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Plant Biology

Modification of *Brassica* seed oil by antisense expression of a stearoyl-acyl carrier protein desaturase gene

(fatty acid biosynthesis/lipid metabolism/rapeseed/saturated fatty acids)

DEBORAH S. KNUTZON, GREGORY A. THOMPSON, SHARON E. RADKE, WILLIAM B. JOHNSON*, VIC C. KNAUF, AND JEAN C. KRIDL†

Calgene, Inc., 1920 Fifth Street, Davis, CA 95616

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ABSTRACT **Molecular gene transfer techniques have been used to engineer the fatty acid composition of *Brassica rapa* and *Brassica napus* (canola) oil. Stearoyl-acyl carrier protein (stearoyl-ACP) desaturase (EC 1.14.99.6) catalyzes the first desaturation step in seed oil biosynthesis, converting stearoyl-ACP to oleoyl-ACP. Seed-specific antisense gene constructs of *B. rapa* stearoyl-ACP desaturase were used to reduce the protein concentration and enzyme activity of stearoyl-ACP desaturase in developing rapeseed embryos during storage lipid biosynthesis. The resulting transgenic plants showed dramatically increased stearate levels in the seeds. A continuous distribution of stearate levels from 2% to 40% was observed in seeds of a transgenic *B. napus* plant, illustrating the potential to engineer specialized seed oil compositions.**

Canola and other temperate vegetable oils are composed predominantly of unsaturated 18-carbon fatty acids: the monounsaturated oleic (18:1) and polyunsaturated linoleic (18:2) and linolenic (18:3) acids. In addition to these fatty acids, most oils also contain small but significant amounts of the saturated palmitic (16:0) and stearic (18:0) acids (1). The plastid-localized enzyme stearoyl-acyl carrier protein (stearoyl-ACP) desaturase (EC 1.14.99.6) catalyzes the initial desaturation reaction in fatty acid biosynthesis (Fig. 1*A*) and thus plays a key role in determining the ratio of total saturated to unsaturated fatty acids in plants (2, 4, 5).

Specialized fatty acid compositions desired for edible and industrial purposes have been produced in oilseed crops through traditional breeding and selection alone or in combination with mutagenesis programs (6–9). Although the molecular basis for the changes is largely unknown, examples such as the removal of erucic acid from rapeseed oil to create canola (10), reduction of linolenic acid content in flax seed (11), and increases in stearate content of up to six times the wild-type level in safflower (up to 12% stearate) (12) and soybean (up to 30% stearate) oil (13, 14) demonstrate the plasticity of fatty acid composition in seed oil. It should also be possible to modify seed oil composition by the use of genetic engineering techniques (15–17). Antisense RNA technology has proven to be an effective means of reducing the level of specific enzymes in plants (18–21). Because fatty acid biosynthesis is an essential metabolic pathway in all tissues of the plant, modification of seed oil biosynthesis may require tissue-specific control of antisense RNA expression. Reduction of stearoyl-ACP desaturase in seeds should alter the ratio of saturated to unsaturated fatty acids and lead to the production of a novel storage oil without compromising the integrity of membrane lipids in leaf and other plant tissues.

We report the isolation of a *Brassica rapa* (syn. *Brassica campestris*, turnip rape) stearoyl-ACP desaturase cDNA‡ and expression of antisense stearoyl-ACP desaturase constructs in seeds of *B. rapa* and *Brassica napus*. The activity and amount of stearoyl-ACP desaturase protein are reduced in developing seeds, resulting in dramatically higher levels of stearate in the mature seed oil.

