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Isolation of EMS-induced mutants in *Arabidopsis* altered in seed fatty acid composition

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Summary. Mutants of *Arabidopsis thaliana* were identified by screening pedigreed M3 seed collections from EMS-treated plants for changes in fatty acid (FA) composition. The FA phenotypes of the most dramatic mutants are as follows: G30 and 1E5 (allelic) lack linolenic acid (18:3) and are elevated in linoleic acid (18:2); 4A5 is deficient in 18:2 and 18:3 and fourfold increased in oleic acid (18:1); 9A1 lacks all FAs > C18 and is twofold increased in 18:1; 1A9 is twofold increased in palmitic acid (16:0) and decreased by one-half in 18:1; 2A11 is two- to threefold increased in stearic acid (18:0) and decreased by one-half in 18:1. Based on segregation of F_2 selfed plants derived from crosses to wild type, all of these phenotypes are the result of single gene mutations.

Key words: *Arabidopsis thaliana* – Lipid biosynthesis – Oilseed breeding – Fatty acid desaturation – Seed lipids

Introduction

The properties of vegetable oils are largely determined by the fatty acid (FA) composition of lipids stored in the seed or fruit of oil crop plants. Changes in FA composition that are the result of blocks in steps of FA biosynthesis have been introduced into several plant species by traditional plant breeding and mutagenesis breeding techniques (Knowles 1989). Eventually, precise customization of vegetable oils will be possible with the development of methods for isolating FA biosynthesis genes concomitant with the establishment of systems for altering expression within a plant or mobilizing genes from one plant species to another.

Our knowledge of biosynthesis of triacylglycerols and other storage lipids in developing seeds has improved substantially in recent years (Slack and Browse 1984; Somerville and Browse 1988; Stumpf and Pollard 1983). In the plastid, the first intermediate is palmitoyl-ACP (16:0-ACP), a product of FA synthetase. 16:0-ACP can be further elongated and desaturated in the plastid to form stearoyl-ACP (18:0-ACP) and oleoyl-ACP (18:1-ACP). Each of the above intermediates can be released from the ACP by acyl ACP hydrolase to form the free FAs, which are transferred across the plastid envelope to the cytoplasm and the eukaryotic pathway. The lipid interconversions in the cytoplasm are via CoA intermediates. 18:0-CoA and 18:1-CoA are elongated to the corresponding C-20 and C-22 FAs. The various FA-CoA's are transferred to different glycerol intermediates by highly specific acyl transferases, to yield phosphatidic acid (PA), phosphatidyl glycerol (PG), diacylglycerol (DAG), phosphatidylcholine (PC) and, eventually, after release of the choline by a phosphatase, triacylglycerol (TAG). Oleoyl-PC can be further desaturated to linoleoyl-PC (18:2-PC) and linolenoyl-PC (18:3-PC) by specific desaturase enzymes. Assays for several of these activities have been developed, although none of the enzymes has been purified to homogeneity. This limitation warrants a genetic approach to gene isolation.

Genes involved in lipid biosynthesis have been described in *Arabidopsis*. Several genes affecting fatty acid desaturation (fad) levels in the leaf have been named *fadA, fadB, fadC,* and *fadD* (Somerville and Browse 1988). *fadA* mutants lack 16:1, which normally occurs only at the *sn*-2 position of PG. *fadB* mutants are enriched in 16:0, lack 16:1, 16:2, 16:3, and have slightly increased levels of all C-18 FAs. The *fadB* gene probably affects desaturase activity and also partitioning of FAs between the plastid and cytoplasm. *fadC* is deficient in 16:2 and 18:2, and probably encodes a chloroplast desaturase that utilizes 16:1 and 18:1. *fadD* mutants are

deficient in 16:3 and 18:3, and are most likely lacking an enzyme that desaturates 16:2 and 18:2. In addition, the *act1* mutation has been described which appears to be deficient in activity of the first enzyme in the plastid pathway of glycerolipid synthesis, acyl-ACP:*sn*-glycerol-3-phosphate acyltransferase (Kunst et al. 1988). Recently, mutations affecting the desaturation and elongation of C18 FAs in the seed have also been reported in *Arabidopsis* (Browse et al. 1989).

