Metabolic Mechanisms Associated with Alleles Governing the 16:0 Concentration of Soybean Oil

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ABSTRACT: Soybean [*Glycine max* (L.) Merr.] oil typically contains *ca.* 11% palmitic acid, but germplasm has been developed with less than 4% to about 35% 16:0. A number of recessive alleles associated with these phenotypes have been described that represent different mutations at *Fap* loci, however, the gene products (enzymes) produced by these alleles are unknown. This work attempts to define the metabolic activities that are regulated by the fap_1, fap_2, and fap_{nc} alleles in soybean. Observation of *de novo* synthesis and metabolic turnover of fatty acids esterified to phospholipids in cotyledons during the period of peak oil accumulation revealed genotypic differences in the supply of 16:0-CoA from plastids. These metabolic studies narrowed the identification of fap_1, fap_2, and fap_{nc} alleles to the genes that encode or regulate the 3-keto-acyl-ACP synthetase II (where ACP is acyl carrier protein), 16:0-ACP thioesterase, 18:0-ACP desaturase, or 18:1-ACP thioesterase enzymes. Kinetic analyses suggested that the fap_2 mutation results in a decreased 3-keto-acyl-ACP synthetase II activity. Deficiencies in 16:0-ACP thioesterase activity represented the most likely explanation of fap_1 and fap_{nc} gene function. This hypothesis was strongly supported by Northern blot assays that revealed a significant reduction in the accumulation of transcripts corresponding to the 16:0-ACP thioesterase in germplasm homozygous for the fap_{nc} allele.

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KEY WORDS: Developing-seed, *fap*-alleles, genetics, glycerolipid composition, *Glycine max*, metabolism, oil, palmitic acid, phospholipid, saturated fat.

Palmitic acid (16:0) concentration in soybean typically averages about 11% of crude oil (1). However, this phenotypic trait may be genetically altered by certain mutations at gene loci designated as *Fap*. Nearly all of the described mutations at *Fap* have been induced by chemical mutagenesis, with the exception of the first recognized low-16:0 soybean [N79-2077-12, (2,3)]. N79-2077-12 is the only germplasm that carries a serendipitous natural mutation, which now is temporarily designated as the recessive fap_{nc} allele. Germplasm with about 150% of normal 16:0 levels may carry homozygous recessive fap_2 [C1727, (4)], fap_{2b} [A21, (5)], fap_4 [A24, (5)], or fap_5 [A27, (6)] alleles. Various combinations of these alleles may elevate 16:0 concentration to 35% of crude oil (6). However, classical Mendelian genetic studies provide the only evidence that fap_2, fap_{2b}, fap_4, and fap_5 might represent independent genetic events or nonallelic mutations at *Fap* loci. The gene products (enzymes) of these alleles also are unknown.

Soybean germplasm exhibiting about 50% of normal 16:0 levels may carry homozygous recessive fap_1 [C1726, (4)], fap_3 [A22, (5)], fap_{nc} [N79-2077-12, (3)], or the temporary designation *fap** [ELLP2, (7)] alleles. Combinations of homozygous fap_1 and fap_3 (8), fap_1 and fap_{nc} (9), or fap_1 and *fap** (7) alleles constitute transgressive segregates, from matings of the respective parental lines, that exhibit less than 4.5% 16:0. The inbred lines C1943 (with northern maturity) and N94-2575 (with southern maturity) are examples of selections in which fap_1 and fap_{nc} are combined (10). Based on this information, it is highly probable that the mutations represented by the fap_3, fap_{nc}, and *fap** descriptors are different from fap_1. However, it is not known whether fap_3, fap_{nc}, and *fap** are independent or allelic to each other, primarily because exclusive licensing agreements restrict access to the germplasm. Although these circumstances impede classical Mendelian genetic studies for uniqueness and allelism among these three alleles, alternate lines of evidence may be used to make these distinctions (11). Indeed, a more precise approach is warranted given the typical ambiguities that are inherent to interpretation of data from progeny segregating for a phenotypic trait. Such need becomes even more important in consideration of the seemingly limitless number of current and potential mutations that may be generated by mutagenic agents at *Fap* loci.

