Transgenic Cotton Plants with Increased Seed Oleic Acid Content

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ABSTRACT: Cottonseed typically contains about 15% oleic acid. Here we report the development of transgenic cotton plants with higher seed oleic acid levels. Plants were generated by *Agrobacterium*-mediated transformation. A binary vector was designed to suppress expression of the endogenous cottonseed Δ-12 desaturase (*fad2*) by subcloning a mutant allele of a rapeseed *fad2* gene downstream from a heterologous, seed-specific promoter (phaseolin). Fatty acid profiles of total seed lipids from 43 independent transgenic lines were analyzed by gas chromatography. Increased seed oleic acid content ranged from 21 to 30% (by weight) of total fatty acid content in 22 of the primary transformants. The increase in oleic acid content was at the expense of linoleic acid, consistent with reduced activity of cottonseed FAD2. Progeny of some lines yielded oleic acid content as high as 47% (three times that of standard cottonseed oil). Molecular analyses of nuclear DNA from transgenics confirmed the integration of the canola transgene into the cotton genome. Collectively, our results extend the metabolic engineering of vegetable oils to cottonseed and should provide the basis for the development of a family of novel cottonseed oils.

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KEY WORDS: Cottonseed oil, fatty acid metabolism, metabolic engineering, oilseeds.

Cottonseed is about 16% oil by weight and ranks third behind soy and canola in world oilseed crushings (1). The United States produced 5.8 million metric tons between October 1999 and September 2000 (www.cottonseed.com). Currently, only about half of the seed is crushed in the United States, leaving a substantial amount of raw material from which to recover added-value processed products. Refined oil is the most valuable cottonseed product (after fiber). It has a distinctive fatty acid composition that is about 26% palmitic (16:0), 2% stearic (18:0), 15% oleic (18:1), and 55% linoleic (18:2) acids (2). Although not often thought of as a food crop, cottonseed has certain flavor-enhancing attributes that make it an excellent cooking oil. However, recent trends in the food-service industry are tending toward oils high in monounsaturates, primarily due to oxidative stability of these oils and some perceived health benefits (3). Consequently, an increased oleic acid content with a compensatory reduction in linoleic acid in cottonseed would represent a potential health benefit and might expand utilization of raw cottonseed.

A number of genetic strategies have been employed to alter the fatty acid profiles of oilseeds (4,5). In some cases natural genetic variation in seed lipid composition has allowed breeders to select for desired fatty acid compositions (e.g., *Brassica*; Ref. 6). In other cases, large-scale mutagenesis programs have led to lines with altered seed fatty acid compositions (e.g., linseed; Ref. 7). Most recently, genetic engineering has been used to add novel genes into oilseed crops or to alter the expression of endogenous genes in lipid biosynthetic pathways (8).

Metabolic engineering of oilseeds has resulted in commercial production of new oils in traditional crops (9–11). Alteration of a single, targeted enzymatic step in storage lipid metabolism can produce dramatic changes in fatty acid profiles of the seed oils, and in many cases, these changes have little effect on total oil yield or overall crop performance (8,12,13). With the tremendous diversity of plant fatty acid structures present in nature (14) and increasingly novel strategies for genetic manipulation of crop plants, there are substantial opportunities for creating new vegetable oils for edible and industrial purposes (15). Indeed, commercialization of high-laurate canola (9) and high-oleic soybeans (3) highlights the potential of this approach to impact agriculture.

Plant lipid metabolism is fairly well characterized, and genes encoding most of the enzymes in the pathway have been isolated from a variety of plant species (16). The enzymatic steps that have received the most attention in terms of altering oilseed fatty acid composition are those catalyzed by the acyl-acyl carrier protein thioesterases (13,17) and the fatty acid desaturases (18). While there are additional factors that are important in regulating the overall accumulation of fatty acids in oilseeds (e.g., high-laurate canola; Ref. 13), simply altering the expression of either (or both) of the above enzyme classes dramatically modifies fatty acid chain length or degree of unsaturation, two key factors that determine the functional properties of plant-derived oils (8).

In this paper we describe the introduction of a "sense suppression DNA construct" into cotton plants to reduce the endogenous activity of the cottonseed Δ-12 desaturase, or fatty

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acid desaturase 2 (FAD2) during seed maturation. The FAD2 enzyme catalyzes the conversion of oleic acid to linoleic acid in the endoplasmic reticulum of plant cells (19), and studies with soy and canola indicate that a reduction in this enzyme activity leads to an increased oleic acid content (with a compensatory decrease in linoleic acid) in the seed oil (10). Here we show that this strategy also is effective in cotton, resulting in the production of a number of transgenic lines with substantially increased seed oleic acid content. In addition, our results indicate that this molecular strategy is a particularly efficient process in cotton, with *ca.* 50% of transgenic lines showing a modified phenotype compared to wild-type oleic-acid levels. In fact, progeny of some of these modified-oleic lines have an oleic acid content three times that of standard cottonseed oil. Overall, these transgenic plants should benefit the cotton industry by supplying novel genetic resources with value-added, seed-quality traits to cotton breeding programs.