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MATERIALS AND METHODS

Isolation of a *B. rapa* Stearoyl-ACP Desaturase cDNA Clone. A cDNA library was constructed in plasmid vector pCGN1703 (22) from poly(A)$^+$ RNA isolated from 17- to 19-day postanthesis (DPA) *B. rapa* cv. R500 embryo tissue. To facilitate screening, the cDNA plasmid bank was linearized with *Eco*RI and ligated into *Eco*RI-digested and dephosphorylated λgt10 arms (Stratagene). Approximately 120,000 recombinant plaques were screened with a 1.1-kilobase (kb) fragment of a castor bean stearoyl-ACP desaturase cDNA clone (23). Six hybridizing clones were plaque purified and one, pCGN3235, was chosen for further characterization. The 1495-base-pair (bp) insert in pCGN3235 contained an open reading frame of 398 amino acids. The identity of this cDNA clone as stearoyl-ACP desaturase was confirmed by homology to the published safflower and castor (4, 5, 23) stearoyl-ACP desaturase sequences.

Binary Plasmid Construction. A 1.16-kb *Hin*dIII–*Pvu* II fragment of pCGN3235 encoding all but four amino acids of the amino terminus and nine amino acids of the carboxyl terminus of the stearoyl-ACP desaturase precursor protein was used in the production of seed-specific chimeric antisense constructs. The stearoyl-ACP desaturase cDNA fragment was inserted in the antisense orientation into expression cassettes consisting of either 1.7 kb of 5′ and 1.25 kb of 3′ regulatory sequences derived from the *B. rapa* napin gene, BcNa1, or 1.5 kb of 5′ and 1.5 kb of 3′ regulatory sequences derived from a *B. rapa* seed ACP gene, Bcg4-4 (24, 25). The two chimeric antisense genes were inserted between the *Xba* I and *Kpn* I sites of pCGN1557 (3) to produce pCGN3242 (Fig. 1*B*).

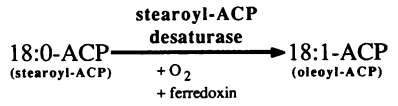
Plant Transformation. The antisense gene construct in the T-DNA region of pCGN3242 was introduced into *B. rapa* cv. Tobin and *B. napus* cv. A112 through *Agrobacterium*-mediated transformation (26). Hypocotyl explants from 7-day-old seedlings were preincubated on tobacco feeder plates made with MS medium containing 1 mg of dichlorophenoxyacetic acid (2,4-D) per liter. Explants were incubated for 10 min in a suspension of *Agrobacterium* as described (26). After 2 days of cocultivation with *Agrobac-*

Abbreviations: ACP, acyl carrier protein; DPA, days postanthesis; T-DNA, transfer DNA.

*Present address: Scripps Research Clinic Foundation, Department of Neuropharmacology, La Jolla, CA 92037.
†To whom reprint requests should be addressed.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. X60978).

A



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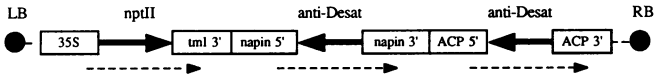

pCGN3242

FIG. 1. Strategy for antisense inhibition of stearoyl-ACP desaturase. (*A*) Reaction catalyzed by stearoyl-ACP desaturase (2), the enzyme targeted for antisense modulation. (*B*) Schematic representation of the transfer DNA (T-DNA) portion of pCGN3242, the antisense stearoyl-ACP desaturase construct used to transform *B. rapa* and *B. napus* plants. Dashed arrows indicate the direction of transcription of the neomycin phosphotransferase (*npt*II) and antisense stearoyl-ACP desaturase (anti-Desat) genes; solid arrows indicate the 5′ → 3′ orientation of the coding sequences within the expression units. The left border (LB) and right border (RB) for T-DNA transfer as well as the 35S-*npt*II-*tml* chimeric gene used as a plant transformation marker are derived from the binary vector pCGN1557 (3).

