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Novel mutations affecting leaf stearate content and plant size in *Arabidopsis*

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Abstract The *fab2-1* mutant of *Arabidopsis* is an extreme dwarf as a direct result of an increase in the levels of stearate (18:0) in membrane lipids. We isolated a series of lines in which second-site suppressor mutations partly alleviate the dwarf phenotype. In all four of the suppressor lines examined, restoration of more normal morphology is accompanied by decreases in leaf 18:0 content. Three of the isolated suppressors suppress the high stearate phenotype in both leaves and seeds. The effects of one of the suppressors, TW2-1, is limited to the leaves. A second allele at the *fab2* locus, *fab2-2*, was also identified and plants homozygous for this allele where intermediate in both plant size and 18:0 content between wild-type *Arabidopsis* and *fab2-1* mutants. The alleles at *fab2* and the suppressor mutations provided a total of nine genotypes which were analyzed to demonstrate a clear-cut relationship between leaf 18:0 content (0.7–19.6% of total leaf fatty acids) and reductions in plant size (24–4 mm). These results illustrate the utility of suppressor analysis for addressing problems in biochemistry and plant biology. They also indicate that the genetic control of plant lipid composition is more complex than previously appreciated.

Key words *Arabidopsis* · Fatty acid · Suppressor · Development · Mutant

Introduction

We have recently described a miniature mutant of *Arabidopsis thaliana*, *fab2,* in which a change in membrane fatty acid composition produces dramatic alterations in plant morphology (Lightner et al. 1994 a). The *fab2* mutant of *Arabidopsis* has increased levels of stearate (18:0) in seeds and leaves and this alteration in fatty acid composition causes numerous cell- and organ-specific changes that result in miniature growth (Lightner et al. 1994 a). Plant developmental processes have been the subject of considerable genetic investigation, and a wide variety of mutations which alter plant development have been identified (Okada and Shimura 1992). The advantages of the higher plant *A. thaliana* as a model for genetic studies have been recognized for more than 40 years (Redei 1992). One important component of biochemical genetic studies has been the isolation and characterization of revertants and suppressors of mutations (Koornneef et al. 1982; Spreitzer and Ogren 1983; Porter et al. 1992). By virtue of the small size of its seeds, and the small space necessary to propagate plants, *Arabidopsis* is uniquely suited for reversion experiments where considerably larger populations must be screened than those required for primary mutagenesis. Recently, Peng and Harberd (1993) have reported the generation of several derivative mutations of the *gai* locus in *Arabidopsis* which restore a wild-type growth habit. The *gai* mutation confers semi-dominant gibberellin insensitivity (Koornneef et al. 1985). All of the reported derivative mutations were allelic to *gai*; this result is not surprising since *gai* is a gain-of-function mutation (Koornneef et al. 1985) and subsequent mutations at this locus can be expected to disrupt the gain-of-function effect. Intergenic suppressors of an *Arabidopsis* nitrate reductase mutant, *ngr*, have also been reported (Braaksma and Feenstra 1982).

The *fab2* mutant was originally isolated in a screen of seed fatty acid composition as having increased levels of 18:0 in seed lipids from a wild-type level of 2–4% to a level of 8% in the mutant (James and Dooner 1990). An increased 18:0 is also observed in the leaves of *fab2* plants and this increase is distributed throughout the

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major membrane-lipid classes (Lightner et al. 1994 b). The most striking feature of the *fab2* mutant is a dramatic miniature phenotype when the plants are grown at 22°C. A large collection of mutations which alter lipid composition have been isolated and characterized in *Arabidopsis* (Browse and Somerville 1991, 1995). Changes in fatty acid composition have been shown to affect chloroplast ultrastructure at normal (McCourt et al. 1987; Kunst et al. 1989) and low (Hugly and Somerville 1992) temperatures. The *fab2* mutant, however, is the only fatty acid mutant which displays an obvious morphological phenotype at normal growing temperatures. Reversion and suppressor mutations provide powerful tools for causally relating two phenotypes in a single mutant line, but they can also provide unique perspectives on biological processes, identifying new loci which influence the process being examined. Here we report the fascile generation of second-site suppressor mutations of the *fab2-1* mutation in *Arabidopsis*, and the preliminary characterization of these suppressors and their role in fatty acid metabolism. We also describe the isolation of a new, leaky allele of the *fab2* mutation, *fab2-2*. The new lines provide quantitative data on the relationship between membrane stearate content and plant size.