MATERIALS AND METHODS

Transformation vectors. A binary vector, designated pZPHM-CFd2, was constructed for use in cotton transformations (Fig. 1). This vector contains the Ti-plasmid left and right border (LB, RB) inverted repeat sequences for integration into cotton genomic DNA. The T-DNA segment harbors the selectable marker *npt*II regulated by the CaMV35S promoter, conferring kanamycin resistance to transgenic plant cells. In addition, a *fad*2 suppression cassette is inserted into the T-DNA segment between unique BamHI and EcoRI sites. This cassette (2860 bp fragment) contains a canola mutant (nonfunctional) *fad*2 allele subcloned between the 5′ and 3′ flanking regions of the phaseolin gene (20). The pZPhMCFd2 binary vector was introduced into *Agrobacterium tumefaciens* (strain LBA4404) by electroporation and maintained with kanamycin selection conferred by *npt*I expression. For transformation/plant regeneration controls, transgenic cotton plants were produced using the binary vector pBI121 (Clonetech) with the same *Agrobacterium* strain.

Cotton transformation. Cotyledon pieces (*ca.* 3 mm^2) were excised from 7- to 14-d-old cotton (*Gossypium hirsutum* L., cv. Coker 312) seedlings that were germinated aseptically (according to Refs. 21 and 22) except that seedlings were grown at 30°C under a 14-h photoperiod, 60 μmol/s/m^2. Cotton explants were co-cultivated with 6×10^8 cells/mL *A. tumefaciens* LBA4404, harboring the binary vector pBI121 for vector-only control experiments, or pZPhMCFd2 for fatty acid modification. *Agrobacterium*-mediated transformation and plant regeneration were based on a combination of published procedures (21,23,24), with minor modifications. Briefly, cotton cotyledon explants were placed in co-cultivation medium (MS medium, 1.5% wt/vol sucrose, 40 μM acetosyringone, 2 mM MES-NaOH, pH 5.5) along with an equal volume of *Agrobacterium* cell suspension. The explant/Agro mixture was placed under vacuum (84.7 kPa) for 8 min, and equilibrated to 25°C in a water bath for an additional 1 h and 15 min. Explants were then blotted on sterile filter paper and placed on G1 medium (MS salts with 3% wt/vol glucose) (21) for 3 d at 25°C. Explants were then transferred to G2 medium [MS medium, 100 mg/L inositol, 1 μM thiamine, 25 μM 6-γ-γ-dimethylallylaminopurine (2iP), 0.5 μM naphthaleneacetic acid, 3% wt/vol glucose, pH 5.8, 0.2% wt/vol Phytagel (Sigma, St. Louis, MO)] supplemented with 400 mg/L carbenicillin and 50 mg/L kanamycin (21). The transformed calli were subcultured every 2 to 4 wk to fresh G2 medium. Mock transformations (co-cultivation of explants with *Agrobacterium* that contained no binary vector) were always conducted to verify selection procedures were adequate. After 2 to 3 mon, proliferating transgenic calli were transferred to a modified MSOB medium (modified from Ref. 21 to contain MS salts, B-5 vitamins, 1.9 mM potassium nitrate, 100 mg/L inositol, 3% wt/vol glucose, pH 5.8, with 0.2% Phytagel) supplemented with 200 mg/L carbenicillin and 50 mg/L kanamycin (21). Developing embryos were recovered after 6 to 8 wk and placed on MSOB medium without antibiotics. Elongated embryos were transferred to MS3 medium (MS salts, 0.4 μM thiamine-HCl, 0.5 μM pyridoxine-HCl, 0.8 μM nicotinic acid, 1% wt/vol glucose, pH 5.8 with 0.8 g/L Phytagel and 4 g/L agar) for root formation (21). Small plantlets were propagated clonally (25), or transferred to soil, hardened off, then transferred to glasshouse conditions (14-h photoperiod, supplemented with high-intensity Na- and Hg-vapor lamps when necessary, 35°C day 25°C night) for production of flowers and bolls. Plants were fertilized biweekly with a dilute solution of Miracle Gro™, and flower production was stimulated with SuperBloom™ (when necessary). Flowers were selfed, tagged at anthesis, and progress of boll development was monitored daily. Any male sterile primary tranformants (less than 5% of plants) were hand-pollinated with Coker 312, wild-type pollen to obtain viable progeny.

Lipid extractions, preparation of fatty acid methyl esters, and gas chromatography. Whole cottonseeds (pooled 8-seed batches or single seeds) were first frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. Ground seeds were then extracted with hexane. Aliquots of hexane extracts were dried under flowing nitrogen and transesterified with acidic methanol essentially as described by Christie (26) except that the $KHCO_3$ wash and Na_2SO_4 drying steps were omitted. Fatty acid methyl esters were analyzed by gas chromatography and quantified by flame-ionization detection (FID) essentially as described by Chapman and Trelease (27), except that a 30 m (0.25 mm i.d.) DB-23 (J&W Scientific) capillary column was employed for separation, and the oven temperature was 200°C.