terium, explants were transferred from feeder plates to a modified callus induction medium (B5 medium containing 1 mg of 2,4-D per liter, 500 mg of carbenicillin per liter, and 25 mg of kanamycin per liter) and cultured at 22–24°C under continuous illumination (75 microeinstein·m^{-2}·s^{-1}). Seven days later, explants were transferred to B5BZ shoot regeneration medium supplemented with 500 mg of carbenicillin per liter and 25 mg of kanamycin per liter. From 5 weeks to 9 weeks after culture initiation, green shoots were excised from kanamycin-selected calli and placed on B5 medium without hormones, supplemented with 300 mg of carbenicillin per liter and 50 mg of kanamycin per liter. After 2 weeks, shoots were transferred to root induction medium containing 50 mg of kanamycin per liter. Leaf samples from green shoots that rooted in the presence of kanamycin were tested for neomycin phosphotransferase activity by dot blot assay (26). Control plants used in these studies were either grown directly from seed in the greenhouse or regenerated from hypocotyl explants as above without *Agrobacterium* treatment. To obtain seeds for study, flowering *B. rapa* cv. Tobin plants were hand-pollinated with a mixed pollen sample collected from wild-type Tobin plants. Flowering *B. napus* cv. A112 plants were self-fertilized. Developing and mature seeds were collected from the transgenic and control plants.

Inheritance Studies. The presence of the kanamycin-resistance gene was determined by germination of seeds on the kanamycin analog G418. Surface-sterilized seeds were placed on filter paper in magenta boxes containing 7 ml of 0.1× MS salts (GIBCO/BRL) containing 100 mg of G418 per liter (Sigma). Seeds were incubated at 24°C for 7 days under continuous illumination (110 microeinstein·m^{-2}·s^{-1}). Seedlings with roots and green cotyledons were scored as resistant to G418. Seedlings scored as sensitive did not elongate and had yellow cotyledons, similar to nontransformed control seedlings.

Fatty Acid Analysis. Samples (mature or immature seeds or seed extracts) were subjected to acidic methanolysis (27), and the resulting fatty acid methyl esters were analyzed by capillary gas chromatography. All separations were achieved under isothermal conditions at 230°C. Average composition data are shown as percent of total peak area. The average fatty acid content (in μg) was determined by comparing total peak area to the area of a 17:0 triglyceride internal standard.

Stearoyl-ACP Desaturase Enzyme Assay. Individual developing seeds at 26 DPA were homogenized in Microfuge tubes containing 125 μl of 0.1 M Pipes (pH 6.0) and 1 mM diethylpyrocarbonate using a motor-driven pestle. The homogenate was incubated on ice for 1 hr to allow diethylpyrocarbonate inactivation of endogenous thioesterases, which compete for substrate in the desaturase assay. One aliquot (62.5 μl) was used to assay stearoyl-ACP desaturase activity as described below, two aliquots (5 μl) were used to determine protein concentration (Bio-Rad protein assay), and the remaining sample was derivatized and analyzed for total lipid composition as described above. Stearoyl-ACP desaturase activity was assayed using 1.2 μM [9,10(n)-^{3}H]stearoyl-ACP substrate in 6 mM dithiothreitol, 0.4 mg of bovine serum albumin per ml, 0.75 mM NADPH, 100 μg of spinach ferredoxin per ml, 0.015 unit of ferredoxin:NADPH oxidoreductase per ml, 24,360 units of catalase per ml, and 50 mM NaPipes (pH 6.0) as described (4).

Detection of Stearoyl-ACP Desaturase Protein. Individual mature seeds were extracted in 100 μl of 20 mM potassium phosphate (pH 7.5). Twenty microliters of extract was electrophoresed through 12% Tris-glycine/acrylamide gels (NOVEX, San Diego) according to the manufacturer's instructions. Proteins were electroblotted to Immobilon-NC transfer membranes (Millipore). The stearoyl-ACP desaturase protein was visualized using an immunodetection kit (GIBCO/BRL) and a 1:5000 dilution of rabbit polyclonal antibody (BAbCo, Berkeley, CA) raised against purified safflower desaturase (4).