Materials and methods

Plant material

Wild-type and mutant *Arabidopsis* were sown on potting mix watered with half-strength GP Blue fertilizer. Pots were covered with plastic wrap and placed in the cold (4°C) overnight. Plants were then transferred to 22°C and the plastic wrap was removed 2 days after germination. Maximum rosette diameter of the true leaves was measured with a ruler at 14 days of age.

Mutagenesis and screening

To guard against pollen or seed contamination of the suppressor screen, a plant of the *fab2-1* line was crossed to a plant carrying the *gl-1* mutation and a *fab2-1 gl-1* double-mutant line was derived. The *gl-1* mutation prevents the formation of leaf trichomes and thus provides a visible marker for the experiment. A total of 40 000 homozygous *fab2-1 gl1* seeds were placed in 100 ml of 0.3% ethylmethanesulfonate in water (v/v) and allowed to imbibe for 12 h with occasional mixing. Seeds were rinsed 20 times over 3 h before planting at high density in 4″ pots (100 seeds/pot). M_1 plants were screened visually for the occurrence of normal growing sectors. M_2 seeds were collected in pools derived from 12 pots each. M_2 seeds were planted at high density and screened for the occurrence of normal growing plants.

Screens for *fab2* alleles were conducted by monitoring seed fatty acid composition as described by James and Dooner (1990).

Lipid analysis

The fatty acid composition of leaves and other tissues was determined by heating samples at 80°C in 1 ml of 2.5% (v/v) H_2SO_4 in methanol for 60 min in screw-capped test tubes (Browse et al 1985). After the addition of 1.5 ml of H_2O and 0.5 ml of Hexane the fatty acid methyl esters were extracted into the organic phase by shaking the tubes and centrifuging at low speed to accelerate separation. Then 1-ml samples were analyzed by gas chromatography on a 15 m × 0.53 mm Supelcowax column (Supelco, Bellefonte, P.) and quantified with flame ionization detection. The chromatograph was programmed for an initial temperature of 140°C for 2 m in followed by a 20°C/min ramp to 160°C and a secondary ramp of 5°C/min to 172°C. The final temperature was maintained for 2 min. Statistical analysis of fatty acid composition was performed using the Microsoft Excel Analysis ToolPak (Microsoft Corporation and Greymatter International, Inc.).

Results

Isolation of a second *fab2* allele

In a screen of a pedigreed M_2 population (James and Dooner 1990), one plant produced M_3 seed with an increased proportion of 18:0 in the oil. Seeds of this plant were kept as line 7b15. The stearate content of 7b15 seeds is only about 1.3 times that of the wild-type (Table 1) compared to the two-fold increase in stearate observed in seeds of *fab2-1*. Notably, the 20:0 content is also increased in the mutant seeds of the 7b15 mutant, as it is in *fab2-1*. The leaf stearate content is increased in the 7b15 line from an average of less than 1% of total fatty acids in the wild-type to almost 6% in the mutant. The 7b15 mutant also showed a miniature growth phenotype that was less extreme than that

Table 1 Seed fatty composition of wild-type and mutant *Arabidopsis*. Values are averages ($n = 5$) (The standard error of the mean was less than 5% of the reported value in all cases

Line	Mole % of total fatty acids							
	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:1
Wild-type	11.6	3.8	13.5	28.4	22.5	1.8	16.5	1.8
fab 2-1	11.4	9.3	7.5	25.7	23.7	4.9	14.2	2.2
fab2-2	8.9	5.1	12.9	27.9	19.7	3.6	16.1	1.2
shs fab2-1	8.8	2.5	16.2	31.1	20.8	1.4	16.1	1.9
TW2-1	9.2	8.5	8.6	27.3	24.1	4.2	15.2	2.0
EF1-1	13.7	6.0	8.5	35.9	17.5	5.5	9.1	2.6
FF4-5	10.3	7.2	7.3	25.7	26.4	4.4	16.3	2.0

observed in *fab2-1*. Reciprocal crosses between *fab2-1* and 7b15 produced F_1 progeny that were miniature plants with leaf 18:0 levels intermediate between those of the two parents. This lack of complementation was confirmed by scoring the F_2 where only the mutant fatty acid phenotype was observed. These results indicate that plants of the 7b15 line contain a mutation at *fab2* and the line is therefore designated *fab2-2*.