As an example, evidence supporting the hypothesis that inheritance of fap_{nc} is independent of fap_1 (9) would be strengthened by identification of the enzyme(s) that are affected by these two alleles. Such a determination has not been made for any *fap* allele in soybean. This issue is complicated by the number of enzyme activities that could be influenced by mutations at *Fap* loci. Within the fatty acid and glycerolipid synthetic pathways in plants (12), changes in the activity or substrate specificity of at least eight enzymes [3-keto-acyl-ACP, (acyl carrier protein) synthetase I (KAS-I), 3-keto-acyl-ACP syn-

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thetase II (KAS-II), 18:0-ACP desaturase (Δ9DES), 16:0-ACP thioesterase (16:0-ACP TE), 18:1-ACP thioesterase (18:1-ACP TE), glycerol-3-phosphate acyltransferase (G3PAT), lysophosphatidic acid acyltransferase (LPAAT), and diacylglycerol acyltransferase (DGAT)], theoretically, could alter the 16:0 concentration of soybean oil. These enzyme activities also may be influenced by modifier genes (genes of minor effect) that are associated with major effects of *fap* loci (13).

Only a few investigations have explored the potential metabolic targets for *fap* and related modifier gene action in soybean (2,14). Those studies tested the hypothesis that fap_1, fap_2, and fap_{nc} alleles altered the activity of glycerolipid acyltransferase enzymes. Based on a comparison of *in vivo* saturation kinetics for triacylglycerol (TG) synthesis from exogenous acetate and 16:0-CoA, it was apparent that these *fap* alleles did not affect the activities of G3PAT, LPAAT, DGAT, or any event downstream of acyl-CoA formation. Rather, 16:0 concentration appeared to be a function of endogenous 16:0-CoA synthesis or supply from plastids of developing soybean cotyledons. This finding focused attention on KAS-II, Δ9DES, 16:0-ACP TE, or 18:1-ACP TE as the prime candidates for fap_1, fap_2, and fap_{nc} gene products. Among enzymes in the fatty acid synthetic pathway, KAS-II catalyzes the elongation of 16:0-ACP to 18:0-ACP, Δ-9DES desaturates 18:0-ACP to 18:1-ACP, 16:0-ACP TE initiates the conversion of 16:0-ACP (and 18:1-ACP to a lesser degree, with little activity on 18:0-ACP) to their respective acyl-CoA derivatives, and 18:1-ACP TE is reported to have a marked preference for 18:1-ACP (15). Hence, alteration of any of these enzyme activities could effect change in 16:0 concentration in soybean seed.

Of these enzymes, the 16:0-ACP TE activity probably displays the highest control in determining the 16:0 content of vegetable oils. For example, the 16:0 content of cottonseed (16) and high-16:0 sunflower germplasm (17) is attributed to elevated 16:0-ACP TE activity (albeit lower KAS-II activity also may be involved in the latter case). Seed of transgenic canola that express a 16:0-ACP TE gene from *Cuphea* are reported to have 35% 16:0 (15), and similar transgenic events appear to increase 16:0 levels in *Arabidopsis* (18). In transgenic soybean embryos (19), it is observed that increased 16:0-ACP TE activity leads to elevated esterification of 16:0 in total lipids (TL) and decreased export of 18:1-CoA from plastids. Conversely, antisense constructs or sense suppression of the 16:0-ACP TE gene produce the opposite effect on the levels of 16:0-CoA and 18:1-CoA that are available for glycerolipid synthesis. In comparison, overexpression of the KAS-II or the Δ9DES gene had little effect on the 16:0 concentration of soybean oil (19). Therefore, it would appear that genetic manipulation of 16:0-ACP TE activity is the most effective way to alter the 16:0 concentration of soybean oil directly.

By implication, we may then speculate that reduced 16:0-ACP TE activity represents a means to obtain a low-16:0 phenotype, and that increased 16:0-ACP TE activity is a viable mechanism for obtaining higher 16:0 concentration in soybean. However, there is no direct evidence that any *fap* allele in soybean represents natural or chemical mutations in the gene(s) that encodes 16:0-ACP TE, or that an association exists between these alleles and expression of 16:0-ACP TE activity. Although 16:0-ACP TE activity must be considered a prime candidate for influencing the 16:0 content of soybean oil, it is likely that some *fap* alleles correspond to genes other than those that govern 16:0-ACP TE activity.