RESULTS

Antisense Stearoyl-ACP Desaturase Constructs. Embryo-specific promoters were combined with a cloned cDNA of *B. rapa* stearoyl-ACP desaturase to make antisense gene constructs designed to lower enzyme levels specifically in the developing seed. The accumulation of stearoyl-ACP desaturase mRNA is developmentally regulated in *B. rapa* embryos. The mRNA can be detected beginning at 19 DPA, is maximal at ≈25 DPA, and is not detected at 35 DPA under our growth conditions (unpublished observations). Gene expression cassettes derived from *B. rapa* Bcg4-4 ACP and BcNa1 napin genes (24, 25) were chosen to achieve a high level of expression and to express the antisense stearoyl-ACP desaturase RNA from the onset of oil synthesis throughout the period of triacylglycerol deposition in seeds. Bcg4-4 ACP mRNA expression is detectable in seeds as early as 11 DPA and peaks at about 21 DPA (24, 25). Expression of the napin storage protein gene BcNa1 in *B. rapa* seeds is first detected at 17 DPA but reaches very high levels between 21 and 30 DPA (24, 25). Thus, the use of the napin and the ACP expression cassettes was anticipated to express antisense mRNA prior to and throughout the time period of high expression of endogenous stearoyl-ACP desaturase mRNA in developing *Brassica* seeds. Antisense gene constructs utilizing the napin and ACP cassettes were combined in one binary vector for plant transformation, pCGN3242 (Fig. 1*B*).

The antisense stearoyl-ACP desaturase gene constructs contained in the T-DNA of pCGN3242 were introduced into the diploid *B. rapa* and the related amphidiploid species, *B. napus*. Due to the self-incompatibility of *B. rapa* cv. Tobin, seeds were obtained by backcrossing with wild-type Tobin. As a result, the segregation ratio of seeds from transgenic Tobin plants containing a single T-DNA insert should be 1:1 (seed hemizygous for the transgene:wild-type seed). In contrast, the expected segregation ratio of seeds from self-fertilized transgenic *B. napus* (cv. A112) plants containing a single T-DNA insert would be 1:2:1 (seed homozygous for the transgene:seed hemizygous for the transgene:wild-type seed).

High-Stearate Phenotype of pCGN3242-Transformed *B. rapa* Plants. Because as many as half of the segregating seeds produced by the backcrossed *B. rapa* transformants were expected to be wild type, oil composition analysis was performed on 10 individual seeds from each plant. Of 22 transgenic plants, 20 produced seeds with stearate levels significantly above the 1.2% stearate present in the nontransformed controls. In the majority of the plants, the highest stearate level observed in individual seeds was in the range of 3–9%. Seeds from plant 3242-T-1 contained the highest percentage composition of stearate observed (up to 32% stearate). Based on oil composition, the seeds from 3242-T-1 were grouped into high-stearate and normal-stearate (composition same as control) classes (Fig. 2). Of 50 mature seeds analyzed, 21 were in the high-stearate class and exhibited stearate levels ranging from 21.5% to 32%, and 29 contained stearate levels equivalent to control Tobin seeds (1.0–1.6%). The increased stearate was accompanied by a decrease in oleic acid, the product of the stearoyl-ACP desaturase reaction. Levels of palmitate, the precursor to stearate, were not significantly affected in seeds of this transformant. The high-stearate 3242-T-1 seeds also contained increased percentages of 18:3 and low but increased levels of long-chain (>18 carbon) saturated fatty acids. The increased percentage of 18:3 is primarily a reflection of reduced oil content in the transgenic seeds. High-stearate seeds contained 393 ± 17 μg of total fatty acid per seed, whereas the normal-stearate seeds contained 721 ± 26 μg of fatty acid per seed. The 21:29 ratio of high-stearate to normal-stearate seeds did not differ significantly from the 1:1 ratio expected for a single functional insertion of the antisense construct (χ^2 test, $P > 0.05$).

