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A Mutation at the *fad8* Locus of *Arabidopsis* Identifies a Second Chloroplast ω-3 Desaturase[1]

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Two independently isolated mutations at the *fad7* locus in *Arabidopsis* produced plants with a temperature-conditional phenotype. Leaves of *fad7* mutants grown at 28°C contained less than 30% of wild-type levels of trienoic fatty acids (16:3 plus 18:3) compared with more than 70% of wild-type levels for plants grown at 15°C. Screening of an M_2 population derived from the *fad7-1* line led to the identification of a line, SH1, in which the proportion of trienoic acids was much less than in *fad7* plants. The segregation pattern of F_2 progeny from a cross between SH1 and wild type indicated that the additional fatty acid mutation in SH1 is at a new locus, designated *fad8*. In a genetic background that was wild type at the *FAD7* locus, the *fad8* mutation had no detectable effect on overall leaf fatty acid composition irrespective of the temperature at which plants were grown. However, fatty acid analyses of individual leaf lipids revealed small decreases in the levels of 18:3 in two chloroplast lipids. In *fad8* plants grown at 22°C, phosphatidylglycerol contained 22.5% 18:3 compared with 33.5% in wild-type *Arabidopsis*. For sulfoquinovosyldiacylglycerol, the values were 31.4 and 44.5%, respectively. Together with information from studies of the cloned *FAD8* gene (S. Gibson, V. Arondel, K. Iba, C. Somerville [1994] Plant Physiol 106: 1615–1621), these results indicate that the *FAD8* locus encodes a chloroplast-localized 16:2/18:2 desaturase that has a substrate specificity similar to the *FAD7* gene product but that is induced by low temperature.

Fatty acids containing three double bonds (trienoic fatty acids) are the dominant acyl components of chloroplast membranes in all higher plants (Harwood, 1982). The major chloroplast glycerolipid, MGD, typically contains more than 90% of α-linolenic acid (18:3) or a combination of α-linolenic and hexadecatrienoic (16:3) acids depending on the plant species (Jamieson and Reid, 1971). These observations have been taken as inferential evidence that trienoic fatty acids have an important, possibly essential, role in assuring photosynthetic competence of the light-harvesting thylakoid membranes. One attractive approach to investigating the role of trienoic fatty acids in photosynthesis and other processes is to isolate mutants that are deficient in 16:3 and 18:3 synthesis. In *Arabidopsis*, two classes of mutants have been isolated that have decreased capacities for conversion of 18:2 to 18:3 and 16:2 to 16:3 (Browse et al., 1986, 1993). However, to date, it has not been possible to identify plants that completely lack trienoic fatty acids.

There are two distinct pathways in leaf cells for the biosynthesis of membrane glycerolipids and the associated production of polyunsaturated fatty acids (Roughan et al., 1980; Browse and Somerville, 1991). Both pathways are initiated in the chloroplast with the synthesis of 16:0-ACP and 18:1-ACP. The "prokaryotic" pathway (Roughan et al., 1980) located in the chloroplast inner envelope uses 18:1-ACP and 16:0-ACP for the sequential acylation of glycerol-3-phosphate to form the glycolipid components (PG, MGD, DGD, and SL) of the chloroplast membranes (Browse and Somerville, 1991). The "eukaryotic" pathway involves the export of 16:0 and 18:1 fatty acids from the chloroplast as CoA thioesters and their incorporation into PC and other phospholipids that are the principal structural lipids of all of the membranes of the cell except for the chloroplasts. In addition, the diacylglycerol moiety of PC can be returned to the chloroplast envelope and used for the production of MGD, DGD, and SL (Browse and Somerville, 1991). In each pathway, further desaturation of the 16:0 and 18:1 occurs only after these fatty acids have been incorporated into the major membrane lipids. Thus, most of the plant desaturases responsible for the synthesis of 18:3 and 16:3 are integral membrane proteins that utilize glycerolipids as substrates.