Assessing genotypic differences in 16:0-ACP TE activity with purified protein would help solve this conundrum. However, most plants contain very low levels of extractable 16:0-ACP TE (20). Thus, it is prudent to learn more about 16:0 metabolism in these genotypes before launching full-scale efforts to purify and assay enzymes involved in this complex process. In that regard, monitoring *in vivo* 16:0 metabolism in phospholipids or total polar lipid (TPL) of developing seed is a practical means to gain such information since combinations of homozygous recessive or dominant fap_1, fap_2, and fap_{nc} alleles produce significant effects on total phospholipid-16:0 concentration in developing cotyledons. In addition, observation of transcript accumulation for genes that encode KAS-II, Δ9DES, 16:0-ACP TE, or 18:1-ACP TE may show differences that are related to genetic effects on 16:0 concentration. These approaches were used in the current report, and led to the conclusion that the both the fap_1 and fap_{nc} alleles influence 16:0-ACP TE activity in soybean. Evidence also is presented that suggests the fap_2 mutation results in a decreased KAS-II activity.

MATERIALS AND METHODS

Plant material. Five highly inbred soybean [*Glycine max* (L.) Merr.] germplasm lines exhibiting homozygous genes at the *Fap* locus were grown at the Central Crops Research Station in Clayton, NC. These lines included: C1726 ($fap_1\,fap_1\;Fap_1Fap_2\;Fap_{nc}Fap_{nc}$), C1727 ($Fap_1Fap_1\,fap_2fap_2\;Fap_{nc}\,Fap_{nc}$), N79-2077-12 ($Fap_1Fap_1\;Fap_2Fap_2\,fap_{nc}fap_{nc}$), N94-2575 ($fap_1fap_1\;Fap_1Fap_2\,fap_{nc}fap_{nc}$), and the cv. Dare ($Fap_1Fap_2\;Fap_2Fap_2\;Fap_{nc}Fap_{nc}$). The stage of seed development was reported in days after flowering (DAF) for each genotype.

Tissue analyses. Representative dry mass and oil content were determined in seed (20 to 30 g fresh weight) harvested at intervals between 30 and 70 (mature) DAF. Extraction and isolation of TPL followed the methods described by Wilson and Kwanyuen (21). Fatty acid methyl esters (FAME) were prepared from TPL by heating with 1 mL 5% (vol/vol) sulfuric acid in methanol at 80°C for 90 min. After cooling to ambient temperature, the reaction mixture was vortexed with 1.5 mL 1.5% NaCl plus 1 mL hexane and held at –20°C to allow phase separation. The hexane phase (top) was removed, dried under nitrogen at 55°C, and resuspended in 100 μL 2:1 chloroform/ methanol prior to analysis by gas chromatography (GC). Individual FAME (16:0, 18:0, 18:1, 18:2, and 18:3) derived from TPL were separated by reversed-phase thin-layer chromatography (TLC) developed using 10% $AgNO_3$ in acetonitrile/1,4-dioxane/acetic acid (80:20:1, vol/vol/vol) (22). Fatty acid composition was determined with a Hewlett-Packard model 5890-II/gas chromatograph (Palo Alto, CA)

equipped with a model 7673 auto sampler, dual flame-ionization detectors, and dual 0.53 mm × 30 m AT-Silar capillary columns (Alltech Associates Inc., Deerfield IL). Operating conditions were: carrier, He (3 mL/min); 25:1 (vol/vol) split injection; injection temperature, 250°C; detector temperature, 275°C; column temperature, 190°C.

In vivo *acetate saturation kinetics.* Whole cotyledons (0.5 g fresh weight) harvested at 35 DAF were incubated at 25°C in 3 mL 0.2 N 2-[*N*-morpholino]ethane-sulfonic acid (MES) buffer, pH 5.5, with 5 μCi [2-^{14}C]acetate (57 mCi mmol^{-1}; American Radiolabeled Chemicals, Inc., St. Louis, MO) plus one of three levels (0.0, 1.0, or 10.0 μmol) of potassium acetate (Fisher Scientific Co., Fair Lawn, NJ). Reactions were terminated at 2 h. Glycerolipid extraction and analysis followed the methods described above. Radioactivity in lipid fractions was measured with a Packard Tri-CARB 2100TR liquid scintillation spectrometer (Meriden, CT) and expressed relative to the specific activity of total acetate added to each reaction. The resultant data emulated sigmoidal curves from which kinetics of the synthetic reactions at saturating substrate levels were interpreted with Hofstee plots (23). All data were reported as means of three replications, and least significant difference ($LSD_{0.05}$) was used to determine treatment effects.