Reduction of Stearoyl-ACP Desaturase Enzyme Activity and Antigen in Seeds of 3242-T-1. To correlate the change in final seed oil composition observed in plant 3242-T-1 with antisense suppression of stearoyl-ACP desaturase, the level of enzyme activity was examined in developing seed tissue. Seeds of *B. rapa* transformants were collected at 26 DPA, a developmental stage corresponding to high levels of expression from the napin promoter and high levels of stearoyl-ACP desaturase activity in wild-type seeds. Extracts of individual seeds of 3242-T-1 and a regenerated Tobin control were divided between oil composition analysis and enzyme activity assay (Table 1). As with mature seeds, two distinct stearate phenotypes were observed in the segregating population of 26-DPA 3242-T-1 seeds. One class of seeds contained from 14.9% to 24.3% stearate, whereas the other class contained <2% stearate, as did the regenerated Tobin controls. The high-stearate seeds contained no detectable desaturase activity, whereas the enzyme activity of the normal-stearate seeds was indistinguishable from Tobin controls.

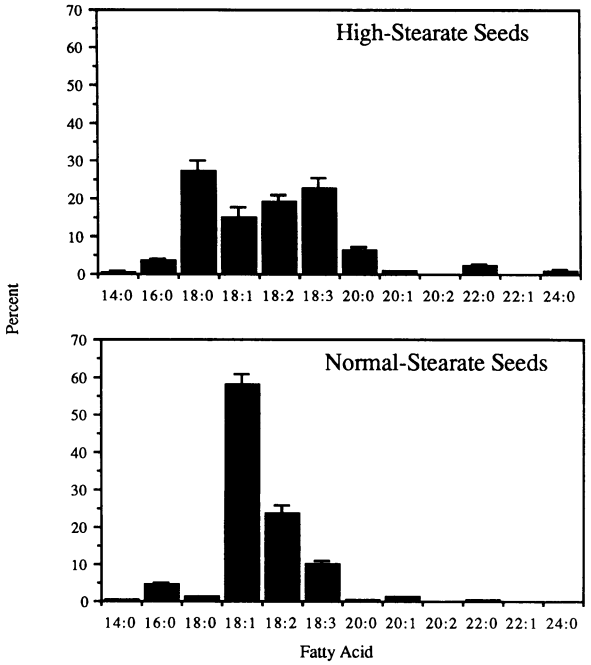

FIG. 2. Fatty acid composition of two classes of seeds from transgenic *B. rapa* plant 3242-T-1. Fifty mature seeds from 3242-T-1 were analyzed for total fatty acids and divided into two classes based on stearate composition (see text). Error bars indicate the standard deviation of each percentage.

Further evidence that the altered stearate profile observed in 3242-T-1 seeds was the result of specific suppression of stearoyl-ACP desaturase was obtained by Western blot analysis. Extracts of individual 26-DPA seeds were divided between Western blot and oil composition analysis (Fig. 3). Seeds displaying the high-stearate phenotype had markedly reduced levels of stearoyl-ACP desaturase antigen compared to normal-stearate 3242-T-1 or control Tobin seeds.

Germination and Kanamycin Resistance of High-Stearate 3242-T-1 Seeds. The segregation of the high-stearate phenotype, depressed stearoyl-ACP desaturase enzyme activity, and reduced desaturase protein levels indicated that transformant 3242-T-1 contained one functional insertion of the antisense genes. When germinated on water-soaked filter paper, 53% of 3242-T-1 seeds germinated compared to 100% of control Tobin seeds. We suspected that the lack of germination might correlate with the high-stearate phenotype of 3242-T-1 seeds. To test this hypothesis and to correlate the high-stearate phenotype with the presence of the kanamycin-resistance gene also contained in the T-DNA, a small chip was taken from each of 60 mature 3242-T-1 seeds and analyzed for oil composition. The remaining portion of the seeds was germinated on 1/10th MS medium as described (26). Germination of many of the high-stearate seeds was poor; the seed coats were uncharacteristically light-colored and the embryos were olive-green compared to the yellow color of wild-type embryos. The high-stearate seedlings did not elongate and it appeared that the cotyledons were not fully developed. To recover plants, the seedlings were transferred to B5 salts and vitamins (28) containing 2% sucrose. Thirty-seven seedlings were recovered and shoot tips were placed on rooting medium containing 50 mg of kanamycin per liter. Of the 22 shoots that rooted on kanamycin, 21 had the high-stearate phenotype, whereas 14 of 15 shoots that did not root on kanamycin had wild-type levels of stearate. Thus, the