Genes affecting FA biosynthesis have been described in other plant systems. In *Brassica napus*, the first major success of breeding for changed oil quality was the discovery of low erucic acid (22:1) lines, which appear to be deficient in elongation of C18 FAs (Stefansson et al. 1961). In corn, gene(s) influencing 18:1 and 18:2 content have been localized on the long arm of chromosome 5 and a recessive gene controlling high 18:2 on the long arm of chromosome 4 (Widstrom and Jellum 1984). In safflower, the gene *ol* governs proportions of 18:1 and 18:2, and the locus *st* largely determines the 18:0 content (Knowles 1972). Mutants with low 18:3 content have been described in flax (Green and Marshall 1984). With soybean, several mutants have been reported, including high 16:0 and 18:0 (Bubeck et al. 1989), high 18:0 (Graef et al. 1985), and low 18:2 and 18:3 (Hammond and Fehr 1983).

We are interested in isolating the genes involved in the late steps of fatty acid elongation and desaturation in the seed by transposon tagging. Towards achieving such an objective, we have obtained FA biosynthetic mutants of *Arabidopsis* trough extensive screening of EMS-treated plants. This paper describes our screening strategy, the characterization of mutants, and the preliminary genetic analysis.

Materials and methods

Plant material

Arabidopsis thaliana (L.) Heynh. cv Columbia (2n=10) was used as the wild type in these studies. Planting was achieved using a 20-µl autopipette: 10 µl of water was taken up along with one seed, expelled on the surface of the soil, and subsequently watered in. Plants were grown in 'cone-tainers' (Cone-tainer Nursery, Canby/OR) in supersoil under cool greenhouse conditions (22°–24 °C). At 6–8 weeks when at least several siliques were mature and turning yellow, plastic pot sleeves were fastened over the cone-tainers and the plants were allowed to dry. Twenty siliques from each plant were collected in vial storage boxes (Cargille Labs, Cedar Grove/NJ), where they were held until analysis or planting.

Mutagenesis

Mutagenesis was carried out by either of two different protocols: (1) dry seeds were incubated overnight in 0.3% (v/v) ethyl methane sulfonate (EMS; Browse et al. 1986); or (2) seeds allowed to imbibe water during incubation at 4 °C for 5 days, and were treated with 0.1% (v/v) EMS for 24 h (Koornneef et al. 1984). Mutagenized seed were rinsed thoroughly in water and planted.

Screening strategy

M_1 plants were allowed o self-pollinate and M_2 seed from each M_1 plant were collected individually. In order to screen the M_2 seeds, we adopted Redei's (1974) minimum sampling strategy: one seed from each plant was planted and plants were selfed. M_3 seed was collected and analyzed for FA composition. This approach enabled us to repeatedly sample our mutagenized population with the confidence that mutants of identical phenotype did not represent duplicates of the same mutational event. Also, having the individual M_2 plants pedigreed allowed us to sample siblings to follow segregation of interesting phenotypes.

Genetic analysis of mutants

M_3 seed was planted to test stability and heritability of the phenotype in the M_4. Reciprocal outcrossing of the putative mutants to wild type and analysis of the selfed F_2 seed were performed to test mode of inheritance. Individual mutants that had similar phenotypes were tested for allelism by crossing them reciprocally and analyzing the FA composition of the resulting seed.

Fatty acid analysis

About 100 seeds (2.0 mg) were simultaneously extracted for lipids, FAs were cleaved from the triacylglycerol and methylated by treatment with 1.0 ml of 1 *N* HCl in 100% methanol for 1 h at 80 °C. After incubation, 1.5 ml of 0.9% (w/v) NaCl and 1.0 ml hexane (spiked with 0.1 mg/ml of heptadecanoic acid methyl ester, an internal standard) was added to the cooled tubes, which were shaken 1 min and centrifuged (1,000 × *g*, 5 min). The hexane phase was transfered to gas chromatography vials for analysis. Samples (1 µl) were injected via autosampler into a 2.0 mm × 3.07 m glass column packed with 3% SP-2310/2% SP-2300 on 100/120 chromosorb WAW (Supelco, Bellefonte/PA). The Perkin-Elmer sigma 300 gas chromatograph was programmed as follows: 160 °C for 2.0 min, ramp to 220 °C at 30 °C/min, held at 220 °C for 12.0 min. Injector and FID detector were held at 250° and 300 °C, respectively. Data were collected by a PE-Nelson Analytical 950 interface, connected to an IBM-compatible personal computer with PC Integrator software (PE-Nelson, Cupertino/CA). The composition of all FAs having chain length greater than C-8 was compared with that of the unmutagenized control. Samples differing from the control by more than four standard errors for any one FA peak were considered putative mutants.

