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# Mutants of *Arabidopsis* with alterations in seed lipid fatty acid composition *

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**Summary.** A diverse collection of mutants of *Arabidopsis* with altered seed lipid compositions was isolated by determining the fatty acid composition of samples of seed from 3,000 mutagenized lines. A series of mutations was identified that caused deficiencies in the elongation of 18:1 to 20:1, desaturation of 18:1 to 18:2, and desaturation of 18:2 to 18:3. In each of these cases the wild type exhibited incomplete dominance over the mutant allele. These results, along with results from earlier studies, point to a major influence of gene dosage in determining the fatty acid composition of seed lipids. A mutation was also isolated that resulted in increased accumulation of 18:3. On the basis of the effects on fatty acid composition, the nature of the biochemical lesion in three of the mutants could be tentatively attributed to deficiencies in activities of specific enzymes. The other mutant classes had relatively less pronounced changes in fatty acid composition. These mutants may represent alterations in genes that regulate lipid metabolism or seed development. The availability of the mutants should provide new opportunities to investigate the mechanisms that control seed lipid fatty acid composition.

**Key words:** Erucic acid – Fatty acid desaturation

## Introduction

Many crop species produce seed oils in which the fatty acid composition is not ideally suited to the intended use. The application of conventional breeding methods, coupled in some cases with mutagenesis, has resulted in the production of new varieties of several species with desirable alterations in the fatty acid composition of seed oil. A notable example is the development of low erucic acid varieties of rapeseed (Stefansson 1983). Similar efforts have resulted in the reduction of the level of polyunsaturated 18-carbon fatty acids in soybean (Wilcox and Cavins 1985; Graef et al. 1988), sunflower (Fick 1989), and linseed oils (Green and Marshal 1984). Most of the genetic variation in seed lipid fatty acid composition appears to involve the presence of an allele of a gene that disrupts normal fatty acid metabolism and leads to an accumulation of intermediate fatty acid products in the seed storage lipids (Downey 1987). However, it seems likely that, because of the inherent limitations of this approach, many other desirable changes in seed oil fatty acid composition may require the directed application of genetic engineering methods. Unfortunately, as in many other aspects of plant biology, the lack of specific information about the biochemistry and regulation of lipid metabolism makes it difficult to predict how the introduction of one or a few genes might usefully alter seed lipid synthesis. An additional problem arises from the fact that many of the key enzymes of lipid metabolism are membrane-bound, and attempts to solubilize and purify them from plant sources have not been successful.

As one approach to these problems, we have isolated a series of mutants of *Arabidopsis* with specific alterations in the fatty acid composition of their seed storage lipids. In this paper we describe the methods used to isolate the mutants, an overview of the kinds of mutants

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Abbreviations: FAMES (fatty acid methyl esters); PC (phosphatidylcholine; 18:1 (oleic acid); 18:2 (linoleic acid); 18:3 (linolenic acid); 20:1 (eicosenoic acid)

recovered, and an outline of the potential utility of the mutants in understanding lipid metabolism of oilseeds. The development of *Arabidopsis* as a model for plant molecular genetics raises the possibility that the mutants may eventually be used as the basis for identifying and cloning the corresponding wild-type genes by methods such as transposon tagging or chromosome walking from restriction fragment length polymorphism (RFLP) sites (Meyerowitz 1989).

## Materials and methods

### *Genetic nomenclature*

We have followed the recommended nomenclature (Bleecker et al. 1988) in assigning symbols to the genetic loci identified in this report. Different symbols were used to designate an "inbred line" (i.e., propagated by self-fertilization) from the genotype of that line. Thus, e.g., the inbred line JB20 carries a mutation at the *fae1* locus. In a preliminary publication (Browse et al. 1989) the *fad2* locus was referred to as *fadE*, and the *fad3* locus as *fadF*.