To date, only one chloroplast membrane-bound desaturase has been purified to homogeneity (Schmidt et al., 1993). In most cases, the techniques required to solubilize integral membrane proteins have led to the loss of desaturase activity. Because of the difficulties involved in studying the desaturases by traditional biochemical techniques, much of our current understanding of desaturation in plants has come from the characterization of six *Arabidopsis* mutants, each one deficient in the activity of a different membrane-bound desaturase. The chloroplast desaturases are responsible for the conversion of 16:0 through 16:1 and 16:2 to 16:3 (Browse et al., 1986, 1989; Kunst et al., 1989). The eukaryotic pathway desaturases located in the ER are responsible for the addition of a second and third double bond to 18:1 (Miquel and Browse, 1992; Browse et al., 1993). However, the chloroplast

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Abbreviations: ACP, acylcarrier protein; DGD, digalactosyldiacylglycerol; EMS, ethylmethane sulfonate; MGD, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; SL, sulfoquinovosyl diacylglycerol (sulfolipid); X:Y, a fatty acid containing X carbon atoms and Y double bonds (*cis* unless specified).

desaturases of the prokaryotic pathway also synthesize 18:3 from 18:1. Because there is reversible exchange of lipids between the chloroplast and ER, the desaturases of both pathways contribute to the production of 18:3 on membrane lipids found throughout the cell (see Browse et al., 1993, for discussion).

In our studies of the original *fad7* mutant (*fad7-1*), we described a temperature-dependent fatty acid phenotype (Browse et al., 1986). When plants were grown in the range of 10 to 18°C, the proportion of trienoic fatty acids (18:3 plus 16:3) in *fad7-1* leaves was similar to wild type. As plants were grown at progressively higher temperatures, the trienoic content decreased sharply so that at a growth temperature of 26°C the proportion of trienoic fatty acids in the mutant was less than 35% of that found in wild-type controls. Our interpretation of these observations was that the *fad7-1* allele represented a temperature-sensitive mutation at the *fad7* locus that gave rise to a thermolabile gene product. To confirm this possibility, we continued to screen M_2 populations of *Arabidopsis* in attempts to find an independent, nonconditional mutation at the *fad7* locus. However, as reported here, this search resulted in the isolation of an additional *fad7* mutant that exhibits the same temperature-sensitive fatty acid profile as *fad7-1*. This suggested that the apparent temperature sensitivity of the *fad7* mutants might represent low-temperature induction of an additional desaturase, which partially compensated for the defect in the *fad7* mutants. Screening of a new M_2 population generated in the *fad7-1* genetic background allowed the identification of a mutation at a new locus, designated *fad8*, that controls the activity of an additional, chloroplast-located 16:2/18:2 desaturase.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Except as noted, the lines of *Arabidopsis thaliana* (L.) Heynh. used here were descended from the Columbia ecotype and are available from the *Arabidopsis* Resource Center, Ohio State University, and Landsberg erecta wild types. The isolation of the mutant line JB101 (*fad7-1*) has been described previously (Browse et al., 1986). The other mutants were isolated from independent M_2 populations obtained after mutagenesis with EMS (Haughn and Somerville, 1986). Line LK9 (*fad7-2*) was isolated following EMS mutagenesis of the Columbia wild type (Kunst, 1988), and SH1 (*fad7-1 fad8*) was isolated from an EMS-mutagenized M_2 population derived from line *fad7-1*. The mutants were identified by directly analyzing the fatty acid composition of single-leaf samples by GC (Browse et al., 1985). All the mutant lines except SH1 were backcrossed to wild type a minimum of two times before use in the experiments described here.

Plants were grown on soil in controlled environment chambers at 22°C and continuous fluorescent illumination (130 μE m^{-2} s^{-2} PAR) for 10 d and then transferred to the various temperatures specified in the figure legends.

Genetic Analysis

Crosses were performed between the mutant lines and the wild type (Browse et al., 1993), and the F_1 progeny were tested for fatty acid composition. Genetic complementation was assessed by performing crosses between the various mutant lines and analyzing the fatty acid composition of the F_1 progeny. Segregation of the *fad7-1* and *fad8* loci was determined by crossing the mutant line, SH1, with wild-type Columbia, allowing the F_1 progeny to self-pollinate, and by scoring individuals from the F_2 population for the fatty acid composition.