Results

Mutagenesis

About 3,000 M_1 seed were sown resulting in 1,946 M_2 seed collections. One M_2 seed from each family was planted, which yielded 1,804 M_3 seed collections. The chief reasons for the decrease in numbers of collections between each successive generation were failure to germinate or develop into viable seedlings, sterility, or immaturity at time of harvest of the rest of the plants. From the 1,804 M_3 seed collections analyzed for FA composition, 153 samples were picked as independent putative mu-

Table 1. Mutants altered in FA composition

Phenotype	Mutant isolation no.
Deficient in 18:1 desaturation	H5, J9, 4A5
Deficient in 18:2 desaturation	G30, 1E5, 4C14, 4F9
Deficient in C18 elongation	G8, H2, 4H5, 5A7, GB15, 9A1, 9C7, 9D9
Elevated in 16:0	D26, 1A9, 5A1, 6J7, 7A19, 7I5, 8G17, 10G13
Elevated in 18:0	D24, 2A11
Reduced in C18, elevated in >C18	K13, K21, 5B4
Elevated in 20:1 and total FA content	3G1, 9D11

Table 2. Percent FA composition of *Arabidopsis* mutants

Sample	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:1
Wild type	7.9	2.9	15.0	29.2	18.8	2.1	17.3	1.3
4A5	4.6	1.7	63.3	0.9	1.3	1.4	23.5	1.1
G30	6.4	2.5	17.1	47.9	1.2	1.2	15.6	4.3
1E5	7.7	3.0	15.6	45.2	1.9	2.1	17.7	5.1
9A1	8.8	3.3	28.2	34.6	22.5	0.3	0.2	0
1A9	18.9	3.4	7.9	21.7	18.8	3.1	17.2	1.4
2A11	7.1	7.6	8.6	26.3	21.4	6.2	16.6	1.7

Table 3. Segregation of siblings of M2 plants

Mutant	Phenotype	FA genotype			
		+/+	m/+	m/m	total
4A5	High 18:1	3	4	1	8
9D9	Low 20:0 and 20:1	3	10	7	20
1A9	High 16:0	2	15	4	21
2A11	High 18:0	1	2	1	4

tants in that they showed a percentage difference of greater than 4 standard errors for any one FA peak. Several putative mutants (G30, 1E5, 9A1) showed almost complete loss of certain FAs, indicating that these were probably homozygous for the mutation. These individuals were planted out and, indeed, these traits were inherited in the M_3 plants.

Of the putative mutants, 76 were chosen (FA phenotypes with marginal differences and some duplicates were eliminated) for planting of M_2 siblings to obtain segregation data. Of the initial 76 putative mutants, 30 were determined to be heritable mutants (Table 1): 9 were heterozygotes producing an intermediate phenotype (homozygotes with a more extreme phenotype were identified among the M_2 siblings), 10 were homozygotes (including the 3 dramatic mutants above), 11 were heritable but without obvious segreation ratios. Of the remaining 46 putative mutants, 33 were not inherited [siblings were wild type (WT)] and 13 were inconclusive (due to low germination or small differences in FA percentages). If our criterion for initial selection of putative mutants had been made more stringent by raising the number of standard errors' difference from the WT, perhaps we might have cut down on the number of noninherited and inconclusive individuals.