Table 1. Stearoyl-ACP desaturase activity and stearate composition in individual *B. rapa* seeds at 26 DPA

3242-T-1		Tobin control	
% 18:0	Desaturase activity, pmol/min per mg of protein	% 18:0	Desaturase activity, pmol/min per mg of protein
20.3	0	1.3	129
19.6	0	1.4	89
14.9	0	1.6	96
22.5	0	1.2	125
24.3	0	1.4	143
1.8	96	1.3	162
1.8	126	1.5	170
20.1	0	1.3	134
1.7	162	1.2	115
23.2	0	1.5	143

Single seeds from transgenic plant 3242-T-1 or a Tobin control plant regenerated from tissue culture were homogenized and divided between enzyme assay, protein determination, and fatty acid analysis.

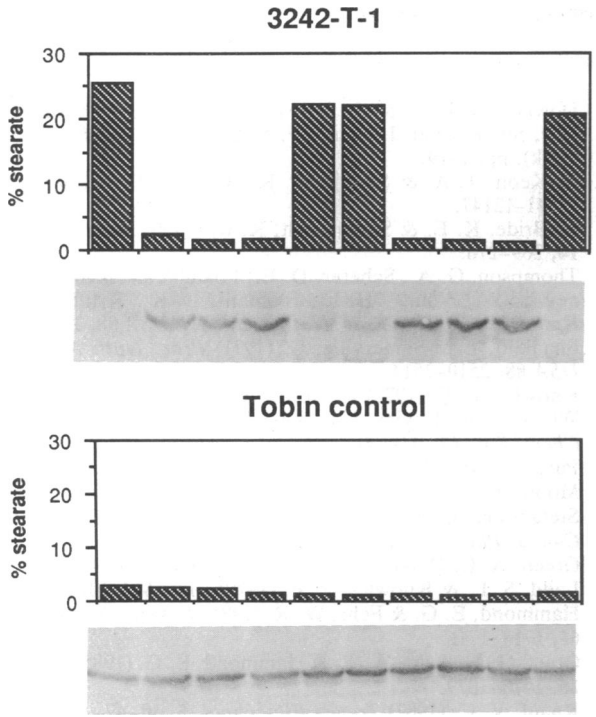

FIG. 3. Stearate content and stearoyl-ACP desaturase antigen in developing seeds of transgenic *B. rapa*. Extracts of 10 individual seeds from transgenic *B. rapa* plant 3242-T-1 and a regenerated Tobin control plant were analyzed for levels of desaturase protein by Western blot analysis and for stearate content by oil composition analysis. The percentage of stearate in each individual seed is represented by the bar graphs above each lane of the Western blot. Only the section of the blot corresponding to the migration of a 40-kDa marker is shown. The position of the dark band corresponds to the position of migration of the safflower stearoyl-ACP desaturase protein expressed in *Escherichia coli* (4).

3242-T-1 seeds with the high-stearate phenotype also contained the kanamycin-resistance marker.