Fatty Acid and Lipid Analysis

Overall, fatty acid compositions of leaves, roots, and seeds were determined by GC after derivitization with 2.5% (v/v) H_2SO_4 in methanol (Miquel and Browse, 1992). Samples of leaf tissue were killed rapidly by immersion in liquid nitrogen and ground under liquid nitrogen before the lipids were extracted and analyzed as previously described (Miquel and Browse, 1992).

RESULTS

Alleles of *fad7* Exhibit a Similar Temperature Dependence

Screening of several, independently derived M_2 populations of *Arabidopsis* led to the isolation of two mutant lines, JB101 (Browse et al., 1986) and LK9 (Kunst, 1988), which contained decreased levels of both 16:3 and 18:3 fatty acids and correspondingly increased levels of 16:2 and 18:2. Crosses between these two lines yielded F_1 progeny with fatty acid compositions similar to those of the two parents (Table I). The lack of genetic complementation in these experiments indicates that the mutations are alleles at the same locus. Therefore, the alleles in JB101 and LK9 are designated *fad7-1* and *fad7-2*, respectively. The F_1 progeny of crosses between wild type and the *fad7* mutants contained levels of trienoic fatty acids that were intermediate between those of the two parents (Table I). Thus, the two alleles demonstrate incomplete dominance with respect to effects on fatty acid composition.

Because the leaf fatty acid composition of *fad7-1* plants is rather similar to wild type at low temperatures but shows a substantial loss of trienoic fatty acids between 18 and 26°C, we previously speculated that this allele might represent a temperature-sensitive lesion (Browse et al., 1986). However, when the fatty acid composition of *fad7-2* plants was exam-

Table I. *Complementation and dominance analysis of fad7 mutants*

Plants were grown at 22°C for 2 weeks and transferred to 27°C for 2 weeks prior to leaf lipid fatty acid analysis. The values represent the averages ± SD of eight independent samples and are presented as mol percent of total leaf fatty acids.

Line	Trienoic Acid Content
	mol %
Wild type	59 ± 5
fad7-1 (JB1)	17 ± 2
fad7-2 (LK9)	16 ± 2
fad7-1 × *fad7-2* F_1	15 ± 1
Wild type × *fad7-1* F_1	28 ± 1
Wild type × *fad7-2* F_1	29 ± 2

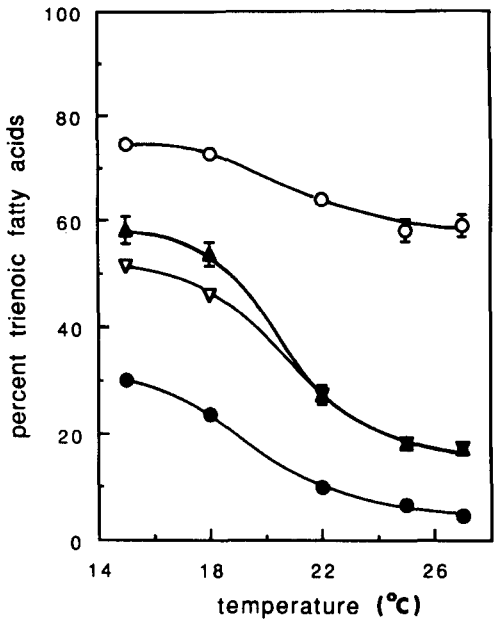

Figure 1. Leaf trienoic acid content of wild-type and mutant *Arabidopsis* at various growth temperatures. Plants were grown at 22°C for 2 weeks and transferred to 15, 18, 22, 25, or 27°C for 2 weeks prior to leaf fatty acid analysis. The values represent the averages ± SE of eight independent samples. O, Wild type; ▲, *fad7–1*; ▽, *fad7–2*; ●, *fad7–1 fad8*.

ined as a function of growth temperature, it became clear that this line also suffered a striking loss of trienoic fatty acids between 18 and 26°C (Fig. 1) but had comparatively normal fatty acid composition below 18°C. Temperature-sensitive mutations are typically missense mutations that mediate a single amino acid substitution in the gene product and thereby produce a protein that is somewhat stable at low (permissive) temperatures but that is denatured at higher, although still physiological (nonpermissive), temperatures. Because such amino acid substitutions are likely to be a small subset of all of the possible mutations at a given locus, the probability of observing two mutant alleles with essentially the same temperature profile is very remote. On this basis, our characterization of the *fad7* mutants provided strong evidence against a temperature-sensitive mutation being the primary cause of the fatty acid lesion in either of the *fad7* lines and raised the possibility that there is a second chloroplast desaturase in *Arabidopsis* that acts on 16:2 and 18:2 acyl groups.