Pulse-chase incorporation of acetate. Whole cotyledons (0.5 g fresh weight) harvested at 35 DAF were incubated at 25°C in 10 mL 0.2 N MES buffer, pH 5.5, with 5 μCi [2-^{14}C]acetate (57 mCi mmol^{-1}; American Radiolabeled Chemicals, Inc.) for 2 h. The incubation media was removed, and cotyledons were washed with three 5-mL vol of deionized water. Incubations were resumed with 1.0 mmol potassium acetate (Fisher Scientific Co.) in 10 mL 0.2 N MES buffer, pH 5.5. Reactions were terminated after 0.0, 0.5, 1, 2, 4, 8, or 12 h. Glycerolipid extraction and TPL analysis followed the methods described above.

Northern blot analysis. Total RNA was extracted from whole soybean cotyledons harvested at 25, 35, and 45 DAF as previously described (24). Poly(A)+ RNA was recovered using the MessageMaker mRNA Isolation system according to the manufacturer's procedures (GibcoBRL, Rockville, MD). Total RNA (30 μg) and Poly(A)+ RNA (5 μg) were electrophoresed through a 1.2% (wt/vol) formaldehyde gel and transferred to Hybond-N+ nylon membrane (Amersham Pharmacia Biotech, Piscataway, NJ) according to standard protocols (25). Soybean cDNA encoding the 16:0-ACP thioesterase (FAT B), 18:1-ACP thioesterase (FAT A), and plastidal Δ-9 desaturase (PDS 1) were ^{32}P-radiolabeled using the Random Primed Labeling Kit (Boehringer Mannheim, Indianapolis, IN). The FAT B, FAT A, and PDS 1 probes used in these experiments were proprietary products of E.I duPont de Nemours & Co. RNA blots were incubated overnight with probe at 42°C in a solution containing 0.9 M NaCl, 60 mM $Na_2H_2PO_4 \cdot H_20$ (pH 7.4), 1 mM EDTA], 50% (vol/vol) formamide, and 10× Denhardt's solution (25). Blots were washed at 42°C in 0.3 M NaCl, 0.03 M sodium citrate, and 0.1% (wt/vol) sodium dodecyl sulfate (SDS) for 15 min, followed by a 15 min wash in 0.15 M NaCl, 0.015 M sodium citrate, and 0.1% (wt/vol) SDS at 65°C. To standardize sample loading or transfer efficiency, each hybridized blot was stripped of its primary probe using boiling water and rehybridized to either ^{32}P-radiolabeled 25S ribosomal RNA gene (for blots probed with PDS 1) or soybean ubiquitin cDNA (for FAT A and FAT B). Hybridization was visualized and quantified using a phosphorimager (Molecular Dynamics, Sunnyvale, CA).

RESULTS AND DISCUSSION

At 35 DAF, the stage of reproductive growth when peak accumulation or synthesis of 16:0 occurs in soybean seed (14), 16:0 concentration in germplasm carrying homozygous dominant or recessive combinations of fap_1, fap_2, and fap_{nc} alleles ranged from *ca*. 9 to 25% of TPL (Table 1). A strong positive relation (R^2, 0.94) between these data and 16:0 concentration in TL at seed maturity (Fig. 1) supports similar observations made earlier (19) and indicates that TPL composition at 35 DAF is predictive of the fatty acid composition at seed maturity. Also, the lack of a relation (R^2, 0.03) between the observed 16:0 and stearic acid (18:0) concentration among these genotypes is consistent with postulates that genetic control of 18:0 concentration in soybean oil is independent of these three *fap* alleles (26).