FA composition of mutants

The FA composition of those mutants showing the greatest departure from wild type is given in Table 2. Mutant 4A5 is almost completely lacking in linoleic acid (18:2) and linolenic acid (18:3), while showing greater than a fourfold increase in oleic acid (18:1) and a slight increase in eicosenoic acid (20:1). Both G30 and 1E5 are dramatically decreased in 18:3, showing a concomitant, stoichiometric increase in 18:2. Reciprocal crossing of these mutants revealed that they are allelic in that the F_1 progeny had a mutant FA composition (data not show). Mutant 9A1 is almost completely deficient in FAs greater than C18 length, while being slightly elevated in 18:1, 18:2, and 18:3 content. Mutant 9A1 is deficient in not only 20:1 but also arachidic acid (20:0), indicating that the same gene/enzyme may be involved in the elongation of 18:0 and 18:1, or that synthesis of 20:1 proceeds via 20:0. Mutant 1A9 is more than doubled in palmitic acid (16:0) content, whereas the percentage of 18:1 is halved. This mutant also contains a peak of palmitoleic acid (16:1) amounting to about 3% of total FAs, not seen in other mutants or the wild type. Finally, mutant 2A11 shows a greater than twofold increase in stearic acid (18:0) and arachidic acid (20:0), while the content of 18:1 is reduced by a half.

Segregation of altered FA phenotypes

The segregation pattern of siblings of several mutants is shown in Table 3. In the case of 1A9 and 9D9, the original M_2 plants were homozygous for the mutation, whereas for 4A5 and 2A11, the plants were heterozygous. Analysis of siblings of the latter two plants resulted in the detection of the more extreme homozygous mutant phenotype. Although the numbers are rather small, this technique also allows speculation that all of these mutants are the result of single gene mutations, based on the approximate 1:2:1 segregation ratio observed. To confirm that the mutant phenotypes were due to single gene mutations, most of these mutants were outcrossed to wild type. F_1 and F_2 progeny from these crosses were planted in succession and allowed to self-pollinate. The seed collections from the F_2 plants were analyzed for FA composition, and the segregation of phenotypes was determined. Table 4 shows an example of F_2 segregation data. An F_1 heterozygote obtained by pollinating the mutant G30 with wild type was selfed to generate these data. Mutant G30 is almost completely lacking 18:3 (1%–

Table 4. F_2 segregation data for FA composition obtained by selfing a G30/+ heterozygote

Fatty acid (% composition)									Genotype
	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:1	
Parentals									
G30	6.4	2.5	17.2	47.8	1.2	1.2	15.6	1.2	m/m
WT	7.9	2.9	15.0	29.2	18.8	2.1	17.8	1.3	+/+
F_2 progeny									
	8.5	3.3	14.2	36.6	9.0	2.8	19.5	1.7	m/+
	9.2	3.2	18.1	42.9	2.4	2.4	16.6	1.3	m/m
	8.3	3.1	14.0	36.2	10.4	2.6	19.2	1.5	m/+
	8.8	2.8	13.5	36.3	9.9	2.5	19.4	1.8	m/+
	9.3	3.3	16.6	36.6	9.2	2.7	17.4	1.5	m/+
	8.9	3.2	14.3	36.1	10.5	2.6	18.4	1.4	m/+
	9.1	3.3	14.7	37.3	9.4	2.8	17.0	1.3	m/+
	8.4	3.3	14.9	35.1	10.9	2.6	18.7	1.5	m/+
	8.7	3.1	15.4	43.9	2.1	2.5	18.3	1.5	m/m
	8.6	3.2	13.4	29.4	16.7	2.8	19.3	1.9	+/+
	9.1	2.9	13.4	29.9	16.9	2.7	19.1	1.8	+/+
	8.5	3.3	15.5	35.8	8.6	2.6	18.7	1.5	m/+
	8.9	3.0	14.0	36.1	11.1	2.4	18.8	1.3	m/+
	8.6	3.0	14.1	28.4	18.5	2.1	19.4	1.5	+/+
	8.4	2.8	14.8	44.3	1.9	2.4	19.6	1.7	m/m
	8.8	3.3	13.8	29.9	16.4	2.8	19.0	1.6	+/+
	9.4	3.0	16.2	37.2	8.1	2.6	17.8	1.8	m/+
	9.2	2.9	16.5	43.9	1.9	2.2	17.2	1.8	m/m
	9.5	3.3	15.5	37.0	8.6	2.6	17.9	1.5	m/+
	9.2	3.4	16.7	29.9	15.7	2.4	17.8	1.4	+/+
	8.9	3.2	14.5	36.8	9.6	2.6	18.2	1.6	m/+
	9.2	3.4	15.1	36.3	9.7	2.9	17.5	1.2	m/+
	8.8	3.1	14.7	45.9	1.8	2.2	17.4	1.4	m/m
	8.3	2.8	11.4	29.2	20.2	2.2	19.3	1.8	+/+