Interestingly, the *fad7–1* plants contained slightly higher levels of trienoic fatty acids at the two lowest growth temperatures (15 and 18°C). This finding suggested to us that *fad7–1* might contain a leaky mutation that permits a small amount of desaturation at low temperatures. The *FAD7* gene has recently been cloned and shown to represent the structural gene encoding a chloroplast desaturase (Iba et al., 1993).

Characterization of a New *fad* Mutant

Extensive screening of M_2 populations derived from mutagenesis of wild-type *Arabidopsis* failed to produce any direct, genetic evidence for an additional desaturase that could mediate the desaturation of 16:2 and 18:2. Therefore, we generated a new M_2 population by EMS mutagenesis of JB101 (*fad7–1*). Screening of 1500 plants by GC identified one plant that contained considerably less trienoic fatty acids than the *fad7–1* parents and correspondingly increased levels of 16:2 and 18:2. Analysis of leaf fatty acids from progeny of this plant demonstrated that the mutation was heritable and the resulting line was designated SH1. Plants of the SH1 line grown at 15°C exhibited a trienoic fatty acid content that was less than half of that found in *fad7–1* plants grown at the same temperature (Fig. 1).

We considered two possible explanations for the decreased trienoic content of SH1 plants relative to plants of the *fad7–1* line. First, the SH1 line might contain a second mutation at the *fad7* locus that is more extreme than those previously identified. Alternatively, the new mutation might be in a separate gene that controls the activity of an isozyme of the FAD7 desaturase. To distinguish between these two possibilities, SH1 plants were crossed to wild type and the leaf fatty acid compositions of individual F_2 progeny, grown at 13°C, were examined. To facilitate analysis of the data, the ratio of 16:2/16:3 was calculated because this ratio clearly distinguished the SH1 parent from homozygous *fad7* and wild-type plants. In this experiment, a more extreme allele of *fad7* would have produced a 1:2:1 segregation; instead, the analysis of 85 F_2 progeny produced a complex distribution (Fig. 2) that suggests the segregation of two independent mutations. The five plants having 16:2/16:3 > 3.0 would correspond to the 1/16th of the sample that were homozygous mutants at *fad7* and the second locus; the 18 plants having 2 > 16:2/16:3 > 0.5 would correspond to the 3/16ths of the sample that are homozygous *fad7* and either wild type or heterozygous at the second locus. The clustering of the remaining

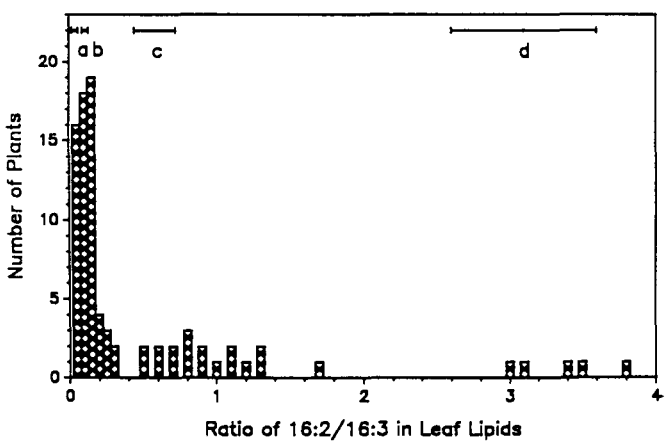

Figure 2. Segregation analysis of the *fad7–1* and *fad8* loci. The SH1 line was crossed to wild type and the resulting F_1 hybrids were allowed to self-pollinate. Eighty-five F_2 progeny were grown at 22°C for 2 weeks and then transferred to 13°C for 2 weeks prior to leaf fatty acid analysis. The ratio of 16:2 to 16:3 was calculated for each individual. Wild type, *fad7–1*, wild type × *fad7–1* F_1, and *fad7–1 fad8* (SH1) plants were also grown under identical conditions and assayed for leaf lipid fatty acid content. The mean ratio of 16:2/16:3 for eight individuals of each genotype ± SD is shown. a, Wild type; b, wild type × *fad7–1* F_1; c, *fad7–1*; d, *fad7–1 fad8*.