Mutations suppressing the *fab2* dwarf phenotype

Approximately 40 000 M_1 seeds, homozygous for *fab2-1* and *gl-1*, were treated with ethyl-methane sulfonate. The *gl-1* mutation was included to provide a marker against contaminating wild-type seeds or pollen. Because of the miniature growth habit of *fab2-1* it was possible to plant the M_1 seed at high density (100 seeds/4″ pot). Approximately one-half of these seeds produced viable M_1 plants which grew to maturity and produced M_2 seed. Because the *fab2* mutation is recessive, reversions at the *fab2* locus should appear as normal growing sectors, heterozygous for *fab2*, in the M_1. The M_1 was screened visually for the occurrence of plants with one or several enlarged leaves. A single M_1 plant was identified as a putative revertant chimera. Seeds from this plant gave rise to a homozygous line in which a second-site suppressor mutation, *shs*, reduces leaf 18:0 and produces plants that are nearly as large as the wild-type (Lightner et al. 1994 a).

For the collection of M_2 seed, pots of M_1 plants were grouped in pools of 12 pots, each representing approximately 60 mature M_1 plants. Pooled seed was mixed thoroughly and one-half of each pool (by weight) was sown out in large 12″ × 16″ flats for M_2 screening. The M_2 was screened visually for large plants against the background of miniatures. Putative revertants were tagged with an identifying name and M_3 seeds were collected for analysis. From the M_2 screen more than 50 putative revertants were identified. Three of these, TW2-1, FF4-6 and EF1-1, were selected for further analysis because they represented three distinctly different morphological categories. All the mutants are considerably smaller than wild-type *Arabidopsis* but larger than the *fab2-1* progenitor.

Genetic characterization of the suppressor lines

Plants from each line were crossed to wild-type and the resulting F_1 progeny were allowed to self-pollinate to produce F_2 seed. These F_2 seeds were planted and the resulting plants scored for the occurrence of the original *fab2* dwarf phenotype. If the mutant parent is homozygous for *fab2* and a second, recessive, unlinked suppressor mutation, then the Mendelian expectation is that 3/16th of the F_2 plants will express the *fab2* dwarf phenotype. In the event, F_2 progeny from the crosses TW2-1 × WT, FF4-6 × WT, and EF1-1 × WT contained *fab2* miniatures at 21.8%, 20.6% and 18.2%, respectively, of the total plants. These results indicate that in all cases the suppression events occurred at loci that are unlinked to the original *fab2* mutation.

Fatty acid composition of suppressors

In *fab2*, the morphological phenotype, miniature growth, is caused by altered fatty acid composition (Lightner et al. 1994 a). Because it might be possible to restore normal growth without affecting fatty acid composition we examined the fatty acid composition of the four lines that carry suppressor mutations. M_3 seeds of the suppressor lines were sown and the leaf fatty acid compositions of the resulting plants were analyzed at 14 days of age. Table 2 shows the leaf fatty acid compositions of the suppressors, wild-type, and the *fab2-1* parent. In the wild-type, 18:0 accounted for less than 1% of the total fatty acids. In contrast, *fab2-1* contained almost 20% stearate, more than 25 times the wild-type level. Of all the suppressor mutations, the *shs* suppressor contained the largest reductions in stearate content, to less than 3% of total fatty acids. The EF1-1 and FF4-5 suppressors had slight reductions in 18:0 content. The TW2-1 suppressor contained about 15% stearate and also contained reduced amounts of 16:3 fatty acids.