### *Mutant isolation*

Plants were grown in growth chambers at 23 °C under continuous light (200 μE $m^{-2}$ $s^{1}$) on a mixture of perlite:vermiculite:sphagnum (1:1:1). Approximately 50,000 *Arabidopsis thaliana* (Columbia wild-type) seeds were mutagenized by soaking them for 12 h in 0.3% (v/v) ethyl methane sulfonate (Haughn and Somerville 1987), then grown to maturity and bulk harvested. Approximately 3,000 randomly chosen $M_2$ seeds were grown to maturity and harvested separately to produce 3,000 $M_3$ families. Samples of 40–100 $M_3$ seeds, with a total weight of approximately 1–2 mg, were screened by direct assay of the fatty acid composition by gas chromatography (Browse et al. 1986a). Although in many cases the $M_2$ progenitors were heterozygous for mutations affecting fatty acid composition, the assay method was sufficiently sensitive to identify $M_3$ families with atypical lipid composition. Homozygous mutant lines were identified by planting ten seeds from each potentially interesting $M_3$ family. The fatty acid composition from a number of individual $M_4$ seeds of each of these ten lines was then assayed in order to identify homozygous individuals. This analysis was repeated in at least one subsequent generation to ensure that the trait was stably inherited.

One of the mutant lines (BL1) was identified as a segregant in a multiply marked line of the Landsberg erecta ecotype originally obtained from M. Koornneef.

### *Fatty acid analysis*

Seed FAMES were prepared by heating seeds or other tissues at 80 °C in 2.5% (v/v) $H_2SO_4$ in methanol for 90 min. The FAMES were then extracted with 1 ml of hexane and 1.5 ml of 0.9% NaCl (w/v) and the organic phase was transferred to autoinjector vials. The gas chromatograph was programmed for an initial temperature of 140 °C for 3 min followed by an increase of 30 °C/min to 190 °C; this final temperature was maintained for a further 12 min. Using a 4 m × 2 mm column packed with DEGS-PS (5% on chromasorb WAW-DCMS) and a 1 μl sample volume, it was possible to separate octanoic acid (8:0) from the solvent peak, while erucic acid (22:1) eluted at about 15 min. Fatty acid methyl esters were identified by comparison of elution times with those of reference compounds. Some analyses

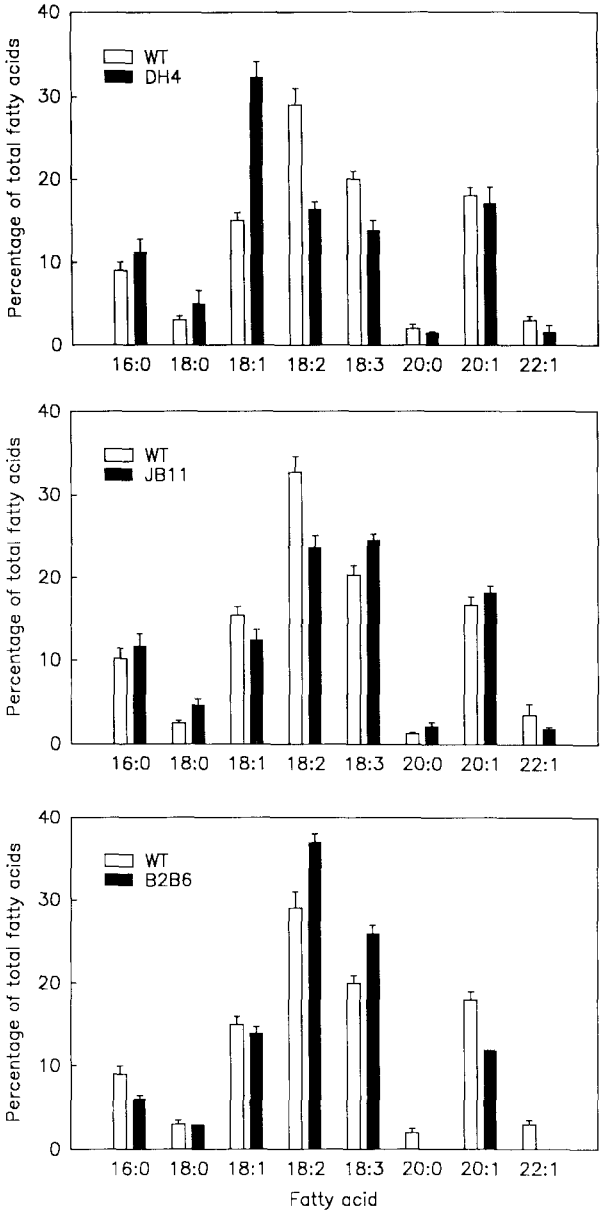


**Fig. 1.** Comparison of the seed fatty acid composition of wild type *Arabidopsis* and three mutant lines. FAMEs were prepared from six samples of 40–100 seeds and analyzed by gas chromatography. Standard deviations are shown