samples (16:2/16:3 < 0.3) suggested that the mutation at the second locus may have no fatty acid phenotype when it is expressed in a genetic background that is wild type at *FAD7*. Such a situation would be consistent with our failure to identify mutations at this locus in M_2 populations derived from mutagenesis of wild-type *Arabidopsis*. We designated this new locus *fad8*.

Since our analysis suggested that plants of the genotype *FAD7/FAD7 fad8/fad8* were indistinguishable from wild type, the 25 plants having the lowest 16:2/16:3 ratios in Figure 2 should include all of the homozygous *FAD7* plants that are segregating 1:2:1 for the two alleles at the *fad8* locus. To isolate and identify a homozygous *FAD7 fad8* line, eight of the plants from this group were crossed with the *fad7-2* mutant. Six individual F_1 seeds from each cross were grown to maturity and F_2 seeds collected. Then F_2 plants of the six families from each cross were grown and scored for the double-mutant phenotype. The basis of this approach is that double-mutant plants will appear in all six families of a cross only if the phenotypically wild-type parent in the cross was homozygous mutant for the *fad8* mutation. A heterozygous *FAD8/fad8* parent will produce double mutants in half of the families, whereas no double mutants will appear in families derived from a homozygous *FAD8* parent. From this experiment, we isolated one homozygous line, which, through its pedigree, unequivocally established the existence of a distinct *fad8* locus. We were also able to produce a *fad7-2 fad8* double mutant.

Temperature Regulation of Leaf Fatty Acid Composition in the Mutants

Analysis of the mutants is complicated to some extent by the strong temperature response of trienoic fatty acid content in *fad7* plants (Fig. 1). In this experiment, the *fad7-1 fad8* double mutant also showed a marked decrease in trienoic content between 15 and 27°C, although some of this decrease probably reflects the leaky nature of the *fad7-1* allele at low temperatures.

To more completely assess the interaction of the *fad7* and *fad8* mutations with temperature, we analyzed the overall leaf fatty acid compositions of wild-type and mutant plants grown at 12 and 28°C (Table II). The *fad8* fatty acid profile was essentially indistinguishable from wild type at either temperature. Both of these lines showed a modest decline from 4.6 to 4.0 in the average number of double bonds per glycerolipid molecule between 12 and 28°C. The effect of the *fad7-2* mutation on the number of double bonds per glycerolipid was much greater at 28°C (3.3) than at 12°C (4.3). This strong temperature effect was substantially reduced when the *fad8* mutation was introduced into the *fad7-2* background (Table II) mainly because the double mutant contains very little 16:3 and only 23% 18:3 in the leaf lipids at 12°C. We attribute the remaining 18:3 in the double mutant to action of the ER desaturase encoded by *FAD3* (Browse et al., 1993).

Fatty Acid Compositions of Individual Lipids

To investigate the lipid phenotypes of the *fad8* and *fad7-2 fad8* lines in more detail, we purified the individual polar lipids from leaf tissues of wild-type and mutant plants and analyzed their fatty acid compositions (Table III). In all of the major leaf lipids, fatty acid composition was essentially unaffected by the *fad8* mutation. However, the data indicate a specific effect of *fad8* in decreasing the 18:3 content of PG and SL, two lipids that are largely confined to the chloroplast membranes. This suggests that there may be some differential substrate specificity exhibited by the desaturases that are controlled by the *FAD7* and *FAD8* genes. When comprehensive lipid analyses were carried out on plants grown at higher (28°C) and lower (12°C) temperatures, it was found that PG and SL were still the only lipids whose fatty acid compositions were significantly affected by the *fad8* mutation (data not presented).