Because mutations affecting fatty acid composition in 16:3 plants like *Arabidopsis* frequently have more pronounced effects on either leaf fatty acid composition or on seed fatty acid composition (Browse and Somerville 1995) the M_4 seed of the chosen putative revertants was also subjected to analysis. Table 1 shows the

Table 2 Leaf fatty acid composition of wild-type and mutant *Arabidopsis*. Values are averages ($n = 5$) (The standard error of the mean was less than 5% of the reported value in all cases. A dash '–' indicates that the acyl group was not detected

Line	Mole % of total fatty acids							
	16:0	16:1 *trans*	16:2	16:3	18:0	18:1	18:2	18:3
Wild-type	12.8	2.4	0.8	12.3	0.7	3.0	15.7	50.9
fab 2-1	9.9	1.4	0.1	6.5	19.6	2.5	16.7	42.4
fab2-2	15.9	2.9	1.0	16.4	5.8	2.4	13.8	41.0
shs fab2-1	12.4	2.54	1.1	15.3	2.9	2.3	12.3	49.1
TW2-1	6.6	2.2	0.3	3.5	15.0	2.3	16.2	50.7
EF1-1	9.1	1.8	–	7.3	16.4	3.3	18.9	42.9
FF4-5	10.2	1.2	0.4	7.8	16.7	3.3	14.5	45.5
act1 fab2-1	5.8	1.5	–	0.8	16.0	5.1	20.8	50.1

fatty acid compositions of seeds from each of the suppressors, *fab2*, and the wild-type. Seeds of the *fab2* mutant contained about a two-fold increase in 18:0 content compared to wild-type. The product of 18:0 elongation, 20:0, showed a similar relative increase from 1.8% of total seed fatty acids in wild-type to 4.9% of total seed fatty acids in *fab2-1*. The *shs* suppressor showed the most complete suppression of the seed fatty acid phenotype with reductions in both 20:0 and 18:0 fatty acids. In fact, the 18:0 content of *shs* seeds was somewhat lower, at 2.5% of total fatty acids, than the wild-type value of 3.8% of total fatty acids. Seeds of the lines FF4-5 and EF1-1 contained reductions in stearate content similar in magnitude to those found in leaves. In contrast, the seed stearate content of seeds of TW2-1 was very similar to that of the *fab2-1* mutant parent.

Morphology of suppressors

In order to more carefully compare the morphology of the suppressors to the *fab2-1* parent and the wild-type, M_3 progeny of the suppressors were sown and photographed at 14 days along with wild-type and *fab2-1 Arabidopsis* (Fig. 1). The *shs* suppressor produced plants that were the most similar in appearance to the wild-type. Plants of the TW2-1 line were larger than the *fab2-1* parent in size but have a chlorotic phenotype. The chlorosis of the TW2-1 suppressor has not been separated from the suppressor phenotype, but we have not definitively established that the two phenotypes are linked. Plants of the FF4-5 and EF1-1 lines showed only small increases in size compared to the *fab2* mutant.

Fig. 1 Morphology of suppressors. Plants of the *fab2-1* parental line (center) and suppressor mutations, photographed at 14 days of age. The suppressor lines are *shs fab2-1* (top left), TW2-1 (lower left) EF1-1 (top right) and FF4-5 (lower right).

Characterization of an *act1 fab2-1 double mutant*

Leaf lipids from TW2-1 plants contain a much lower proportion of 16:3 than the *fab2-1* progenitor. Mutations at the *act1* locus reduce leaf 16:3 because they limit the flux of fatty acids into the "prokaryotic" pathway (Browse and Somerville 1991) that leads to 16:3 synthesis on chloroplast galactolipids (Kunst et al. 1988). To test the possibility that the suppressor mutation in the TW2-1 line was an allele at *act1*, we first crossed plants of *act1* and *fab2-1*, allowed the F_1 progeny to self and screened the resulting F_2 plants by gas chromatography. Homozygous double-mutant plants were identified because their leaf lipids contained less than 1% 16:3 and 16% 18:0. These data indicate that the introduction of an *act1* mutation into the *fab2-1* background does partially suppress the *fab2* fatty acid phenotype. The TW2-1 line was also crossed with *act1*. The F_1 plants from this cross contained an average of 10.5% 16:3 considerably higher than either of the parents (Table 2; Kunst et al. 1988). Furthermore, an F_2 population obtained from selfing the F_1 segregated for 16:3 content and contained both wild-type ($> 12.5\%$ 16:3)and homozygous *act1* ($< 1\%$ 16:3) individuals. These results demonstrate that the suppressor mutation in TW2-1 plants is not an allele of *act1* but that it may represent a lesion at another locus that regulates fatty acid flux into the prokaryotic pathway.