DNA isolations. Small amounts of cotton genomic DNA (30–100 μg) were isolated from *ca.* 100 mg fresh, young rapidly expanding cotton leaf tissue using plant DNAZOL reagent (GibcoBRL Life Technologies) per the manufacturer's instructions. Larger amounts of genomic DNA (2–4 mg) were extracted from the nuclei isolated from 3–4 g of young leaf tissues as described by Paterson *et al.* (28), and quantified by ultraviolet (UV) spectroscopy (29).

Polymerase chain reactions (PCR). Cotton genomic DNA was used as template for PCR analysis with two canola-

specific *fad*2 primers, forward—5′-ATGCAAGTGTCTC-CTCCCTCC-3′ and reverse—5′-CGTTAACATCACGGT-GCGTC-3′, which specifically amplified a 528 bp fragment of the canola *fad*2. The PCR reaction mixtures contained *ca.* 1 μg template DNA, 1 μM of each primer, 0.2 mM of deoxy nucleotide triphosphate, and 1.25 units of AmpliTaq (Perkin-Elmer, Norwalk, CT) DNA polymerase. The amplification was performed on a GeneAmp PCR System 2400 (PerkinElmer) with 32 cycles of 95°C (45 s), 60°C (45 s), and 72°C (2 min). PCR amplification products were electrophoresed in 2% agarose gels (BioRad MiniSub submarine apparatus) according to manufacturer's instructions, and visualized following staining with ethidium bromide (29).

Genomic blot analyses. To prepare the canola *fad*2-specific cDNA probe, the pZPhMCFd2 plasmid, propagated in *Escherichia coli* DH5a, was isolated and purified (Wizard Plus Miniprep DNA Purification System, Promega). The plasmid DNA was digested with BamHI and EcoRI and fractionated on a 0.8% agarose gel. A 2.9 kilobase (kb) pair fragment corresponding to the phaseolin-canola *fad*2 fusion cassette (see Fig. 1) was extracted (QIAquick Gel Extraction Kit; Qiagen) and used as template to generate ^{32}P-labeled DNA fragments by random priming (30) for use as hybridization probe.

Cotton nuclear DNA were digested with BamHI and/or EcoRI, and the fragments were separated on a 0.8% agarose gel. DNA fragments were transferred to nylon membranes (Amersham Hybond N+) by alkaline blotting in 0.4 M NaOH (31). Immobilized DNA were prehybridized at 60°C for 4 h in 6× SSC [150 mM sodium chloride and 15 mM sodium citrate (pH 7.0)] (29), 0.5% sodium dodecylsulfate (SDS), 5× Denhardt's reagent, and denatured sheared salmon sperm DNA (0.1 mg/mL). Hybridization was overnight at the same temperature and in the same solution except including 20 mM Tris-HCl (pH 8.0), 2 mM EDTA, 2.5 mM Na pyrophosphate (pH 8.0), and the ^{32}P-labeled canola *fad*2-specific probe. After hybridization, nylon membranes were rinsed at 60°C in 2× SSC, 0.1% SDS (once for 5 min and once for 30 min), followed by two rinses (30 min each) in 1× SSC, 0.1% SDS (29).

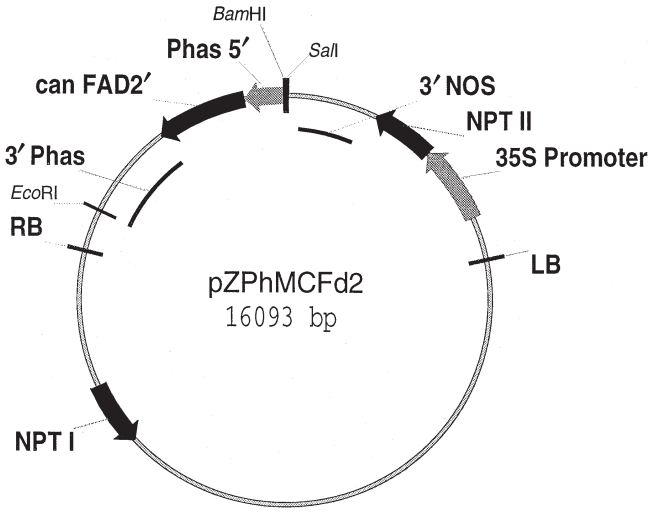

FIG. 1. Diagram of the binary vector, pZPhMCFd2, identifying several salient features. The T-DNA region is defined by the position of the LB and RB (left and right border sequences required for excision from the plasmid and integration into the host nuclear genome). Expression of the selectable marker in transgenic plant cells (*npt*II) is controlled by CaMV35S promoter and the 3′ nos (nopaline synthase) terminator region. The suppression cassette (*ca.* 2.9 kb) was subcloned directionally into unique BamH1 and EcoR1 sites. A mutant canola *fad*2 allele (can FAD2) was cloned downstream from the 5′-flanking region of the phaseolin gene (containing seed-specific regulatory elements). The 3′-flanking region containing the transcription termination sequence is immediately downstream from the canola *fad*2 sequence. Expression of *npt*I allows for selection of the binary vector in bacterial hosts; fad, fatty acid desaturase; kb, kilobase pair; bp, base pair; npt, neomycin phosphotransferase.