**Table 1.** Mutants with altered seed lipid composition

| Line | Gene symbol | Phenotype |
|---|---|---|
| JB9 | *fad2*-1 | Deficient in desaturation of 18:1 |
| JB12 | *fad2*-2 | Deficient in desaturation of 18:1 |
| BL1 | *fad3* | Deficient in desaturation of 18:2 |
| JB11 | *ela1* | Enhanced linolenate accumulation |
| JB20 | *fae1* | Deficient in elongation of 18:1 |
| B2B6 | *fae2* | Reduced elongation of 18:1 |
| DH4 | *rod1* | Reduced oleate desaturation |

**Table 2.** Overall fatty acid composition of seeds, leaves, and roots of wild-type *Arabidopsis* and mutant line JB9. WT = wild type

| Fatty acid | Seeds | | | Leaves | | Roots | |
|---|---|---|---|---|---|---|---|
| | WT | $F_1$ | JB9 | WT | JB9 | WT | JB9 |
| 16:0 | 10.2±1.2 | 10.5±0.7 | 8.6±2.7 | 13.7±0.1 | 13.9±2.3 | 24.7±2.9 | 14.0±0.6 |
| 16:1 | – | – | – | 2.4±0.4 | 2.2±0.4 | 1.2±0.2 | 2.3±0.2 |
| 16:3 | – | – | – | 16.0±1.0 | 18.5±3.4 | – | – |
| 18:0 | 2.5±0.3 | 2.6±0.4 | 4.3±1.9 | 0.4±0.1 | 0.5±0.2 | 3.2±0.4 | 1.7±0.3 |
| 18:1 | 15.4±1.1 | 21.4±0.7 | 53.5±2.9 | 2.3±0.2 | 20.9±4.5 | 6.8±1.2 | 55.9±2.2 |
| 18:2 | 32.7±1.9 | 25.2±1.2 | 3.2±2.6 | 14.5±1.6 | 3.8±1.3 | 29.8±2.1 | 6.4±0.6 |
| 18:3 | 20.3±1.2 | 20.5±1.2 | 5.5±1.2 | 50.8±0.8 | 39.6±2.6 | 29.1±2.5 | 12.8±1.2 |
| 20:0 | 1.3±0.1 | 1.4±0.7 | 1.0±0.3 | – | – | 1.2±0.3 | 1.9±0.3 |
| 20:1 | 16.7±1.0 | 17.2±1.1 | 23.9±1.5 | – | – | 0.2±0.2 | 0.7±0.1 |
| 22:1 | 3.5±1.3 | 2.5±0.9 | 2.8±2.3 | – | – | – | – |

The values are mol% ± SD (n = 12 for seeds; n = 4 for leaves; n = 9 for roots)

**Table 3.** Overall fatty acid composition of seeds, leaves and roots of wild-type *Arabidopsis* and mutant line BL1. WT = wild type