Significantly, the *act1 fab2-1* double mutants are very similar in appearance to TW2-1 plants. Leaves are several times larger than those of *fab2-1* plants, but are noticeably chlorotic. Thus, two separate mutations that restrict lipid synthesis by the prokaryotic pathway suppress the *fab2* phenotype.

The relationship of plant size to 18:0 content

The morphological phenotypes of the suppressors varied from the nearly complete suppression of miniature growth in *shs* to the fairly modest changes in plant size associated with the EF1-1 and FF4-5 suppressors. In order to examine the relationship between plant size and leaf stearate content we measured the maximum rosette diameter of 14-day-old plants of the suppressor lines, *fab2*, and wild-type Arabidopsis. Figure 2 shows the relationship between 18:0 content and the rosette diameter of the plant. Rosette diameter declines rapidly

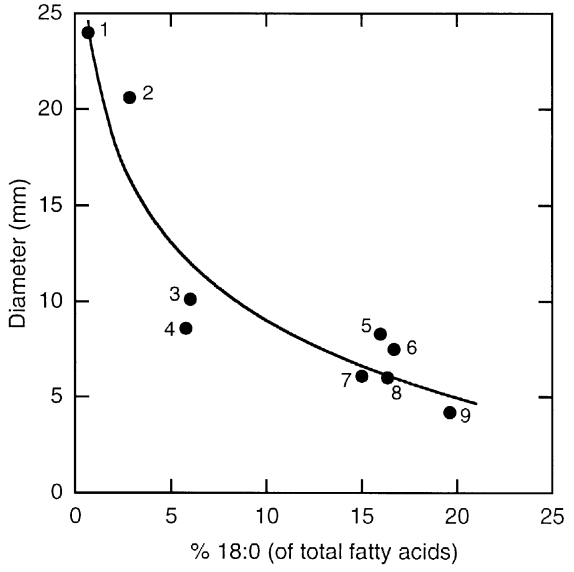

Fig. 2 Relationship of 18:0 content to plant size. The average rosette diameter of five plants from each line was measured when the plants were 14 days old and the plant leaves were then analyzed for fatty composition. The data points are for the genotypes/lines *1*, wild-type; *2*, *fab2-1 shs*; *3*, *fab2-1 shs* (+/−); *4*, *fab2-2*; *5*, TW2-1; 6, *act1 fab2-1*; *7*, FF4-5; *8*, EF1-1; *9*, *fab2-1*. The fitted curve has the equation: rosette diameter = 22.5–13.5 log (% 18:0)

as the proportion of leaf 18:0 rises above the very low level, 0.7%, found in the wild-type. There is a greater than 50% reduction of rosette diameter in the *shs* heterozygote which contains an 8-fold increase in 18:0 content relative to the wild-type and in plants carrying the *fab2-2* allele. Further increases in 18:0 continue to decrease rosette size although at a slower rate. In all of the suppressor mutants, increased plant size was associated with a decrease in stearate content compared with the *fab2* mutant. The data are a close fit ($R^2 = 0.902$) to a relationship in which rosette diameter declines in proportion to the logarithm of leaf 18:0 content.

Discussion

The isolation and characterization of suppressor mutations provides new information for biochemical genetic investigations. *A. thaliana* is particularly well suited to the requirements of suppressor and revertant isolation. By virtue of its small seed size tens of thousands, or even hundreds of thousands, of seeds can be treated with relatively small volumes of chemical mutagens. The reduced size of the plant allows the propagation of very large M_1 populations and the short life cycle of the plant allows M_2 screening to begin within as little as 2 months after mutagen treatment.

Our isolation of several new loci that influence stearate desaturation suggest a new layer of genetic complexity in the control of fatty acid desaturation. Although our M_1 screen identified only one putative revertant from the 40 000 M_1 seeds treated, the M_2 screen produced more putative revertants than could be easily evaluated. The four suppressors we describe in this report represent a range of morphological variation from a nearly complete suppression of miniature growth in the *shs* suppressor to relatively subtle changes in leaf form like those in EF1-1. In all cases, restoration of more normal growth by the suppressors was associated with a reduction of leaf stearate level, with the most normal growth in the lines with the lowest levels of leaf stearate. Previous studies (Lightner et al. 1994 a) have established the causal relationship between increased 18:0 in leaf lipids and the extreme dwarf phenotype of *fab2-1* plants. Here, we have used the suppressor mutants, the newly isolated *fab2-2* allele, and the *act1 fab2-1* double mutant to extend and quantify the effect of increased 18:0 on plant size (Figs. 1, 2).