TPL also has been shown to be the most metabolically active glycerolipid fraction in developing soybean cotyledons (27), therefore, genotypic differences in endogenous 16:0 incorporation in TPL at 35 DAF should provide metabolic indicators of *fap* allele function. *In vivo* saturation kinetics of exogenous acetate metabolism in developing seed at 35 DAF were used to test this hypothesis. Over all genotypes, TPL at 35 DAF accounted for 72.7 ± 1.9% of the total lipid radioactivity in 16:0 synthe-

TABLE 1
Effect of *Fap* Alleles on Total Phospholipid Composition in Developing Soybean Cotyledons

		16:0	18:0	Other[a]	TPL[b]	
Germplasm	Alleles	(% of TPL at 35 DAF)			(% of total lipid)	(mg/seed)
C1727	Fap_1 fap_2 Fap_{nc}	25.2	4.9	69.9	11.6	1.5
N79-2077-12	Fap_1 Fap_2 fap_{nc}	11.2	4.7	84.1	11.0	2.1
C1726	fap_1 Fap_2 Fap_{nc}	17.1	6.1	76.8	13.4	1.8
N94-2575	fap_1 Fap_2 fap_{nc}	8.9	5.6	85.5	10.7	1.6
DARE	Fap_1 Fap_2 Fap_{nc}	17.5	4.9	77.6	14.5	1.6
$LSD_{0.05}$		5.7	1.0	6.3	2.0	0.3

[a]Other: 18:1 + 18:2 + 18:3.
[b]TPL, total polar lipid or total phospholipid; DAF, days after flowering; LSD, least significant difference.

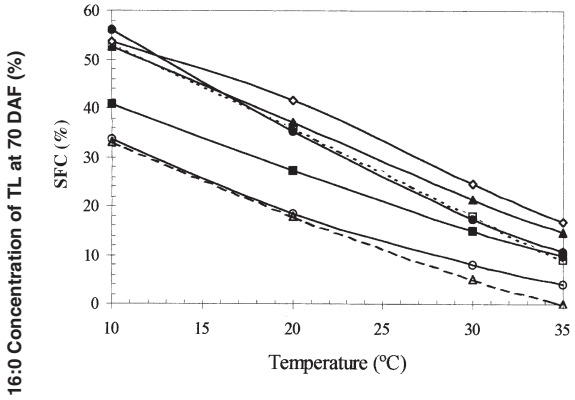

FIG. 1. Relation of 16:0 concentration in total polar lipid (TPL) at 35 days after flowering (DAF) and total lipid (TL) at seed maturity. C1726 (fap_1fap_1 Fap_2Fap_2 $Fap_{nc}Fap_{nc}$), C1727 (Fap_1Fap_1 fap_2fap_2 $Fap_{nc}Fap_{nc}$), N79-2077-12 (Fap_1Fap_1 Fap_2Fap_2 $fap_{nc}fap_{nc}$), N94-2575 (fap_1fap_1 Fap_2Fap_2 $fap_{nc}fap_{nc}$), and the cv. Dare (Fap_1Fap_1 Fap_2Fap_2 $Fap_{nc}Fap_{nc}$).

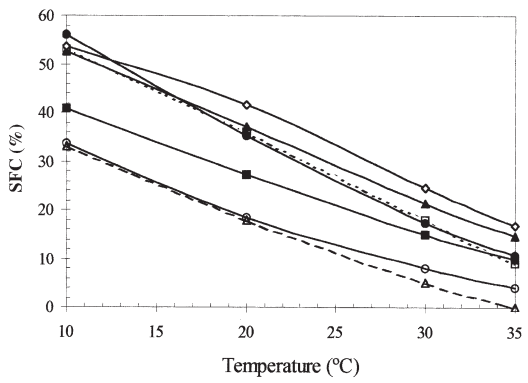

FIG. 2. Effect of *fap* alleles on *de novo* 16:0 synthesis in TPL of developing soybean cotyledons. Velocity for 16:0 synthesis in TPL was estimated from acetate saturation kinetics at 35 DAF. Germplasm lines exhibited mutations at *Fap* loci. For abbreviations see Figure 1.