| Fatty acid | Seeds | | | Leaves | | Roots | |
|---|---|---|---|---|---|---|---|
| | WT | $F_1$ | BL1 | WT | BL1 | WT | BL1 |
| 16:0 | 10.2±1.2 | 8.8±0.8 | 7.7±0.9 | 13.7±0.1 | 10.4±2.8 | 24.7±2.9 | 16.7±0.6 |
| 16:1 | – | – | – | 2.4±0.4 | 3.1±1.0 | 1.2±0.2 | 2.5±1.0 |
| 16:3 | – | – | – | 16.0±1.0 | 18.3±3.0 | – | – |
| 18:0 | 2.5±0.3 | 2.4±2.0 | 2.6±0.5 | 0.4±0.1 | 0.4±0.1 | 3.2±0.4 | 2.3±0.5 |
| 18:1 | 15.4±1.1 | 20.2±1.5 | 23.0±1.3 | 2.3±0.2 | 1.1±0.5 | 6.8±1.2 | 12.0±2.9 |
| 18:2 | 32.7±1.9 | 36.2±2.8 | 46.0±2.2 | 14.5±1.6 | 19.5±2.0 | 29.8±2.1 | 55.3±0.6 |
| 18:3 | 20.3±1.2 | 12.5±1.1 | 2.3±0.9 | 50.8±0.8 | 47.2±2.6 | 29.1±2.5 | 10.5±1.3 |
| 20:0 | 1.3±0.3 | 1.2±0.4 | 1.7±0.9 | – | – | 1.2±0.3 | – |
| 20:1 | 16.7±1.0 | 15.6±2.3 | 17.4±1.4 | – | – | 2.0±0.2 | 1.0±0.5 |
| 22:1 | 3.0±1.3 | 3.0±0.9 | 3.0±1.1 | – | – | – | – |

Values are mol% ± SD (n = 12 for leaf; n = 4 for root; n = 7 for seed)

were carried out using a Supelcowax 15 m × 0.53 mm widebore column (Supelco, Bellefonte/PA) using similar conditions to those for the packed column.

## Results

### *Mutant isolation*

Direct analysis of the fatty acid composition of 3,000 families of $M_3$ seed resulted in the identification of a number of families that exhibited one of three distinct mutant phenotypes. These phenotypes included pronounced deficiencies in the desaturation of oleic acid (18:1), the desaturation of linoleic acid (18:2), and the elongation of 18:1 to eicosenoic acid (20:1) (Table 1). Several lines with less pronounced alterations in seed fatty acid composition were also obtained for all three classes of phenotypes (i.e., three lines with slightly reduced 18:1 elongation). An unusual mutant which accumulated elevated levels of 18:3 was also obtained. The seed fatty acid composition of three mutant lines and wild-type *Arabidopsis* is shown in Fig. 1. A more detailed analysis of the fatty acid composition of three additional mutant lines is presented in Tables 2, 3, and 4.

**Table 4.** Overall fatty acid composition of seeds of wild-type *Arabidopsis* and mutant line JB20. WT = wild type

| Fatty acid | Seeds | | |
|---|---|---|---|
| | WT | $F_1$ | JB20 |
| 16:0 | 10.2±1.2 | 9.7±1.0 | 9.4±0.5 |
| 18:0 | 2.5±0.3 | 3.7±1.0 | 3.4±0.5 |
| 18:1 | 15.4±1.1 | 17.4±1.0 | 25.6±1.5 |
| 18:2 | 32.7±1.9 | 33.2±2.8 | 37.4±2.6 |
| 18:3 | 20.3±1.2 | 22.2±2.2 | 21.2±0.8 |
| 20:0 | 2.1±0.1 | 1.1±0.4 | 0.6±0.2 |
| 20:1 | 16.7±1.0 | 11.2±2.0 | 0.2±0.1 |
| 22:1 | 3.0±1.3 | 1.5±1.4 | – |

Values are mol% ± SD (n = 7)

In order to examine the inheritance of the altered fatty acid composition in the various lines with abnormal fatty acid composition, ten $M_3$ seeds from each family were planted, grown to maturity, and harvested sepa-

rately. The fatty acid compositon of samples of the $M_4$ seed of each subline was then determined. In those families that were homozygous for a mutation affecting fatty acid composition, the fatty acid composition of samples of the seed from the sublines was always found to be indistinguishable from one another and from the $M_3$ parental line. In several cases, $M_3$ lines were judged to be heterozygous because the $M_4$ sublines were heterogeneous with respect to fatty acid composition. In these instances, the process was repeated with $M_5$ lines descended from the several $M_4$ sublines with the most extreme phototype. This resulted in the recovery of lines that were judged homozygous for a mutation affecting fatty acid composition in at least two generations.