RESULTS AND DISCUSSION

Fatty acid profiles of seeds of 43 independent lines were analyzed for changes in the relative percentages of oleic acid (18:1) in total lipids extracted from pooled seed samples (Fig. 2). In general, an increase in seed oleic acid content was noted (compared with wild type; see Table 1), and this appeared to be at the expense of linoleic acid (18:2) content. Palmitic (16:0) and stearic (18:0) acid levels remained relatively unchanged (combined as total saturates). These results strongly support a targeted suppression of endogenous cottonseed FAD2 enzyme activity in many of the transgenic cotton plants. Unmodified cottonseed fatty acid profiles from a variety of sources are summarized in Table 1, which shows that seed oleic acid content is consistently below 18% of the total seed fatty acid composition. Approximately half of the transgenic cotton lines had seed oleic

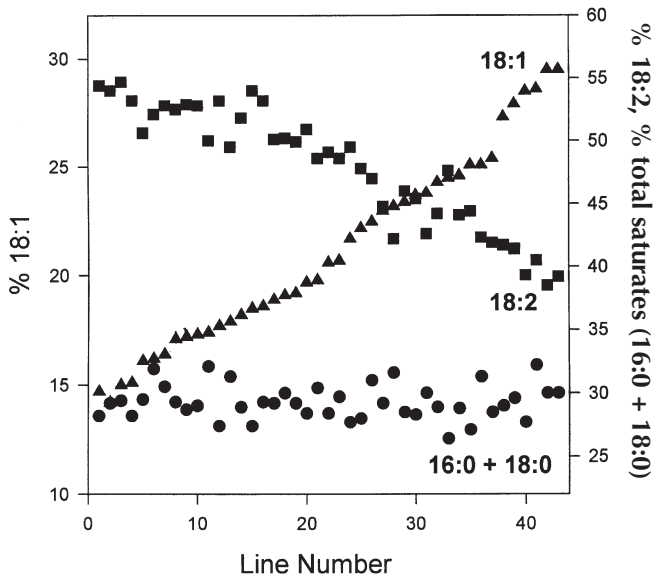

FIG. 2. Fatty acid compositions of various lines of transgenic cotton. Eight-seed batches of randomly selected seeds from 43 independent lines of transgenic cotton were pooled and analyzed for their fatty acid compositions. Data shown represent mass percentages and are ranked in order of increasing amounts of oleic acid (18:1, ▲). The percentage of total saturates (●, sum of palmitic and stearic acids) was relatively unchanged in transgenic lines, while the relative percentage of linoleic acid (■) declined as oleic acid was increased.

TABLE 1
Fatty Acid Compositions of Various Control Cottonseed Oils

Control line	Fatty acid[a] (%)				
	16:0	18:0	18:1	18:2	Others[b]
Texas-grown[c]	25.2	2.7	17.5	52.6	2.0
California-grown[c]	22.7	2.3	17.3	55.8	1.9
Untransformed Coker 312	25.8	2.4	14.7	55.9	1.2
pBI-121 Vector-only control					
Individual no. 1	27.9	2.8	17.4	50.5	1.4
Individual no. 2	27.4	3.2	15.9	53.5	ND[d]

[a]Palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids.
[b]"Others" represents trace amounts of myristic, palmitoleic, linolenic, and arachidonic acids.
[c]Data from Jones and King (2).
[d]None detected.

acid content (pooled seed samples) above 20%, with several lines approaching 30% (Fig. 2). These results indicated that (i) the molecular manipulation of endogenous FAD2 activity was an efficient process in cotton, (ii) changes in oleic acid content were manifested in the appropriate plant part, and (iii) the modified oleic acid content was heritable in the progeny of the primary transformants.

To evaluate transformation at the molecular level, the genomic DNA of transgenic plants were screened by PCR with canola-*fad*2-specific primers (see Fig. 3 for representative results). Amplification of a predictable 528 base pair (bp) fragment was generated from DNA samples of transgenic lines (lanes 2 through 8), but not from DNA samples of vector-only controls (lane 1; pBI121 plant) or from untransformed Coker 312 (not shown). The transgenic lines analyzed in Figure 3 are representative of individuals showing maximum modified oleic

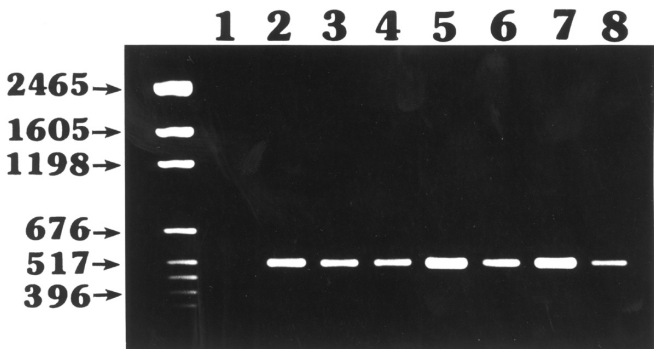

FIG. 3. Separation of polymerase chain reaction (PCR) products amplified from cotton genomic DNA with canola-*fad*2-specific primers (see methods for sequence). DNA molecular size standards derived from pGEM (Promega) are shown on the left. For lane 1, template was genomic DNA from vector-only transgenic control plant, pBI121-2. Template for ZPh *fad*2 transgenics was genomic DNA isolated from ZPh84a (lane 2), ZPh83a (lane3), ZPh18a2 (lane 4), ZPh11a3 (lane 5), ZPh11f (lane 6), ZPh11h (lane 7), or ZPh11m (lane 8). A 528 bp fragment is present in *fad*2 transgenics only and is marked with an arrow at the right; no other PCR products were generated from the canola *fad*2-specific primers. DNA sequence analyses of the 528 bp PCR products confirmed these were identical to the canola *fad*2 DNA sequence (not shown).

