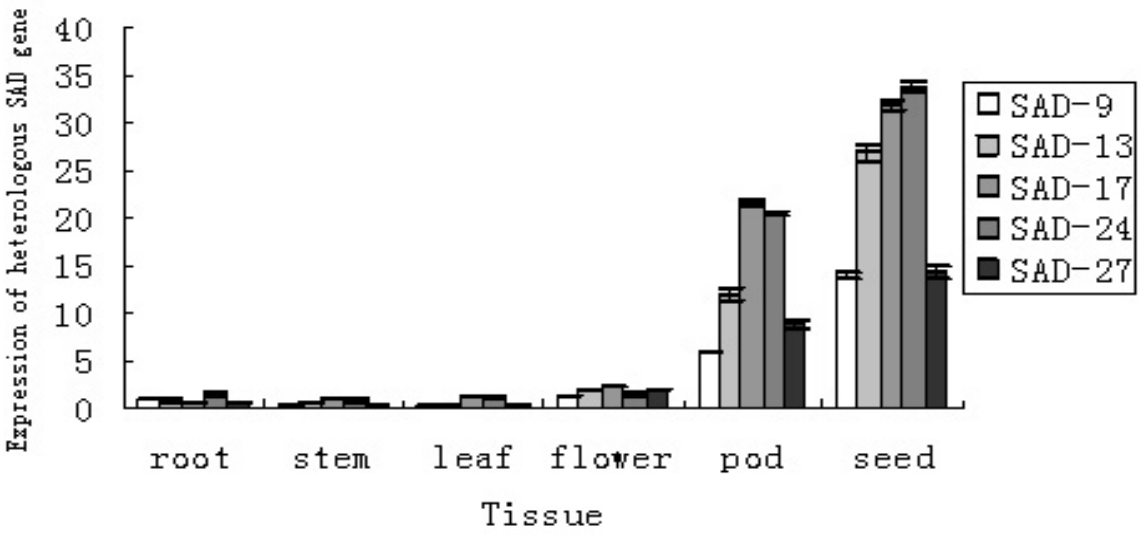

Figure 6. Expression levels of heterologous *SAD* gene in the organs of transgenic *Arabidopsis* with FAE1-SAD constructs. Total RNA isolated from roots, stems, leaves, flowers, and pods of transgenic *Arabidopsis* were used in quantitative real-time PCR. The relative transcript levels were calculated by using the *Arabidopsis actin1* gene (NM179953) as an internal control. The results shown are mean values ± SD of three independent analyses.

driven by the FAE1 promoter in different tissues was investigated by real-time PCR. The results show that the SAD gene expression level varied in different organs. The highest gene expression was detected in seeds, followed by pods and flowers. The maize SAD gene exhibited the lowest expression level in the roots, stems, and leaves (Figure 6). In the SAD-13 transgenic line, maize SAD gene expression in seeds and pods was 99 and 58 fold higher, respectively compared to that in the roots. Similar results were obtained for the SAD-17, SAD-24, SAD-27, and SAD-30 transgenic lines. The expression level of the SAD gene driven by the FAE1 promoter was still high in pods and flowers in transgenic lines, demonstrating that FAE1 promoter is highly active in these organs. The highest gene expression was found in seed. Therefore, the FAE1 promoter is seed preferred, and not embryo or seed specific.

Three (3) FAE1pro:GUS transgenic lines and seven (7) FAE1pro:SAD transgenic lines were selected to determine the fatty acid content and composition of seeds by gas chromatography. The FAE1pro:GUS transgenic lines were used as the control plants. Compared to the control plants, in the transgenic plants, *SAD* gene expression controlled by the FAE1 promoter led to the desired phenotype. The mean content of stearic acid in the controls was 3.37%, while in the SAD transgenic lines it significantly decreased to 2.84% (Table 2). The analysis also revealed a significantly decreased long-chain saturated fatty acid content (2.88%). In addition, we observed a decreased ratio of total saturated to unsaturated fatty acid (0.1675%). The fatty acid composition was significantly altered when FAE1 promoter-driven *SAD* gene expression was transferred into *Arabidopsis*. Therefore, although the FAE1 promoter is seed-preferred, it still is adequate for the genetic engineering of seed oil composition.

DISCUSSION

The FAE1 promoter is active as early as the torpedo stage in embryos, and its activity persist throughout subsequent embryonic development, indicating that the FAE1 promoter is indeed an embryo-specific promoter (Rossak et al., 2001). The results agree with Rossak et al. (2001), but also show that the FAE1 promoter is not specific for seed. FAE1 promoter activity was also observed in young leaves, young stems, flowers, and pods (Figure 5a, e and f). Therefore, the FAE1 promoter isolated from *Arabidopsis* is not embryo specific or seed specific, but may be seed preferred.