Comparisons of the *fad7-2 fad8* double mutant with the wild type reveals that every lipid is affected by the combined mutations but that each lipid also retains a considerable proportion of 18:3 (but not 16:3) fatty acid. Although these data cannot rule out the existence of additional chloroplast desaturases, they are also consistent with the possibility that 18:3, synthesized on the ER by the *FAD3* gene product, is supplied to the chloroplast lipids via the eukaryotic pathway (Browse and Somerville, 1991; Browse et al., 1993).

DISCUSSION

The isolation of a new mutant allele at the *fad7* locus (previously *fadD*) caused us to reevaluate our earlier hypothesis (Browse et al., 1986) that the *fad7-1* mutation in line

Table II. *The total fatty acid composition of leaves of wild-type and mutant Arabidopsis grown at 12 and 28°C*

The values represent the averages of eight independent samples. Standard errors were approximately 3% of the mean values shown.

Fatty acid	12°C				28°C			
	Wild type	*fad8*	*fad7-2*	*fad 7-2 fad8*	Wild type	*fad8*	*fad7-2*	*fad7-2 fad8*
16:0	14.8	14.2	15.1	13.8	16.5	16.8	17.1	16.3
16:1	1.4	1.4	1.3	2.2	3.9	4.2	4.5	4.4
16:2	0.1	0.6	7.1	10.6	1.2	1.2	9.7	9.3
16:3	12.4	11.6	5.5	0.5	9.4	8.1	0.6	—
18:0	0.7	0.6	0.7	0.5	1.8	2.0	2.5	1.9
18:1	2.8	2.8	3.6	4.0	4.2	4.6	6.0	6.0
18:2	14.1	17.6	20.7	44.8	22.3	25.9	46.2	51.9
18:3	53.2	51.2	46.1	23.5	40.6	37.2	13.2	9.8

Table III. *Fatty acid compositions of leaf lipids from wild-type and mutant Arabidopsis grown at 22°C*

Values represent averages of two samples (four replicates each) and are presented as mol percent. Standard errors were approximately 5% the mean values shown.

Lipid Class	Genotype	Percentage of Total Polar Lipids	Fatty Acid Composition							
			16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3
MGD	Wild type	37.5	1.3	0.8	1.8	32.4	0.1	0.8	2.9	59.7
	fad8	34.7	1.1	0.7	1.7	32.5	0.1	0.8	3.3	59.7
	fad7-2 fad8	35.1	1.0	1.7	29.9	—[a]	0.2	2.0	50.5	14.4
DGD	Wild type	15.5	12.3	0.6	0.9	2.4	1.5	1.3	4.9	77.6
	fad8	16.5	9.7	0.8	1.0	2.6	0.7	1.1	5.4	78.8
	fad7-2 fad8	17.4	8.0	0.4	4.2	—	0.4	1.7	55.1	28.9
PG	Wild type	8.9	29.3	18.8[b]	—	—	1.3	5.2	11.9	33.5
	fad8	8.5	27.8	18.1	—	1.2	2.3	3.3	22.8	22.5
	fad7-2 fad8	9.0	30.1	17.6	1.1	—	1.1	8.5	37.1	4.8
SL	Wild type	2.9	36.3	1.0	—	—	2.3	4.1	12.0	44.5
	fad8	3.2	36.2	2.2	—	—	2.2	5.8	21.7	31.4
	fad7-2 fad8	3.2	36.0	1.0	1.0	—	2.2	6.5	38.0	16.4
PC	Wild type	20.4	20.4	—	—	—	1.9	5.4	37.2	34.4
	fad8	21.9	19.3	—	—	—	2.4	6.4	36.1	31.4
	fad7-2 fad8	20.6	19.3	—	—	—	1.3	8.4	45.6	24.0
Phosphatidylethanolamine	Wild type	10.6	27.3	—	—	—	2.3	3.3	36.9	28.5
	fad8	10.7	27.6	—	—	—	1.8	4.2	37.6	28.0
	fad7-2 fad8	10.7	27.1	—	—	—	1.4	4.7	44.4	20.6
Phosphatidylinositol	Wild type	4.1	36.9	—	—	—	3.8	3.3	25.1	26.8
	fad8	4.4	38.5	—	—	—	3.3	3.8	26.6	25.9
	fad7-2 fad8	4.0	38.5	—	—	—	3.6	4.1	33.1	18.2

[a] Dashes indicate that the acyl group was not detected or was less than 1%. [b] The isomer in PG is 16:1 *trans*.