phenotypes (27 to 30%, lanes 2, 3, 5, 7, 8), as well as those showing modest (22%, lane 6) or no phenotype (16%, lane 4).

Fatty acid profiles of single seeds from several cotton lines (selfed in the greenhouse) were analyzed. Eighteen individual seeds of the untransformed Coker 312 wild-type are shown in Figure 4A for comparison, indicating essentially no seed-to-seed variation in oleic acid content. By contrast, the elevated oleic acid phenotype in several of the progeny of transgenic lines approached 40 to 45% by wt (Fig. 4B–E), although there was some variability in the extent of "suppression" at high-oleic acid levels. As expected, some individual progeny of the transformants exhibited no change in oleic acid content from wild-type suggesting these were segregating null individuals. While a clear understanding of the precise molecular mechanisms responsible for this elevated oleic trait must await a comprehensive genetic characterization of appropriately crossed populations, it seems likely that new cotton lines with modified oleic acid content can be developed from progeny of these primary transformants.

For further characterization of the transgenic cotton lines at the molecular level, alkaline blot analyses of genomic DNA were performed. Representative profiles of transgenic DNA are shown in Figure 5. As expected, genomic DNA from control samples (group A) did not contain any bands that hybridized with the phaseolin/canola *fad*2 cassette, indicating that this probe did not cross-hybridize under these conditions with any endogenous cotton DNA sequences. High molecular weight bands were evident in single digests of DNA from trangenics (groups B and C), and in both cases, an EcoRI/BamHI double digest generated the expected 2.9 kb phaseolin/canola-*fad*2 cassette intact (compare to lane 1), clearly demonstrating that the T-DNA (containing the transgene) was integrated into the cotton genome.

Here we demonstrate the development of new transgenic cotton lines with heritable increases in seed oleic acid content. More than 40 lines were recovered from transformation experiments with the *Agrobacterium*-introduced ZPhMCFd2 suppression DNA construct (Fig. 1). All lines examined were harboring transgenes, and about half showed a modified seed oleic acid phenotype (Fig. 2). It will be a priority to verify that other cottonseed quality factors are unaltered in these transgenics, once stable, back-crossed individual lines can be selected for field trials. Of paramount importance will be to examine fiber yield and quality; however, this is not likely to be affected since fiber development and oil accumulation are temporally separated events in cotton boll development (32). Other factors to be examined will be oil and protein content. It also is important to ensure that negative attributes such as gossypol content or cyclopropenoid fatty acid levels remain unchanged in transgenic seeds. This likely will be the case because these metabolites are either produced by other biochemical pathways (polyphenolic metabolism; Ref. 32) or accumulate in organs other than cotyledons (cyclopropenoid fatty acid occurs exclusively in the axis of mature seeds; Ref. 34). It should be pointed out here that the changes we noted in fatty acid composition targeted selectively oleic acid and