All of the identified suppressors result in significant reductions ($P < 0.05$) in leaf stearate content relative to the *fab2-1* parent. The *shs* suppressor produced very normal plant morphology. By contrast, the suppressor mutation in the FF4-5 line resulted in relatively small increases in plant size. The EF1-1 suppressor resulted in elongated, narrow leaves that, as the plants age, become significantly longer than leaves of the *fab2* parent. The TW2-1 mutant has a highly chlorotic appearance and leaves that are much broader than those of *fab2*. The *fab2* mutant, wild-type, and isolated suppressor mutations, in combination with the heterozygote of the semi-dominant *shs* suppressor, provide a wide range of leaf 18:0 content and plant size. We have shown a significant ($P < 0.01$) negative correlation between 18:0 content (log of percent 18:0) and plant size as measured by maximum rosette diameter. Plant size is somewhat reduced even in the *fab2-1 shs* double mutant demonstrating that even small increases in stearate content have negative effects on plant size.

Genetic analysis indicates that all four of the suppressor mutations are at sites distinct from the *fab2* locus. One common form of such intergenic suppressors are the informational, or direct, suppressors. In microorganisms, where informational suppression has been extensively studied, suppressors mutations frequently alter transfer RNAs (reviewed by Smith 1972) or other aspects of the translational machinery (Gorini 1970). Another type of suppression phenomenon is termed indirect suppression. In this case the suppressor mutation may obviate the need for the original mutant function. Indirect suppressor mutants have been particularly well exploited in the nematode *Caenorhabditis elegans* (Herman and Horvitz 1980). The incomplete suppression of both fatty acid phenotypes and morphological phenotypes by all four analyzed suppressor mutations suggests that these

mutations are indirect suppressors, rather than informational suppressors. One question we have been unable to address, as yet, is the possible allelism among the suppressors. The flowers of the *fab2* mutant are severely reduced in size compared to the wild-type (Lightner et al. 1994 a). Although *fab2* is self-fertile, attempts to use *fab2* as a pollen donor in crosses are rarely successful. The flowers of the suppressors, with the exception of *shs*, were similar in size to those of *fab2* and attempts to perform complementation crosses between the suppressors were unsuccessful. We are continuing our efforts to obtain these results, but the differences in fatty acid composition and plant appearance described here suggest that the suppressor mutations are unlikely to be allelic.

Seed fatty acid composition can be an important diagnostic test for characterizing fatty acid mutations. While the *fab2* mutant affects both leaf and seed fatty acid composition, many fatty acid mutations affect either the leaf or seed fatty acid composition more profoundly. Generally, mutations affecting steps in the eukaryotic pathway will more substantially affect seed fatty acid composition while those affecting the prokaryotic pathway alter leaf fatty acid composition most significantly (Browse and Somerville 1991, 1995). The seed fatty acid compositions of both FF4-5 and EF1-1 show proportional 18 : 0 reductions similar to those found leaves. The seed 18 : 0 composition of the TW2-1 line is substantially the same as that of the *fab2* parent ($P > 0.25$). This is consistent with other results in suggesting that the suppressor mutation in the TW2-1 line affects the prokaryotic pathway, which has essentially no influence over seed fatty acid composition (Kunst et al. 1988). Seeds of the *shs* suppressor actually have significantly ($P < 0.001$) lower seed 18 : 0 content than wild-type seeds.

Research with other genetic models (Smith 1972; Herman and Horvitz 1980) has demonstrated that both informational and indirect suppressor mutations can provide valuable insights into genetically tractable processes. In a relatively short time we were able to isolate several intergenic suppressors of loss-of-function mutation. The *fab2* fatty acid phenotype is suppressed by three of the suppressors, *shs*, FF4-5 and EF1-1, in both leaves and seeds. In contrast, the TW2-1 suppressor only functions in the leaves. The isolation of these suppressors has provided a collection of mutants with increases in leaf stearate content which vary from 4- to 28-fold relative to wild-type. The isolation of second-site suppressors of *fab2*, provides valuable insights in the role of one membrane component, stearate, in plant growth, and also demonstrates the utility of reversion studies in *Arabidopsis* biochemical genetics. Compared with the contemporary understanding of the genetics of plant lipid metabolism (Browse and Somerville 1991, 1995) the identification of so many new loci which can influence the abundance of a particular fatty acid suggests the existence of an additional layer of complexity to the genetic determination of plant fatty acid composition.