Genetic engineering is an efficient way to improve the content and composition of plant fatty acids in plant seeds. Overexpression of the *AtFATB1* gene resulted in accumulation of high amounts of palmitate in seeds, while antisense expression resulted in a reduction of seed and flower palmitate content (Dörmann et al., 2000). The concentrations of oleic acid in Brassica seeds, hydroxyl fatty acid in *Arabidopsis* seeds, and eicosenoic in soybeans were improved by genetic engineering (Stoutjesdijk et al., 2000; Smith et al., 2000; Cahoon and Shanklin, 2000). The expression of Δ6 desaturase driven by CaMV35s promoter in tobacco and flax resulted in a high-level accumulation of GAL in vegetative tissue, but the level in seeds was still low (Sayanova et al., 1999; Qiu et al., 2002).

Table 2. Fatty acid composition of *Arabidopsis thaliana* T2 seeds from transgenic lines harboring the FAE1 or FAE1-SAD gene constructs.

Material	C18:0					Long-chain saturated fatty acid					Ratio of total saturated to unsaturated fatty acid				
	1	2	3	Mean		1	2	3	Mean		1	2	3	Mean	
FAE-2	3.35	3.3	3.36	3.34±0.03		2.86	3.23	3.08	3.06±0.19		0.1744	0.1751	0.1748	0.1748±0.0004	
FAE-8	3.24	3.33	3.28	3.28±0.05	3.37±0.10	3.29	2.96	3.07	3.11±0.17	3.07±0.03	0.1762	0.1769	0.1773	0.1768±0.0006	0.1768±0.002
FAE-11	3.42	3.54	3.47	3.48±0.06		2.91	3.1	3.12	3.04±0.12		0.1781	0.1794	0.1792	0.1789±0.0007	
SAD-9	2.63	2.89	2.73	2.75±0.13		3.01	2.72	2.94	2.89±0.15		0.1674	0.1662	0.1645	0.166±0.0015	
SAD-11	2.94	2.72	2.85	2.84±0.11		2.74	2.81	2.87	2.81±0.07		0.1671	0.1683	0.169	0.1681±0.0010	
SAD-12	2.92	3.01	2.88	2.94±0.07		2.79	2.85	2.93	2.86±0.07		0.1566	0.1631	0.1647	0.1615±0.0043	
SAD-15	2.64	2.78	2.69	2.70±0.07	2.84±0.13**	2.78	2.89	2.72	2.80±0.09	2.88±0.06**	0.1689	0.1634	0.1658	0.1660±0.0028	0.1675±0.004**
SAD-21	3.11	2.99	2.91	3.00±0.10		3.09	2.91	2.82	2.94±0.14		0.1689	0.1671	0.1649	0.1670±0.0020	
SAD-22	2.62	2.67	2.74	2.68±0.06		3.04	2.87	2.95	2.95±0.09		0.1778	0.1657	0.1645	0.1693±0.0074	
SAD-27	3.07	2.84	3.01	2.97±0.12		2.86	3.08	2.77	2.90±0.16		0.1743	0.1732	0.1764	0.1746±0.0016	

Mean values ± standard errors of 3 replicates selected fatty acids in relation to the total fatty acid content.
*Significantly different from the control (P<0.05).

Therefore, seed-preferred promoters are superior to constitutive promoters for the genetic engineering of plant fatty acids. In the present study, we have showed that the FAE1 promoter was highly active and seed-preferred (Figures 5g and 6). SAD, as a key gene in fatty acid metabolic pathway, was driven by FAE1 promoter, and transferred into *Arabidopsis*. The content of stearic acids and long-chain saturated fatty acids in seeds was significantly decreased in transgenic *Arabidopsis* (Table 2). Seed oil composition in *Arabidopsis* has been modified effectively in present study, and suggesting that the FAE1 promoter can be useful for fatty acid genetic engineering of oil crops, such as canola, camelina and soybean.

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REFERENCES

An G, Ebert PR, Mitra A, Ha SB (1988). Binary vectors. In: Gelvin SB, Schilperoot RA (eds) Plant Mol Biology Manual, Matinus Nijhoff, Dordrecht, The Netherlands, ppA3: 1-19.

Bäumlein H, Boerjan W, Nagy I, Panitz R, Inzé D, Wobus U (1991a). Upstream sequence relating legumin gene expression in heterologous transgenic plants. Mol. Gen. Genet., 225: 121-128.

Bäumlein H, Boerjan W, Nagy I, Bassüner R, Van Montagu M, Inzé D, Wobus U (1991b). A novel seed protein gene from *Vicia faba* is developmentally regulated in transgenic tobacco and *Arabidopsis* plants. Mol. Gen. Genet., 225: 459-467.

Brusslan JA, Tobin EM (1995). Isolation of new promoter-mediated co-suppressed lines in *Arabidopsis thaliana*. Plant Mol. Bio., 27: 809-813.

Cahoon EB, Shanklin J (2000). Substrate-dependent mutant complementation to select fatty acid desaturase variants for metabolic engineering of plant seed oils. Proc. Natl. Acad. Sci. USA, 97: 12350-12355.

Cheng BF, Wu GH, Vrinten P, Falk F, Bauer J, Qiu X (2010). Towards the production of high levels of eicosapentaenoic acid in transgenic plants: the effects of different host species, genes and promoters. Transgenic Res., 19: 221-229.