Transformation of *B. napus* with Antisense Stearoyl-ACP Desaturase Constructs. The antisense stearoyl-ACP desaturase gene constructs in pCGN3242 were also introduced into the amphidiploid rapeseed species, *B. napus*. Fatty acid analysis of mature self-pollinated seed from 30 transgenic plants revealed that, as with the *B. rapa* transformants, the majority of plants produced seeds with stearate levels in the range of 3–10%. A dramatic increase in stearate composition was observed in mature self-pollinated seeds of *B. napus* plant 3242-A-3. In contrast to the discrete high-stearate and normal-stearate classes observed in 3242-T-1 seeds, a wide range of stearate content from 1.8% to 39.8% was observed in 240 individual seeds of transformant 3242-A-3 (Fig. 4). Overall, the fatty acid profile of the increased-stearate 3242-A-3 seeds resembled that of high-stearate 3242-T-1 seeds; increased stearate was accompanied by a decreased percentage of 18:1 and increased percentages of 18:3 and long-chain saturated fatty acids (data not shown). The oil content of high-stearate 3242-A-3 seeds was variable; some seeds containing >30% stearate had an oil content comparable to control A112 seeds. Mature seeds of 3242-A-3 were germinated on G418; the observed ratio of 165 resistant to 4 sensitive seedlings did not differ from an expected ratio of 63:1 (χ^2 test, $P > 0.05$), suggesting that this plant may contain functional T-DNA inserts at three loci. Southern blot analysis was consistent with this result (data not shown). The germination rate of 3242-A-3 seeds was about 90% and, unlike the 3242-T-1 *B. rapa* plant, 3242-A-3 seeds containing as much as 40% stearate appeared to germinate normally.

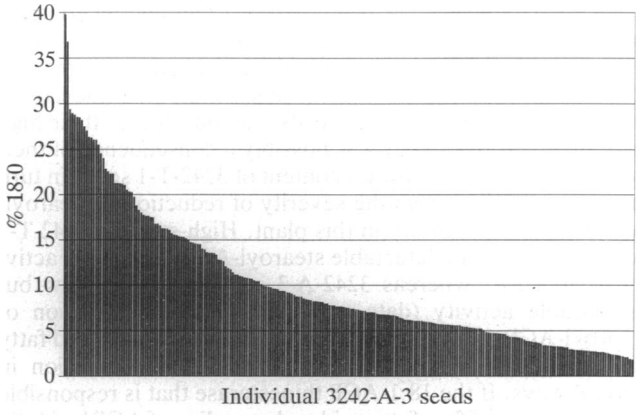

FIG. 4. Percent stearate in individual segregating seeds from a transgenic *B. napus* plant. Chips containing dried cotyledon tissue from 240 mature seeds of plant 3242-A-3 were analyzed for oil composition, and the remaining seed tissue was germinated for future studies of gene inheritance.

DISCUSSION

This work demonstrates alteration of the oil composition of a commercial oilseed species by genetic engineering. We have shown that antisense stearoyl-ACP desaturase gene expression constructs are effective in increasing the stearate content in seeds of two canola species, *B. rapa* and *B. napus*, by up to 25-fold. The increased stearate level in plant 3242-T-1 was specific to the seed oil; no change was observed in the leaf lipid composition (data not shown). Preliminary results from transgenic plants containing a constitutively expressed antisense stearoyl-ACP desaturase gene suggest that reduction of the enzyme level in leaves can be detrimental to plant growth (unpublished observations). The large decrease in stearoyl-ACP desaturase activity and protein observed in developing seeds of 3242-T-1 suggests that the tissue-specific ACP and napin expression cassettes used in the antisense gene constructs were effective in depressing stearoyl-ACP desaturase levels at an appropriate time in embryo development. Further investigation would be necessary to ascertain the effects of the antisense constructs on the steady-state levels of sense and antisense stearoyl-ACP desaturase RNA in these seeds.