*Mutants deficient in oleate desaturation*

Three lines were recovered that had reduced levels of 18:2 and 18:3 fatty acids in their seed lipids. The mutant line JB9, which had the most extreme phenotype in this class, contained only 7% polyunsaturated 18-carbon fatty acids and had an approximately threefold greater amount of 18:1 (Table 2). The fatty acid composition of the $F_1$ seed, from a cross of JB9 with wild type, also contained slightly elevated levels of 18:1 relative to the wild type (Table 2). In a sample of 107 individual $F_2$ seeds from the same cross, 26 had >50% 18:1 and 81 had <25% 18:1. The frequency of segregation of high and low oleic acid seeds is in close agreement ($\chi^2 = 0.02$; $P \geq 0.9$), with a 3:1 segregation ratio expected for the segregation of a single nuclear mutation at a locus we have designated *fad2*. Because $F_1$ seeds contain slightly elevated levels of 18:1 compared with wild type, the wild-type allele should be considered incompletely dominant. Another mutant (JB12) isolated from an independent $M_2$ population exhibited a seed fatty acid composition that was very similar to that of JB9 (data not shown). Seeds of the $F_1$ progeny from a cross between JB9 and JB12 had the same fatty acid composition as the parental lines. The lack of genetic complementation indicates that JB12 also carries a mutation at the *fad2* locus. As 18:2 and 18:3 are normally derived from 18:1 by the sequential desaturation of 18:1 and 18:2 (Stymne and Stobart 1987), respectively, the fatty acid composition of JB9 is most consistent with a deficiency in an 18:1 desaturase.

It has been demonstrated in several oilseeds that the desaturation of 18:1 in the developing cotyledons occurs on PC of the endoplasmic reticulum (Stymne and Appelqvist 1978; Stymne and Stobart 1987) and a similar 18:1-PC desaturase has been described in microsomal preparations from plant leaves (Slack et al. 1976). To determine whether the same gene controls 18:1 desaturation in different tissues of *Arabidopsis*, we compared the overall fatty acid composition of seeds, leaves, and roots of plants from the JB9 and wild-type plants (Table 2). The level of 18:1 in leaf tissue was increased, and there was a concomitant decrease in 18:2 and 18:3. Thus, the *fad2* mutation is expressed in leaves. Similarly, the root fatty acid composition of the JB9 line had an eightfold increase in the amount of 18:1 and a substantially reduced level of polyunsaturated 18-carbon fatty acids when compared to the wild type.

Our previous characterization of a mutant, designated *fadC*-deficient in activity of the chloroplast 18:1/16:1 desaturase, indicated that this pathway of 18:1 desaturation is favored in leaf tissue (Browse et al. 1989). All the chloroplast lipids appear to be accessible to the chloroplast 18:1/16:1 desaturase, and we would therefore predict that chloroplast lipids in the JB9 line would not be affected to a significant extent by a deficiency in the endoplasmic reticulum desaturase. Consistent with this prediction and the observation that over 70% of leaf membrane lipids are in the chloroplasts (Browse et al. 1986b), the JB9 leaves exhibit much higher levels of 18:3 than do the seeds. However, the increased 18:1 in leaves of the JB9 line is consistent with a block in the desaturation of 18:1 on the extrachloroplast lipids in this tissue. In contrast, roots of JB9 plants show a very substantial decrease in the amount of 18:2 and 18:3 compared with wild type and a concomitant increase in the level of 18:1 (Table 2). In our opinion, the more pronounced phenotype of JB9 in root tissue can be explained by a greater flux of lipid through the eukaryotic pathway in nonphotosynthetic tissue. All of the above observations are consistent with the hypothesis that the mutation in JB9 affects the activity of an endoplasmic reticulum 18:1 desaturase that is constitutively expressed in all tissues.