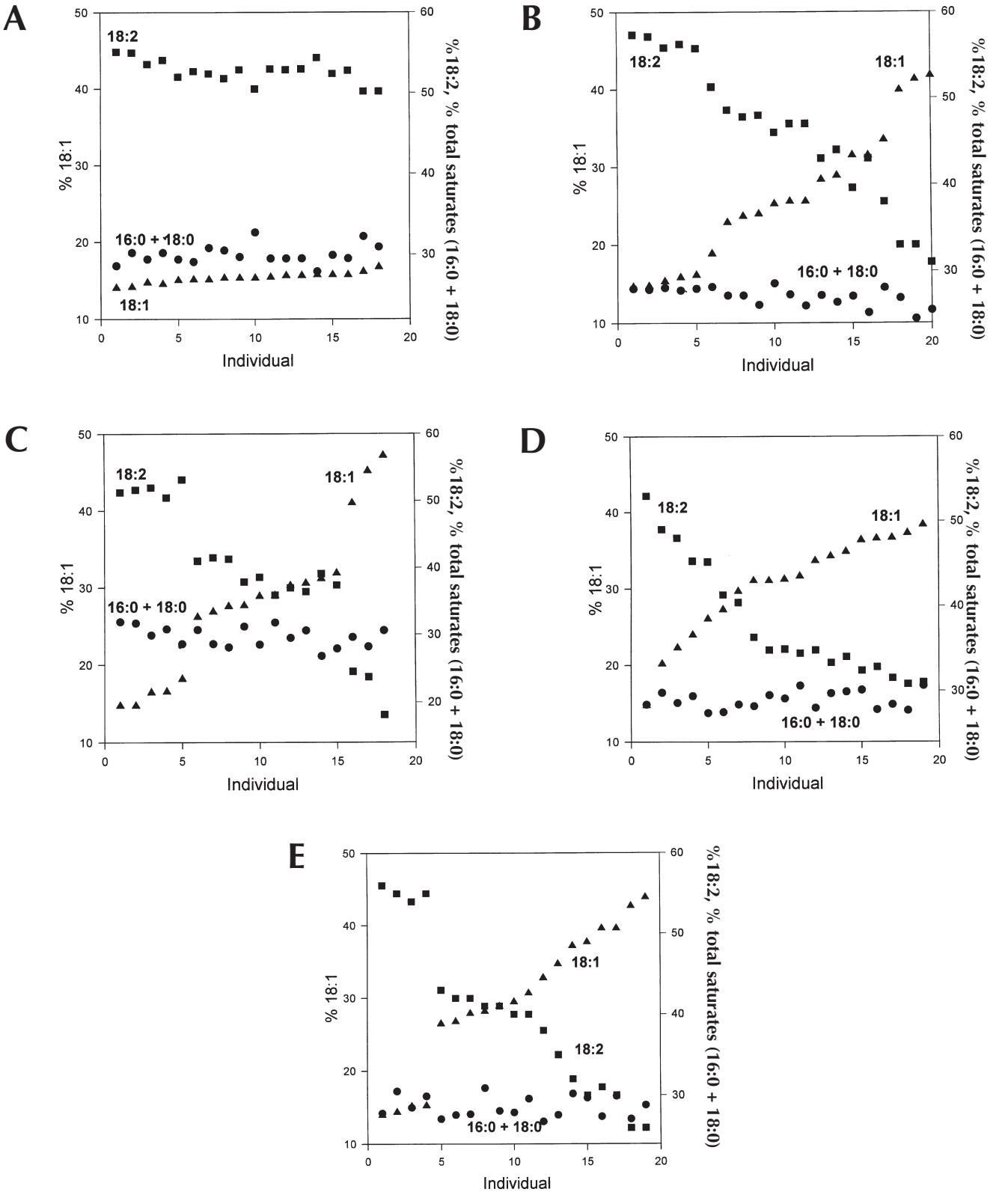

FIG. 4. Fatty acid compositions of individual seeds of several lines of cotton. Of each line, 18 to 20 seeds were individually analyzed for their fatty acid compositions. Data shown represent mass percentages and are ranked in order of increasing amounts of oleic acid (18:1, ▲). Content of total saturates (sum of palmitic and stearic acids) is represented by ●, while content of linoleic acid (18:2) is represented by ■. (A) Untransformed Coker 312 wild-type; (B) ZpH 11f (line number 24 from Fig. 2); (C) ZpH 11a3 (line number 38 from Fig. 2); (D) ZpH 84a (line number 40 from Fig. 2); (E) ZpH 83a (line number 43 from Fig. 2).