Table 5. F_2 segregation data for the mutations 9A1, 4A5, and G30

Cross	No. of individuals			
	+/+	m/+	m/m	Total
WT × 9A1	16	37	17	70
9A1 × WT	4	20	3	27
WT × 4A5	14	27	3	44
WT × G30	13	44	17	74
G30 × WT	6	13	5	24

2%), and 18:2 is increased to the approximate stoichiometric sum of what 18:2 and 18:3 would be in the WT (45%). In the heterozygotes the concentration of 18:3 is intermediate between the homozygotes and WT (8–10%). The segregation ratio of these progeny is 6:13:5, which is approximately 1:2:1, indicating that the original mutant is the result of a mutation of one gene and that the wild-type allele is incompletely dominant over the new mutant.

The situation appears to be much the same for 9A1 and 4A5. Table 5 shows a summary of the F_2 segregation data for three mutations, 9A1, 4A5, and G30. The crosses, WT × 9A1, 9A1 × WT, and WT × G30, and G30 × WT, yielded ratios in the F_2 that statistically approximate 1:2:1. Within 4A5, however, repeated crosses of the mutant used as the female were not successful, and F_2 segregation data from the reciprocal cross yielded a deficit of mutant phenotypes. With this particular mutant, about 50% of the number of seeds planted fail to either germinate or to reach maturity. At this time it is not known whether these features are a consequence of the FA mutation or of some other unlinked gene.

Discussion

By using a screening strategy involving limited sampling of individual M_2 pedigreed plants, we were able to obtain a large number of independent FA mutants with a minimum of FA analyses. Of the mutants in Table 2, half were initially found to be of intermediate phenotype (indicating heterozygosity). Additional sampling of siblings of these individuals allowed us to identify the more extreme phenotype (the homozygotes) without having to grow out and self the original mutants.

Our mutants appear to be similar in phenotype to those described by Lemieux et al. (1990) in the accompanying paper. Mutant G30 resembles *fad3* in that it is deficient in 18:3 and elevated in 18:2. The mutation most likely involves the loss of a cytoplasmic 18:2 desaturase (although enzyme activities have not been tested). The phenotype for 4A5 resembles *fad2* in that both 18:2 and 18:3 are almost completely lacking and 18:1 is increased. The *fad2* mutation probably involves the loss of a cytoplasmic 18:1 desaturase. Mutant 9A1 lacks FAs greater than C-18, indicating a lesion similar to that of the *fae1* mutation. Based on segregation data from crosses to WT, the 9A1 mutation involves a single gene. However, both 20:1 and 20:0 are decreased, which may mean that the elongase that is deficient in this mutant is capable of elongating 18:0- and 18:1-CoA in the cytoplasm. However, in view of a recent report of other types of FA elongation mutants in *Arabidopsis*, a minimum of two genes may be involved in the synthesis of FAs of > C18 chain length (Kunst et al. 1989).

Mutant 1A9 is elevated two- to threefold in 16:0, halved in 18:1, slightly decreased in 18:2, and increased in 16:1 and 20:0. This particular mutation also involves a single gene. We propose therefore to call this mutant *fab1* (fatty acid biosynthesis). A similar mutant, the result of a single recessive gene, has been reported in *Brassica campestris* var. *annua*, with two- to threefold increase in 16:0, reduced 18:1, slightly decreased 18:2, and increased in 16:1 (Persson 1985). It is obvious that more

biochemical data is needed before this type of mutation can be assigned to a specific step in the pathway.

Mutant 2A11 is elevated two- to threefold in 18:0 and 20:0 and halved in 18:1. Again, more biochemical data is needed to determine the basis of this mutation. This mutant will be refered to as *fab2*.