*Mutants deficient in linoleate desaturation*

Two mutant lines were found to be deficient in the desaturation of linoleic acid (18:2). Of these, mutant line BL1 had the most extreme phenotype (Table 3), with only about 2% 18:3 as compared to the wild-type level of about 20%. The decreased accumulation of 18:3 was accompanied by increased levels of 18:1 and 18:2 (Table 3), as expected of a mutant deficient in desaturation of 18:2 to 18:3 (Browse et al. 1986c). $F_1$ seeds from a cross of BL1 to wild-type *Arabidopsis* were found to have levels of 18:3 intermediate to that in the parents (Table 3). Analysis of the fatty acid composition of 35 individual $F_2$ seeds revealed a 10:17:8 segregation consistent with the expected 1:2:1 ratios ($\chi^2 = 0.26$; $P > 0.9$). The deficiency in 18:3 desaturation is, thus, the result of a single nuclear mutation in a gene designated *fad3* that controls the level of 18:3 in seeds. Previous studies of oilseed lipid metabolism indicate that desaturation of 18:2 to 18:3 probably takes place on PC of the endoplasmic reticulum (Browse and Slack 1981; Stymne and

Stobart 1987). Further characterization of the BL1 mutant will help confirm this hypothesis.

By analogy with available information concerning the properties of the endoplasmic reticulum 18:1 desaturase and our observations on the *fad2* mutant described above, we considered it possible that the *fad3* locus would affect 18:2 desaturation in other plant tissues. In fact seeds, leaves, and roots of the BL1 mutant do show an 18:3 deficient phenotype (Table 3). The fatty acid composition of seeds and roots is more strongly altered than that of leaf tissue and, again, this observation is consistent with the existence of alternative desaturation reaction in leaves involving the *fadD* gene product (Browse et al. 1986c).

*A mutant with increased levels of linolenate*

One exceptional mutant, JB11, had increased levels of 18:3 and decreased levels of 18:2 and 18:1 (Fig. 1). In terms of our current understanding of seed lipid metabolism (Stymne and Stobart 1987), the mutant cannot be explained in terms of a deficiency in any known step of fatty acid desaturation. Nevertheless, genetic analysis indicates that it is heritable and is due to a single recessive mutation (data not shown) at a locus we have provisionally designated *ela1* (enhanced linolenate accumulation). The phenotype exhibits a relatively subtle change in seed fatty acid composition. Nevertheless, the mutant plants were always readily distinguishable from wild type by calculating the ratio of 18:3 to 18:2 in their seed lipids. This ratio is $0.63 \pm 0.05$ for wild type and $1.04 \pm 0.10$ for the JB11 line.

When plants of the JB11 line and wild type were grown side by side, the fatty acid compositions of leaf and root tissues of the JB11 plants showed small but statistically significant ($P < 0.05$) increases in the 18:3/18:2 ratio relative to wild type (data not shown). However, it will be necessary to further characterize this mutant before it is possible to conclude whether or not the mutation definitely affects lipid metabolism in these tissues other than the seeds.

*Mutants deficient in the elongation of oleate*

*Arabidopsis* seed triglycerides contain about 20% of very long chain fatty acids (i.e., approximately 17% 20:1 and 3% 22:1). We have isolated two mutants deficient in the elongation of 18:1. One of these, JB20, has only 0.2% 20:1 in its seed oil (Table 4). The $F_1$ seed from a cross of JB20 and wild-type plants was found to have an intermediate level of 20:1 relative to that of the two parents (i.e., 8%–14% 20:1). The fatty acid composition of 61 $F_2$ seeds segregated 15:29:17 with respect to these three phenotypic classes. This is an excellent fit to the 1:2:1 hypothesis ($\chi^2 = 0.27$; $P > 0.9$), indicating that the phenotype of JB20 is caused by a single nuclear mutation in a gene we designate *fae1*. The incomplete dominance shown by the wild-type allele at *Fae1* indicates that the level of its expression is rate-limiting to elongation. Significantly, synthesis of 20:0 is also decreased in the mutant (Table 4), although the effect is less dramatic than for the monounsaturated fatty acids. Thus, the product of the *fae1* gene appears to regulate the elongation of both 18:0 (or 16:0) and 18:1 fatty acids. As leaf lipids of *Arabidopsis* do not contain significant amounts of long chain fatty acids, the fatty acid profile of JB20 leaves was indistinguishable from that of the wild type (data not shown).