Chung M, H, Chen MK, Pan SM (2000). Floral spray transformation can efficiently generate *Arabidopsis* transgenic plants. Transgenic Res., 9: 471-476.

Clough SJ, Bent AF (1998). Floral dip: a simplified method for agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J., 16(6): 735-743.

Dörmann P, Voelker TA, Ohlrogge JB (2000). Accumulation of palmitate in arabidopsis mediated by the acyl-acyl carrier protein thioesterase FATB1. Plant Physiol., 123: 637-643.

Du HW, Zhang ZX, Li JS (2010). Isolation and functional characterization of a waterlogging-induced promoter from maize. Plant Cell Rep., 29: 1269-1275.

Fujisawa M, Takita ET, Harada H, Sakurai N, Suzuki H, Ohyama K, Shibata D, Misawa N (2009). Pathway engineering of *Brassica napus* seeds using multiple key enzyme genes involved in ketocarotenoid formation. J. Exp. Bot., 60(4): 1319-1332.

Han J, Lühs W, Sonntag K, Zähringer U, Borchardt DS, Wolter FP, Heinz E, Frentzen M (2001). Functional characterization of beta-ketoacyl-CoA-synthase genes from *Brassica napus* L. Plant Mol. Biol., 46: 229-239.

Jefferson RA, Kavanagh TA, Bevan MW (1987). GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J., 6: 3901-3907.

Knutzon DS, Thompson GA, Radke SE, Johnson WB, Knauf VC, Kridl JC (1992). Modification of *Brassica* seed oil by antisense expresion of a stearoyl-acyl carrier protein desaturase gene. Proc. Natl. Acad. Sci. USA, 89: 2624-2628.

Lindqvist Y, Huang WJ, Schneider G, Shanklin J (1996). Crystal structure of Δ^9 stearoyl-acyl carrier protein desaturase from castor seed and its relationship to other di-iron proteins. EMBO J., 15(16): 4081-4092.

Liu Q, Singh SP, Green AG (2002). High-stearic and high-oleic cottonseed oils produced by hairpin RNA-mediated post-transcriptional gene silencing. Plant Physiol., 129: 1732-1743.

Park YD, Papp I, Moscone EA, Iglesias VA, Vaucheret H, Matzke AJM (1996). Gene silencing mediated by promoter homology occurs at the level of transcription and results in meiotically heritable alterations in methylation and gene activity. Plant J., 9: 183-194.

Qiu X, Hong HP, Datla N, MacKenzie SL, Tayler DC, Thomas TL (2002). Expression of borage Δ6-desaturase in *Saccharomyces cerevisiae* and oilseed crops. Can. J. Bot., 80: 42-49.

Rossak M, Smith M, Kunst L (2001). Expression of the FAE1 gene and FAE1 promoter activity in developing seeds of *Arabidopsia thaliana*. Plant. Mol. Biol., 46: 717-725.

Sayanova OV, Davies GM, Smith MA, Griffiths G, Stobart AK, Shewry PR, Napier JA (1999). Accumulation of Δ6-unsaturated fatty acids in transgenic tobacco plants expressing a Δ6-saturase from *Borago officinalis*. J. Exp. Bot., 50: 1647-1652.

Seffens WS, Aloguera C, Wilde HD, Haar RAV, Thomas TL (1990). Molecular analysis of a phylogenetically conserved carrot gene: developmental and environmental regulation. Dev. Genet., 11: 65-76.

Smith M, Moon H, Kunst L (2000). Production of hydroxyl fatty acids in the seeds of *Arabidopsis thaliana*. Biochem. Soc. Trans., 28: 947-950.

Stoutjesdijk PA, Hurlestone C, Singh SP, Green AG (2000). High-oleic acid Australian *Brassica napus* and *B.juncea* varieties produced by co-suppression of eddogenous delta-12 desaturases. Biochem. Soc. Trans., 28: 938-940.

Sukhija PS, Palmquist DL (1988). Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem., 36: 1202-1206.

Töpfer R, Martini N (1994). Molecular cloning of cDNAs or genes edcoding proteins involved in de novo fatty acid biosynthesis in plants. Plant Physiol., 143: 416-425.

Weigel D, Glazebrook J (2002). *Arabidopsis*: a laboratory manual, pp.165-166.Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Yang XH, Guo YQ, Yan JB, Zhang J, Song TM, Rocheford T, Li JS (2010). Major and minor QTL and epistasis contribute to fatty acid compositions and oil concentration in high-oil maize. Theor. Appl. Genet., 120: 665-678.

Zarhloul MK, Stoll C, Lühs W, Syring-Ehemann A, Hausmann L, Töpfer R, Friedt W (2006). Breeding high-stearic oilseed rape (*Brassica napus*) with high-and low-erucic background using optimized promoter-gene constructs. Mol. Breed, 18: 241-251.

Zhou Y, Zhu JY, Li ZY, Gu F, Zhang HG, Tang SZ, Gu MH, Liang GH (2009). Fine mapping and cloning of *MT1*, a novel allele of *D10*. Progress in Natural Sci., 19: 1683-1689