In summary, our analysis has uncovered at least five loci affecting the seed FA composition in *Arabidopsis*. Mutations in loci affecting 18:1 desaturation, 18:2 desaturation, and C18 elongation, respectively, produce phenotypes similar to the *fad2*, *fad3*, and *fae1* mutations described by Lemieux et al. (1990). We have shared our mutants with J. Browse, and allelism tests are in progress to determine if the mutations that condition similar phenotypes belong to the same gene. With a view to minimizing potential conflicts in nomenclature, we have refrained from assigning specific gene designations to our G30, 4A5, and 9A1 mutations until the allelism tests are completed. In addition, we have obtained mutations at two new loci, *fab1* and *fab2*, that cause increases in the content of saturated FAs. The identification and subsequent mapping of these genes will greatly aid in their subsequent isolation by procedures such as chromosome walking or transposon tagging.

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References

Browse J, McCourt PJ, Sommerville CR (1986) Fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue. Anal Biochem 152:141–145

Browse J, Lemieux B, Guerra D, Sommerville C (1989) Strategies for modifying plant lipid composition. In: Lamb C, Beachy R (eds) Plant gene transfer. Alan R. Liss, New York

Bubeck DM, Fehr WR, Hammond EG (1989) Inheritance of palmitic and stearic acid mutants of soybean. Crop Sci 29:652–656

Graef GL, Fehr WR, Hammond EG (1985) Inheritance of three stearic acid mutants of soybean. Crop Sci 25:1076–1079

Green AG, Marshall DR (1984) Isolation of induced mutants in linseed (*Linum usitatissimum*) having reduced linolenic acid conent. Euphytica 33:321–328

Hammond EG, Fehr WR (1983) Registration of A5 germ plasm line of soybean. Crop Sci 23:192–197

Knowles PF (1972) The plant geneticist's contribution toward changing lipid and amino acid composition of safflower. J Am Oil Chem Soc 49:27–29

Knowles PF (1989) Genetics and breeding of oil crops. In: Robbelen G, Downey RK, Ashri A (eds) Oil crops of the world. McGraw-Hill, New York, pp 260–282

Koornneef M, Reuling G, Karssen CM (1984) The isolation and characterization of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. Physiol Plant 61:377–383

Kunst L, Browse J, Somerville C (1988) Altered regulation of lipid biosynthesis in a mutant of *Arabidopsis* deficient in chloroplast glycerol-3-phosphate acyltransferase activity. Proc Natl Acad Sci USA 85:4143–4147

Kunst L, Taylor D, Giblin M, Underhill E (1989) Analysis of seed lipid elongation using *Arabidopsis thaliana* mutants. Conf Genet Mol Biol *Arabidopsis*, Indiana University, Bloomington/IN, Poster Abstr 1:4

Lemieux B, Miquel M, Somerville C, Browse J (1990) Mutants of *Arabidopsis* with alterations in seed lipid fatty acid composition. Theor Appl Genet (in press)

Persson C (1985) High plamitic acid content in summer turnip rape (*Brassica campestris* var. *annua* L.). Cruciferae Newslett. 10, Scottish Crop Research Institute, Invergowrie, Dundee, p 137

Redei G (1974) Economy in mutation experiments. Z Pflanzenzuecht 73:87–96

Slack CR, Browse JA (1984) Synthesis of storage lipids in developing seeds. In: Murray DR (ed) Seed physiology, vol 1. Academic Press, New York, pp 209–244

Somerville CR, Browse J (1988) Genetic manipulation of the fatty acid composition of plant lipids. Recent Adv Phytochem 22:19–44

Stefansson BR, Hougen FW, Downey RK (1961) Note on the isolation of rape plants with seed oil free from erucic acid. Can J Plant Sci 41:218–219

Stumpf PK, Pollard MR (1983) Pathways of fatty acid biosynthesis in higher plants with particular reference to developing rapeseed. In: Kramer JKG, Sauer FD, Pigden WJ (eds) High and low erucic acid rapeseed oils. Academic Press, New York, pp 131–141

Widstrom NW, Jellum MD (1984) Chromosomal location of genes controlling oleic and linoleic acid composition in the germ oil of two maize inbreds. Crop Sci 24:1113–1115