sized from acetate, but there were genotypic differences (Fig. 2) in the rate of 16:0 incorporation in TPL, where: C1727 (Fap_1Fap_1 fap_2fap_2 $Fap_{nc}Fap_{nc}$) > Dare (Fap_1Fap_1 Fap_2Fap_2 $Fap_{nc}Fap_{nc}$) > C1726 (fap_1fap_1 Fap_2Fap_2 $Fap_{nc}Fap_{nc}$) = N79-2077-12 (Fap_1Fap_1 Fap_2Fap_2 $fap_{nc}fap_{nc}$) > N94-2575 (fap_1fap_1 Fap_2Fap_2 $fap_{nc}fap_{nc}$). Estimates of V_{max} for 16:0 synthesis derived from these data reflected major genetic effects of *fap* alleles that were positively correlated (R^2, 0.80) with 16:0 concentration in TPL at 35 DAF (Table 2). Significant genotypic differences also were found in the estimated first-order rate constant for 16:0 synthesis from acetate, which may be used to calculate the approximate half-life ($t_{0.5}$) or time required for esterification of 50% of the 16:0-CoA exported from plastids into phospholipid. Based on these calculations, N94-2575 (fap_1fap_1 Fap_2Fap_2 $fap_{nc}fap_{nc}$) exhibited the lowest capacity for *de novo* 16:0 synthesis, and C1727 (Fap_1Fap_1 fap_2fap_2 Fap_{nc} Fap_{nc}) mediated the greatest capacity for *de novo* 16:0 synthesis among these genotypes (Table 2).

The acetate saturation kinetics also showed a significantly lower V_{max} for 18:0 synthesis and a considerably longer $t_{0.5}$ for 18:0 incorporation into TPL of C1727. This observation suggested that the apparent fap_2fap_2-mediated increase in 16:0 synthesis might be related to reduced KAS-II activity rather than or in addition to increased 16:0-ACP TE activity. Furthermore, pulse-chase experiments with exogenous acetate removed the possibility that C1727 exhibited a greater rate of 18:0 metabolism or transfer from TPL toward final deposition in TG. The $t_{0.5}$ for 18:0 decay from pulse-chase experiments was not significantly different among genotypes, averaging 8.7 ± 0.9 h (Fig. 3). These results strongly suggest that increased accumulation of 16:0 in glycerolipids of C1727 germplasm is a consequence of reduced conversion of 16:0-ACP to 18:0-ACP, a function of the KAS-II enzyme.

Similarly, little difference was found among genotypes in the first-order decay rates for 16:0 (Fig. 4). The $t_{0.5}$ for 16:0 turnover averaged 12.7 ± 1.2 h among these lines. These findings supported previous work showing an apparent lack of major genetic effects of these *fap* alleles on glycerolipid acyltransferase activi-

TABLE 2
***In Vivo* Acetate Saturation Kinetics for Fatty Acid Incorporation into Phospholipids of Developing Soybean Cotyledons**

	16:0	18:0	Other[a]	Total	16:0	18:0	Other[a]	16:0	18:0	Other[a]
Genotype	V_{max} (nmol acetate h^{-1} g dwt^{-1})[b]				[acetate]$_{90}$ (mM)[b]			$[t]_{0.5}$ (h)[b]		
Fap_1 fap_2 Fap_{nc}	670.0	27.6	1057.2	1754.8	11.6	12.5	11.7	4.0	104.6	2.6
Fap_1 Fap_2 fap_{nc}	427.9	51.1	1649.7	2128.7	14.2	14.8	14.8	7.7	66.9	2.1
fap_1 Fap_2 Fap_{nc}	455.2	54.4	1346.9	1856.5	12.8	14.6	13.1	6.5	62.0	2.2
fap_1 Fap_2 fap_{nc}	323.4	55.1	1591.4	1969.9	14.5	15.0	14.2	10.4	62.9	2.1
Fap_1 Fap_2 Fap_{nc}	609.2	66.0	1524.2	2199.4	12.8	13.0	12.8	4.9	45.5	1.9
$LSD_{0.05}$	189.7	19.1	322.6	248.9	1.6	1.5	1.6	3.4	29.5	0.3

[a]Other; 18:1 + 18:2 + 18:3.
[b]V_{max}, maximal rate of acetate incorporation into fatty acids of TPL, from Hofstee plot analysis; [acetate]$_{90}$, (-9K_m^{app}) substrate concentration required for V_{max} acetate incorporation into TPL fatty acids; $t_{0.5}$, estimated half-life or time required for 50% of substrate to be converted to product under first-order reaction kinetics.

ties or any event downstream of acyl-CoA formation (14). Thus, the amount of 16:0 that entered and passed through TPL was a function of the 16:0-CoA produced by plastids.