JB101 represented a temperature-sensitive mutation. Ironically, detailed temperature profiles for *fad7-1* and *fad7-2* leaf fatty acid compositions suggest that *fad7-1* may indeed have some FAD7 desaturase activity at low temperature (Fig. 1), although *FAD7* transcript was not detectable on northern blots of RNA from *fad7-1* plants grown at 22°C (Iba et al., 1993). Remutagenesis of the *fad7-1* line allowed us to identify a new mutant locus, *fad8*, that also controls 16:2 and 18:2 desaturation. Segregation analysis indicated that the *fad8* mutation is in a nuclear gene that is not linked to *fad7*. Because *fad8* has no easily discernible phenotype in *FAD7* background, identification of a homozygous *fad8* line required confirmation through pedigreed crosses to *fad7-2* mutant plants. The subsequent cloning of the *FAD8* gene (Gibson et al., 1994) has permitted the identification of a mutation-induced restriction enzyme polymorphism in the mutant *fad8* allele that permits tracking of the mutation as a restriction fragment length polymorphism.

Only the chloroplast lipids MGD and DGD contain 16:3; therefore, the observation that the *FAD8* gene product appears to mediate both 16:2 and 18:2 desaturation indicates that *FAD8* is a second chloroplast ω-3 desaturase. We found that the overall fatty acid compositions of roots and mature seeds of *fad7 fad8* mutant plants did not differ significantly from wild type (data not presented). Since plastids are a minor component of the membrane complement in root and seed tissues, this observation also supports the proposal that the *fad8* locus controls a chloroplast desaturase.

Comparison of the temperature profiles of trienoic acid content in *fad7* and *fad7 fad8* mutant plants indicates that the *FAD8* gene has a greater role in lipid desaturation at low temperatures. This observation is consistent with data showing that the *FAD8* transcript is considerably more abundant in plants grown at low temperature than in plants grown at temperatures above 20°C (Gibson et al., 1994). A decrease in temperature has been shown to increase transcript levels of the *desA* gene of *Synechocystis* PCC6803, which encodes a 16:1/18:1 desaturase in this cyanobacterium (Vigh et al., 1993). Furthermore, the effect of low temperature could be mimicked by limited catalytic hydrogenation of the cell membranes of the cyanobacterium (Vigh et al., 1993). The small effect of the *fad8* mutation on the fatty acid compositions of PG and SL and the lack of any effect on other leaf membrane lipids (Tables II and III) indicate that within the temperature range examined (12° to 28°C) the *FAD7* gene product is largely capable of maintaining wild-type levels of membrane unsaturation. From these data, it might be concluded that *FAD8* is largely irrelevant to lipid metabolism in *Arabidopsis*. However, the results do not preclude a role either under

particular conditions, e.g. very low temperatures, or at a particular stage of plant development.

We have not yet evaluated growth of the *fad8* and *fad7–2 fad8* mutants at very low temperatures, but within the range of temperatures used in this study (12–28°C), plants of both lines exhibited robust growth and were indistinguishable from wild type except for a slight decrease in leaf Chl content caused by the *fad7* mutation (McCourt et al., 1987; Iba et al., 1993). The *fad7–2 fad8* double-mutant plants contain no 16:3 and only 10 to 25% (depending on temperature) of the total trienoic fatty acids found in wild-type plants (Table II). The 18:3 that is found in the double mutant may be synthesized by the *FAD3* gene product (Arondel et al., 1992; Browse et al., 1993), although the existence of other desaturases cannot be ruled out. In any case, the production of a *fad3 fad7 fad8* triple mutant is predicted to provide plants with levels of 18:3 much lower than those reported here for *fad7–2 fad8* plants. The triple mutant will supply a new means to investigate the roles of trienoic fatty acids in the structure and function of cellular membranes, particularly the light-harvesting thylakoid membranes of the chloroplasts. Because 18:3 is the precursor of jasmonate and methyl jasmonate in plants (Farmer and Ryan, 1992; Sembdner and Parthier, 1993), the triple mutant may also provide an opportunity to study the synthesis and functions of these signaling molecules.

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