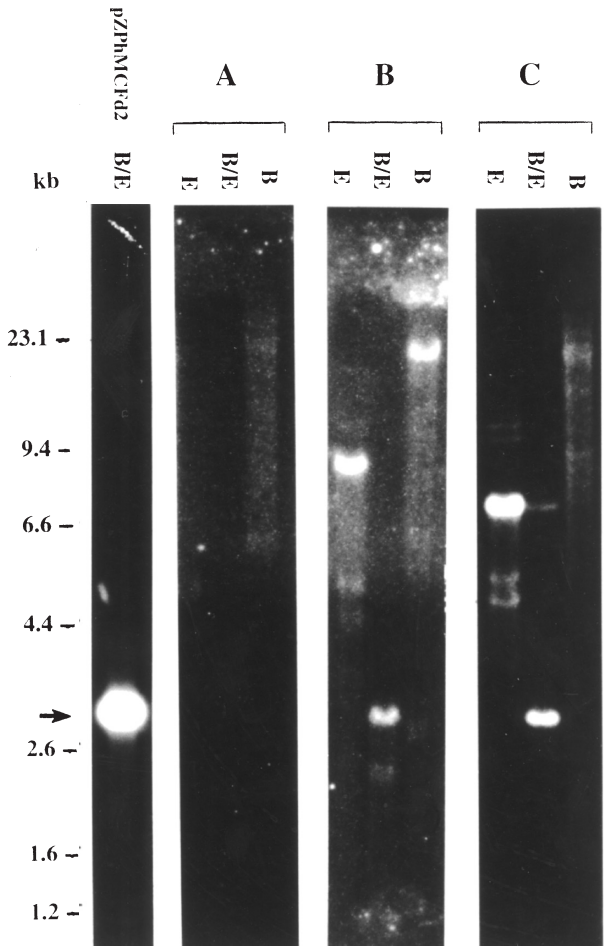

FIG. 5. Alkaline blot hybridization of transgenic cotton genomic DNA with the phaseolin/canola-*fad*2 probe. Restriction enzymes BamHI (B) and EcoRI (E) were used to digest pZPhMCFd2 plasmid DNA and cotton genomic DNA. The DNA fragments were resolved along with DNA size markers derived from HindIII-digested lambda DNA and pGEM DNA (Promega) on a 0.8% agarose gel. (A) Represents analyses of genomic DNA from control plants transformed with pBI121, showing the absence of bands hybridizing to the phaseolin/canola-*fad*2 cassette. (B) and (C) represent analyses of genomic DNA isolated from transgenic plants ZPh83a and ZPh84a, respectively, showing bands hybridizing to the phaseolin/canola-*fad*2-specific probe. Autoradiography times were as follows: plasmid pZPhMCFd2, 35 min room temperature; (A) and (B), 6 d with an intensifying screen at –70°C; (C), 18 h with an intensifying screen at –70°C. The figure is a composite of autoradiograms (negative images) from the same hybridization membrane developed after different exposure times.

linoleic acid content in transgenics. Even other storage fatty acids such as palmitic or stearic acid content were unchanged, emphasizing the likelihood that metabolic changes are specific for FAD2 alone. Consequently, it should be possible to identify individual lines with promising characteristics for advancement.

While stability of this trait in the field must be addressed, the results presented here have several important implications. First, the targeted reduction of endogenous FAD2 is a feasible strategy for metabolic engineering of seed oil in cotton, which could be important in attempting to modulate the levels of other metabolites. Second, vectors with heterologous elements developed for modification of other oilseeds are useful for the modification of cottonseed, which increases the number of molecular tools available for cotton engineering. Third, manipulation of seed oleic acid content in cotton highlights the opportunity for the targeted elevation of other desirable products in cottonseed as a means of increasing the overall value of the cotton crop. Fourth, because several unusual fatty acids are synthesized from oleic acid precursors (e.g., ricinoleic acids), these high-oleic acid lines represent novel genetic resources from which to create new oleochemicals in cottonseed. In all, this work should provide the basis for cotton germplasm development focused on oil-quality traits in this undervalued oilseed crop.

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REFERENCES

1. Cottonseed: World's No. 3 Oilseed Yields Diverse Products, *inform 11*:820–839 (2000).
2. Jones, L.A., and C.C. King, Cottonseed Oil, in *Bailey's Industrial Oil and Fat Products, Vol. 2. Edible Oil and Fat Products: Oils and Oil Seeds,* 5th edn., edited by Y. Hui, John Wiley & Sons, New York, 1996, pp. 159–240.
3. Kinney, A.J., and S. Knowlton, Designer Oils: The High-Oleic Soybean, in *Genetic Modification in the Food Industry*, edited by S. Roller and S. Harlander, Blackie Academic and Professional, New York, 1998, pp. 193–213.
4. Kinney, A.J., Genetic Engineering of the Storage Lipids of Plants, *Curr. Opinion Biotechnol. 5*:144–151 (1994).
5. Browse, J., J. Spychalla, J. Okuley, and J. Lightner, Altering the Fatty Acid Composition of Vegetable Oils, in *Plant Lipid Biosynthesis: Fundamentals and Agricultural Applications*, edited by J.L. Harwood, Society for Experimental Biology Seminar Series 67, Cambridge University Press, Cambridge, 1998, pp. 131–153.
6. Downey, R.K., and G. Röbbelen, *Brassica* Species, in *Oil Crops of the World: Their Breeding and Utilization*, edited by G. Röbbelen, R.K. Downey, and A. Ashri, McGraw Hill, New York, 1989, pp. 339–362.
7. Green, A.G., and D.R. Marshall, Isolation of Induced Mutants in Linseed *(Linum usitatissimum)* Having Reduced Linolenic Acid Content, *Euphytica 33*:321–328 (1984).
8. Kinney, A.J., Genetic Engineering of Oilseeds for Desired Traits, in *Genetic Engineering,* edited by J.K. Setlow, Plenum Press, New York, 1997, Vol. 19, pp. 149–166.
9. Del Vecchio, A.J., High-Laurate Canola, *inform 7*:230–243 (1996).
10. Kinney, A.J., Production of Specialised Oils for Industry, in *Plant Lipid Biosynthesis: Fundamentals and Agricultural Applications*, edited by J.L. Harwood, Society for Experimental Biology Seminar Series 67, Cambridge University Press, Cambridge, 1998, pp. 273–285.