Changes in the steady-state accumulation of mRNA encoding the Δ9DES, 16:0-ACP TE, KAS-II, or 18:1-ACP TE enzymes could represent one of the mechanisms by which 16:0 concentration is altered in soybean germplasm containing the fap_1, fap_2, or fap_{nc} mutations. To test this possibility, RNA purified from cotyledons at 35 DAF was hybridized to cDNA clones corresponding to these four enzyme activities. In using Δ-9DES cDNA (PDS 1) as a hybridization probe, total cellular RNA was sufficient to provide the necessary sensitivity to interpret the Northern blotting data. However, purified PolyA+ RNA was required to visualize and interpret Northern blots with the 16:0-ACP TE (FAT B) and 18:1-ACP TE (FAT A) probes. Unfortunately, even PolyA+ RNA provided insufficient hybridization sensitivity to obtain reliable data for the KAS-II cDNA probe.

Reduction in the steady-state mRNA levels in germplasm possessing the fap_{nc} allele was seen consistently in Northern blots hybridized to FAT B, whereas no apparent differences were noted in the hybridization intensity with the PDS 1 or FAT A probes. A typical blot with mRNA extracted at 35 DAF (when the peak rate of oil accumulation occurred) is shown in Figure 5. After quantifying the hybridization signals of several Northern blotting experiments (using independent mRNA preparations), it was determined that the germplasm lines N79-2077-12 (Fap_1Fap_1 Fap_2Fap_2 $fap_{nc}fap_{nc}$) and N94-2575 (fap_1fap_1 Fap_2Fap_2 $fap_{nc}fap_{nc}$) accumulated approximately 50% of the relative level of FAT B transcript observed in the control genotype (cv. Dare) or C1726 or C1727 germplasm (Fig. 6). In contrast, no significant differences were detected in steady-state mRNA accumulation among these five genotypes when PDS 1 or FAT A was used as hybridization probe.

These results provide evidence that the major genetic effect of the fap_{nc} allele is a significant decrease in 16:0-ACP TE mRNA accumulation. Such a response suggests that the fap_{nc} allele results in an overall decrease in the synthesis of the 16:0-ACP TE, as opposed to production of a 16:0-ACP TE enzyme that displays reduced activity. The latter possibility, however, still could represent a viable explanation for the fap_1 allele, especially in consideration of the fact that fap_1 was generated by ethylmethane sulfonate mutagenesis (a procedure that typically gives rise to point mutations). Finally, kinetic analyses suggest that the fap_2 mutation represents a defect that reduces the ability of the KAS-II enzyme to elongate 16:0-ACP to 18:0-ACP.

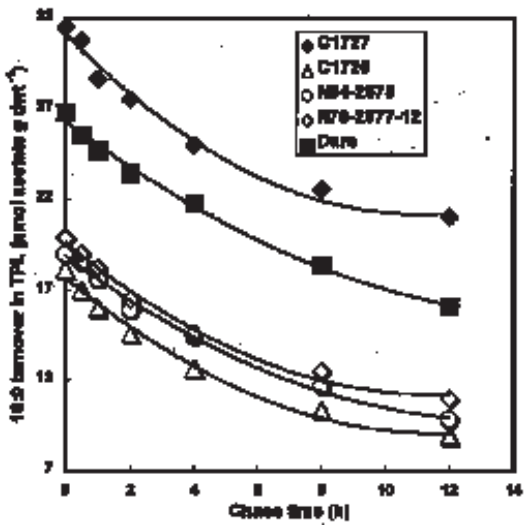

FIG. 4. Genotypic differences in metabolic turnover of 16:0 in TPL of developing soybean seed. Velocity for 16:0 turnover or decay in TPL was estimated from pulse-chase incorporation of exogenous acetate at 35 DAF. For abbreviations see Figure 1.

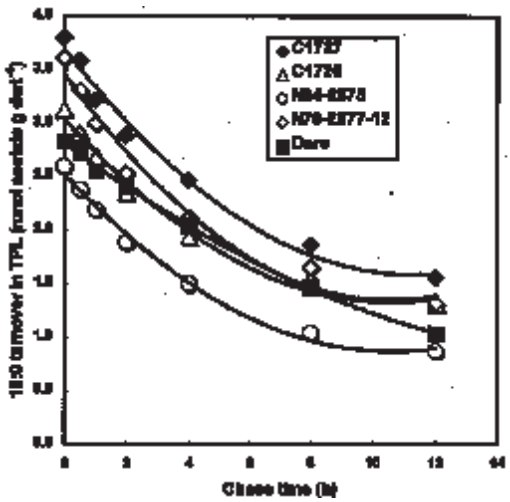

FIG. 3. Genotypic differences in metabolic turnover of 18:0 in TPL of developing soybean seed. Velocity for 18:0 turnover or decay in TPL was estimated from pulse-chase incorporation of exogenous acetate at 35 DAF. For abbreviations see Figure 1.