The increased stearate observed in the seeds of 3242-transformed rapeseed plants was associated primarily with a decreased percentage of oleate, the product of the stearoyl-ACP desaturase reaction (Fig. 1). This was also reported in the previously described high-stearate safflower and soybean lines (12–14). However, in the high-stearate soybean lines the percentages of linoleic and linolenic acids did not change appreciably, whereas linolenic acid appeared to increase in seeds of 3242-T-1. We do not yet know if this reflects an inherent difference in fatty acid synthesis between these species or is related to the overall low oil accumulation of the high-stearate 3242-T-1 seeds. We also noted an increase in long-chain saturated fatty acids in high-stearate 3242-T-1 seeds and to a lesser extent in 3242-A-3 seeds. Two explanations are possible: either the decreased stearoyl-ACP desaturase activity results in pools of stearoyl-ACP in plastids sufficiently high to allow elongation by β-ketoacyl-ACP synthase II or pools of stearoyl-CoA accumulate and are elongated in the cytoplasm. However, elongation by a cytoplasmic system seems unlikely in a low-erucic acid canola variety that lacks the elongation pathway from 18:1-CoA to 22:1-CoA (29). Also, the fact that levels of 20:0 increased in the high-stearate soybean mutants (13) favors the former explanation. Further investigation of acyl-ACP and acyl-CoA pool sizes in the developing and mature seeds of the transgenic plants may clarify this point.

High-stearate seeds of plant 3242-T-1 had low oil contents and did not germinate. In contrast, seeds of plant 3242-A-3 with as much as 39.8% stearate had relatively normal amounts of oil and germinated well. These data suggest that the lack of germination of the 3242-T-1 seeds was not due to their high stearate levels *per se* but was possibly a consequence of their low oil contents. The low oil content of 3242-T-1 seeds in turn may have resulted from the severity of reduction in stearoyl-ACP desaturase activity in this plant. High-stearate 3242-T-1 seeds contained no detectable stearoyl-ACP desaturase activity at 26 DPA, whereas 3242-A-3 seeds contained low but measurable activity (data not shown). The elimination of stearoyl-ACP desaturase during the peak of normal seed fatty acid biosynthesis may have inhibited oil accumulation in several ways. If the 18:1-ACP thioesterase that is responsible for production of free fatty acid and recycling of ACP had little activity on stearoyl-ACP (2), pools of stearoyl-ACP would accumulate. All of the available ACP might be sequestered in this pool, limiting fatty acid biosynthesis. Alternatively, the lack of stearoyl-ACP desaturase activity may severely limit the availability of unsaturated fatty acids for further membrane and triacylglycerol biosynthesis. Indeed, the average oil content of high-stearate 3242-T-1 seeds increased only 10% from 26 DPA to maturity, whereas normal-stearate seeds increased 65%. Since the triacylglycerols in canola oil contain almost exclusively unsaturated fatty acids in the *sn*-2 position (30) and rapeseed lysophosphatidic acid acyltransferases display specificity for unsaturated fatty acids (31), lack of sufficient 18:1 may have resulted in a block in triacylglycerol biosynthesis. The total amount of 18:1 in the high-stearate seeds of plant 3242-T-1 actually decreased from 80 μg at 26 DPA to 60 μg at maturity, possibly as a result of further desaturation to 18:2 and 18:3. We suggest that developing high-stearate seeds from plant 3242-A-3 retained a small amount of stearoyl-ACP desaturase activity and that either sufficient 18:1 was produced to allow continued production of storage lipid or there is a long-chain thioesterase in *B. napus* that has a higher specificity for a stearoyl-ACP substrate than the thioesterase of *B. rapa*.

The 1:1 segregation ratio of stearate phenotype, reduced enzyme activity, reduced protein, and the correlation of high stearate with kanamycin resistance in 3242-T-1 seeds all indicate the presence of one T-DNA insert. In contrast, Southern blot analysis and segregation of kanamycin resistance in seeds of 3242-A-3 suggest T-DNA inserts on three different chromosomes. We have not yet assessed the individual contributions of each antisense gene insertion to the phenotype, but we believe that the independent segregation of multiple antisense genes displaying various levels of expression may account for the range of stearate levels observed in the self-pollinated 3242-A-3 seeds. We anticipate that breeding of the 3242-A-3 progeny will lead to a number of stable lines, each with a different level of stearate.

A temperate oilseed crop such as canola with the ability to make a high-stearate oil could have considerable commercial utility as well as providing material for studies on regulation of fatty acid metabolism. Finally, the alteration of stearate levels in canola oil by genetic engineering predicts the success of other direct genetic modifications of vegetable oil composition.

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