11. Mazur, B., E. Krebbers, and S. Tingey, Gene Discovery and Product Development for Grain Quality Traits, *Science 285*: 372–375 (1999).
12. Ohlrogge, J.B., Design of New Plant Products: Engineering of Fatty Acid Metabolism, *Plant Physiol. 104*:821–826 (1994).
13. Voelker, T., Plant Acyl-ACP Thioesterases: Chain-Length Determining Enzymes in Plant Fatty Acid Biosynthesis, in *Genetic Engineering,* edited by J.K. Setlow, Plenum Press, New York, 1996, Vol. 18, pp. 111–133.
14. Somerville, C., J. Browse, J. Jaworski, and J.B. Ohlrogge, Lipids, in *Biochemistry and Molecular Biology of Plants*, edited by B. Buchanan, W. Gruissem, and R. Jones, American Society of Plant Physiologists, Rockville, MD, 2000, pp. 456–527.
15. Harwood, J.L., Recent Advances in the Biosynthesis of Plant Fatty Acids, *Biochim. Biophys. Acta 1301*:7–56 (1996).
16. Mekhedov, S., O. Martínez de Ilárduya, and J.B. Ohlrogge, Toward a Functional Catalog of the Plant Genome. A Survey of Genes for Lipid Biosynthesis, *Plant Physiol. 122*:389–402 (2000).
17. Voelker, T.A., A.C. Worrell, L. Anderson, J. Bleibaum, C. Fan, D.J. Hawkins, S.E. Radke, and H.M. Davies, Fatty Acid Biosynthesis Redirected to Medium Chains in Transgenic Oilseed Plants, *Science 257*:72–74 (1992).
18. Shanklin, J., and E.B. Cahoon, Desaturation and Related Modifications of Fatty Acids, *Annu. Rev. Plant Physiol. Plant Mol. Biol. 49*:611–642 (1998).
19. Okuley, J., J. Lightner, K. Feldmann, N. Yadav, E. Lark, and J. Browse, *Arabidopsis* FAD2 Gene Encodes the Enzyme That Is Essential for Polyunsaturated Lipid Synthesis, *Plant Cell 6*:147–158 (1994).
20. Burow, M.D., P. Sen, C.A. Chlan, and N. Murai, Developmental Control of the beta-Phaseolin Gene Requires Positive, Negative, and Temporal Seed-Specific Transcriptional Regulatory Elements and a Negative Element for Stem and Root Expression, *Plant J. 2*:537–548 (1992).
21. Thomas, J.C., D.G. Adams, V.D. Keppenne, C.C. Wasmann, J.K. Brown, M.R. Kanost, and H.J. Bohnert, Protease Inhibitors of *Madhuca sexta* Expressed in Transgenic Cotton, *Plant Cell Rep. 14*:758–762 (1995).
22. Trolinder, N.L., and J.R. Goodin, Somatic Embryogenesis in Cotton *(Gossypium)*. II. Requirements for Embryo Development and Plant Regeneration, *Plant Cell Tissue Org. Cult. 12*:43–53 (1988).
23. Umbeck, P., G. Johnson, K. Baron, and W. Swain, Genetically Transformed Cotton (*Gossypium hirsutum* L.), *Plants Biotechnol. 5*:263–266 (1987).
24. Firoozabady, E., D.L. DeBoer, D.J. Merlo, E.L. Halk, L.N. Amerson, K.E. Rashka, and E.E. Murray, Transformation of Cotton (*Gossypium hirsutum* L.) by *Agrobacterium tumefaciens* and Regeneration of Transgenic Plants, *Plant Mol. Biol. 10*:105–116 (1987).
25. Hemphill, J.K., C.G.A. Maier, and K.D. Chapman. Rapid *in vitro* Regeneration of Cotton (*Gossypium hirsutum* L.), *Plant Cell Rep. 17*:273–278 (1998).
26. Christie, W.W., *Lipid Analysis,* 2nd edn. Pergamon Press, New York, 1982, pp. 52–54.
27. Chapman, K.D., and R.N. Trelease, Acquisition of Membrane Lipids by Differentiating Glyoxysomes: Role of Lipid Bodies, *J. Cell Biol. 115*:995–1007 (1991).
28. Paterson, A.H., C.L. Brubaker, and J.F. Wendel, A Rapid Method for Extraction of Cotton (*Gossypium* spp.) Genomic DNA Suitable for RFLP or PCR Analysis, *Plant Mol. Biol. Reporter 11*:122–127 (1993).
29. Sambrook, J., E.F. Fritsch, and T. Maniatis, *Molecular Cloning: A Laboratory Manual,* 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
30. Feinberg, A.P., and B. Vogelstein, A Technique for Radiolabeling DNA Restriction Endonuclease Fragments to High Specific Activity, *Anal. Biochem. 132*:6–13 (1983).
31. Reed, K.C., and D.A. Mann, Rapid Transfer of DNA from Agarose Gels to Nylon Membranes, *Nucl. Acids Res. 13*:7207–7221 (1985).
32. Trelease, R.N., J.A. Miernyk, J.S. Choinski, Jr., and S.J. Bortman, Synthesis and Compartmentation of Enzymes During Cottonseed Maturation, in *Cotton Physiology*, edited by J.M. Stewart, and J.R. Mauney, Cotton Foundation, Memphis, TN, 1986.
33. Croteau, R., T.M. Kutchan, and N.G. Lewis. Natural Products (secondary metabolites), in *Biochemistry and Molecular Biology of Plants*, edited by B. Buchannan, W. Gruissem, and R. Jones, American Society of Plant Physiologists, Rockville, MD, 2000, pp. 1250–1318.
34. Wood, R., Comparison of the Cyclopropene Fatty Acid Content of Cottoneed Varieties, Glanded and Glandless Seeds, and Various Seed Structures, *Biochem. Arch. 2*:73–80 (1986).

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