## Discussion

Many genetic studies of lipid composition have been initiated by plant breeders in order to identify useful natural or induced variation in seed fatty acid composition. Among the best known example is the development of rapeseed cultivars containing low levels of erucic (22:1) and eicosenoic (20:1) acids as a result of a deficiency in one or more of the enzymes required for the elongation of 18:1-CoA (Stumpf and Pollard 1983). Variation in the levels of 18:0, 18:1, 18:2 and 18:3 has been observed in soybean (Wilcox and Cavins 1985; Graef et al. 1988; Martin and Rinne 1986), sunflower (Fick 1989), rapeseed (Röbbelen and Nitsch 1975; Diepenbrock and Wilson 1987), flax (Green and Marshal 1984), maize (Widstrom and Jellum 1984), and safflower (Knowles 1972). Many of these variants have substantial changes in fatty acid composition, but they have not been characterized in sufficient detail to indicate the biochemical basis of the mutant phenotype.

The series of *Arabidopsis* mutants described here collectively represent most of the changes in seed fatty acid composition that have been identified in the crop plants noted above. In addition, we have isolated a mutant that shows increased levels of 18:3 compared with wild type. Because of the complementary information available from *Arabidopsis* mutants deficient in various aspects of leaf lipid metabolism (Browse et al. 1985, 1986a, b, c; 1989; Kunst et al. 1988), we believe that the collection of mutants described here will contribute to our understanding of the reactions and regulation of lipid metabolism in oilseeds. Most of the fatty acid desaturases and other enzymes of triacylglycerol synthesis are membrane-bound proteins, and attempts to solubilize and purify them from plant sources have not been successful. For this reason, analysis of mutants has been particularly useful in characterizing the desaturase enzymes (Somerville and Browse 1988). In addition, the development of *Arabidopsis* as a model for molecular genetics

(Meyerowitz 1989) raises the possibility that genetically mapped mutations can provide a basis for identifying and cloning the corresponding wild-type genes.

Two steps of the fatty acid desaturation pathway in seeds have been marked by mutations with pronounced phenotypes. We suggest that the loci designated *fad2* and *fad3*, respectively, encode the 18:1-PC and 18:2-PC desaturase enzymes of the endoplasmic reticulum. It should be possible to test this hypothesis by direct assays of the desaturase activities in microsomal preparations from seeds or other plant tissues (Stymne and Appelqvist 1978; Browse and Slack 1981).

The mutation at the *fae1* locus in JB20 controls elongation of both saturated (16:0 or 18:0) and monounsaturated (18:1) fatty acids in the seeds. It is not certain at present if the same gene product is involved in elongation of 20:1 to 22:1, since the substrate for this reaction is not formed in the *fae1* mutant. The elongation of 18:1 involves four reactions (besides those required for synthesis of malonyl-CoA): condensation of 18:1-CoA with malonyl-CoA and the subsequent reduction, dehydration, and reduction steps. If each step is catalyzed by a separate enzyme (Bessoule et al. 1989), then in principle any one of them might be the target for the mutation in the JB20 line. Indeed, analysis of a larger set of *Arabidopsis* mutants deficient in 18:1 elongation indicates that mutations in at least three complementation groups affect this pathway (Kunst et al. 1989; and personal communication).

Several other mutants with less pronounced phenotypes were obtained in our seed fatty acid screen. As such reduced phenotypes were seldom seen in our previous leaf fatty acid mutant screen (Somerville and Browse 1988), we believe these secondary loci may provide additional insight into the regulation of seed lipid synthesis in crop species. Indeed, most of the variations in fatty acid composition seen in agronomic species are also partial reductions similar to those seen in these *Arabidopsis* mutants. Although the altered lipid composition of some of the mutants could be secondary effects of mutations at loci affecting other cellular functions, such as deficiencies in hormone biosynthesis and transport (Finkelstein and Somerville 1989), others will no doubt be at loci directly involved in lipid biosynthesis. In this respect, the mutant line JB11 is particularly interesting for the study of the regulation of the level of polyunsaturated 18-carbon fatty acids, because it represents an unusual case in which activity of an enzymatic step, the conversion of 18:2 to 18:3, is apparently increased by mutation. The relatively large number of mutants we have obtained with alterations in seed lipid composition suggests that many products are involved in the regulation of seed lipid composition. The characterization of these genes and their effects on lipid metabolism should provide new insights into the regulation of seed lipid metabolism.

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