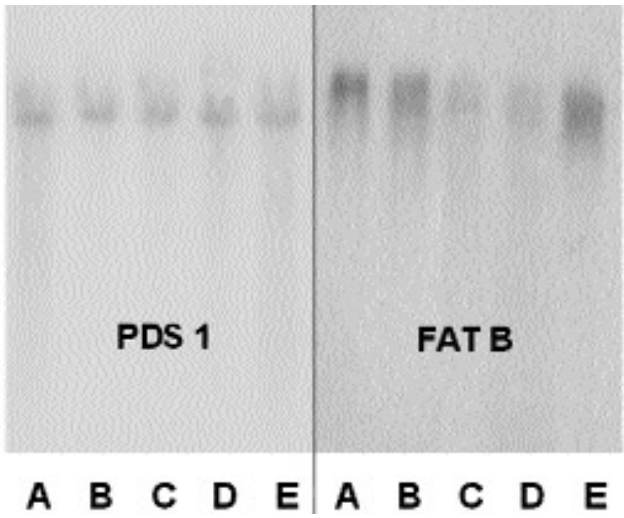

FIG. 5. Northern blots of RNA from developing soybean cotyledons at 35 DAF. Steady-state mRNA levels visualized after hybridization with cDNA probes for the 18:0-acylcarrier protein (ACP) desaturase [Δ9DES: plastidal Δ-9 desaturase (PDS 1)] and the 16:0-ACP thioesterase (TE) (FAT B). Lane A: C1726 (fap_1fap_1 Fap_2Fap_2 $Fap_{nc}Fap_{nc}$), Lane B: C1727 (Fap_1Fap_1 fap_2fap_2 $Fap_{nc}Fap_{nc}$); Lane C: N79-2077-12 (Fap_1Fap_1 Fap_2Fap_2 fap_{nc}-fap_{nc}), Lane D: N94-2575 (fap_1fap_1 Fap_2Fap_2 $fap_{nc}fap_{nc}$), Lane E: cv. Dare (Fap_1Fap_1 Fap_2Fap_2 $Fap_{nc}Fap_{nc}$). For abbreviations see Figure 1.

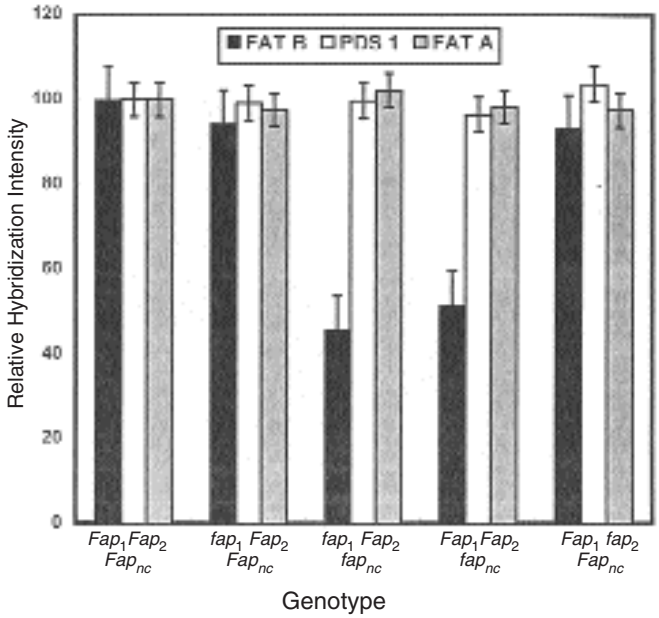

FIG. 6. Relative mRNA transcript levels from developing soybean cotyledons at 35 DAF. Transcriptional expression of soybean cDNA probes for 18:1-ACP TE (FAT A), 16:0-ACP TE (FAT B), and Δ9DES (PDS1) is shown relative to the cv. Dare = 100%. For abbreviations see Figure 5.

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