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References

Braaksma FJ, Feenstra WJ (1982) Reverse mutants of the nitrate reductase-deficient mutant B25 of *Arabidopsis thaliana*. Theor Appl Genet 61 : 263–271

Browse J, Somerville C (1991) Glycerolipid synthesis: biochemistry and regulation. Annu Rev Plant Physiol Plant Mol Biol 42 : 467–506

Browse J, Somerville C (1995) Glycerolipids. In: Meyerowitz E, Somerville C (eds) *Arabidopsis*. Cold Spring Harbor Press, New York, pp 881–912

Browse J, McCourt P, Somerville CR (1985) Overall fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue. Anal Biochem 152 : 141–146

Gorini L (1970) Informational suppression. Annu Rev Genet 4 : 107–134

Herman RK, Horvitz HR (1980) Genetic analysis of Caenorhabditis elegans. In: Zuckerman BM (ed) Nematodes as biological models. Academic Press, New York, pp 227–261

Hugly S, Somerville C (1992) A role for membrane lipid polyunsaturation in chloroplast biogenesis at low temperature. Plant Physiol 99 : 197–202

James DW, Dooner HG (1990) Isolation of EMS-induced mutants in *Arabidopsis* altered in seed fatty acid composition. Theor Appl Genet 80 : 241–245

Koornneef M, Jorna ML, Brinkhorst-van der Swan DLC, Karssen CM (1982) The isolation of abscisic acid (ABA)-deficient mutants by selection of induced revertants on non-germinating gibberellin-sensitive lines of *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet 61 : 385–393

Koornneef M, Elgersma A, Hanhart CJ, van Loenen-Martinet EP (1985) Induction and analysis of gibberellin-sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet 58 : 257–263

Kunst L, Browse J, Somerville C (1988) Altered regulation of lipid biosynthesis in a mutant of *Arabidopsis* deficient in chloroplast glycerol phosphate acyltransferase activity. Proc Natl Acad Sci USA 85 : 4143–4147

Kunst L, Browse J, Somerville C (1989) A mutant of *Arabidopsis* deficient in de-saturation of palmitic acid in leaf lipids. Plant Physiol 90 : 943–947

Lightner J, James DW Jr, Dooner HK, Browse J (1994 a) Altered body morphology is caused by increased stearate levels in a mutant of *Arabidopsis*. Plant J: 401–412

Lightner J, Wu J, and Browse J (1994 b) A mutant of *Arabidopsis* with increased levels of stearic acid. Plant Physiol 106 : 1443–1451

McCourt P, Kunst L, Browse J, Somerville C (1987) The effects of reduced amounts of lipid unsaturation on chloroplast ultrastructure and photosynthesis in a mutant of *Arabidopsis*. Plant Physiol 84 : 353–360

Okada K, Shimura Y (1992) Aspects of recent developments in mutational studies of plant signalling pathways. Cell 70 : 369–372

Peng J, Harberd NP (1993) Derivative alleles of the gibberellin-insensitive (*gai*) mutation confer a wild-type phenotype. Plant Cell 5 : 351–360

Porter ME, Power J, Dutcher, SK (1992) Extragenic suppressors of paralyzed flagellar mutations in *Chlamydomonas reinhardtii* identify loci that alter the inner dynein arms. J Cell Biol 118 : 1163–1176

Redei GP (1992) A heuristic glance at the past of *Arabidopsis* genetics. In: Koncz C (ed) Methods in *Arabidopsis* research. World Scientific, Singapore, pp 3–15

Smith JD (1972) Genetics of transfer RNA. Annu Rev Gen 6: 235–256

Spreitzer RJ, Ogren WL (1983) Nuclear suppressors of the photosensitivity associated with photosynthesis in *Chlamydomonas reinhardii*. Plant Physiol 71 : 35–39