PERGAMON

Phytochemistry 52 (1999) 1029–1033

PHYTOCHEMISTRY

The natural genetic variation of the fatty-acyl composition of seed oils in different ecotypes of *Arabidopsis thaliana*

Anthony A. Millar, Ljerka Kunst*

Department of Botany, University of British Columbia, Vancouver, BC, Canada V6T 1Z4

Received 1 December 1998; received in revised form 21 June 1999

Abstract

The fatty-acyl composition of the seed oil was determined for 100 ecotypes of *Arabidopsis thaliana*. Despite coming from diverse geographical locations, seed fatty-acyl profiles of all ecotypes were remarkably similar. They contained identical fatty acids, including the characteristic C_{20} and C_{22} very-long-chain fatty acids (VLCFAs). The total proportions of seed VLCFA varied between 22% and 35% w/w of the total seed fatty acid content.

Keywords: *Arabidopsis thaliana*; Brassicaceae; Ecotypes; Seed oil composition; Very-long-chain fatty acids

1. Introduction

Seed oils in nearly all plant species are stored as triacylglycerols (TAGs), which act as an energy reserve for the germinating seed. These oils are also an important component of the human diet, and of interest as renewable materials for industrial applications. Thus, seed oil composition has become a major target for modification by plant breeding and genetic engineering (Murphy, 1996).

Over the last 10 years, the crucifer *Arabidopsis thaliana* has been developed as a model system for the genetic analysis of lipid biosynthesis (Browse & Somerville, 1994). Several independent genetic screens resulted in the isolation of mutants of *A. thaliana* with specific alterations in fatty-acyl composition of seed storage lipids. Mutants were found that were deficient in fatty acid desaturation (*fad2* and *fad3*) (Lemieux, Miquel, Somerville & Browse, 1990; James & Dooner, 1990; Miquel & Browse, 1992), fatty acid elongation (*fae1*) (Lemieux et al., 1990; James & Dooner, 1990; Kunst, Taylor & Underhill, 1992), or TAG assembly (*tag1*) (Katavic et al., 1995). These mutants demonstrated that lesions in single genes have profound effects on the *A. thaliana* seed oil fatty-acyl composition. Furthermore, many of these mutants were semi-dominant, suggesting that the gene products encoded by the mutated genes were rate limiting. The genes corresponding to several of these mutants have since been isolated (Arondel, Lemieux, Hwang, Gibson, Goodman & Somerville, 1992; Okuley, Lightner, Feldmann, Yadav, Lark & Browse, 1994; James, Lim, Keller, Plooy, Ralston & Dooner, 1995) and now provide the means to manipulate the fatty acid composition and accumulation in seed oils. For instance, over-expression of the *fae1* gene in the seeds of *A. thaliana*, led to increased proportions of very-long-chain fatty acids (VLCFAs; C_{20} or longer) accumulating in the seed oil of *A. thaliana* (Millar & Kunst, 1997).

Encouraged by the results obtained with chemically-induced mutants, we wished to explore whether significant genetic differences in seed fatty-acyl composition

* Corresponding author.

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00403-3

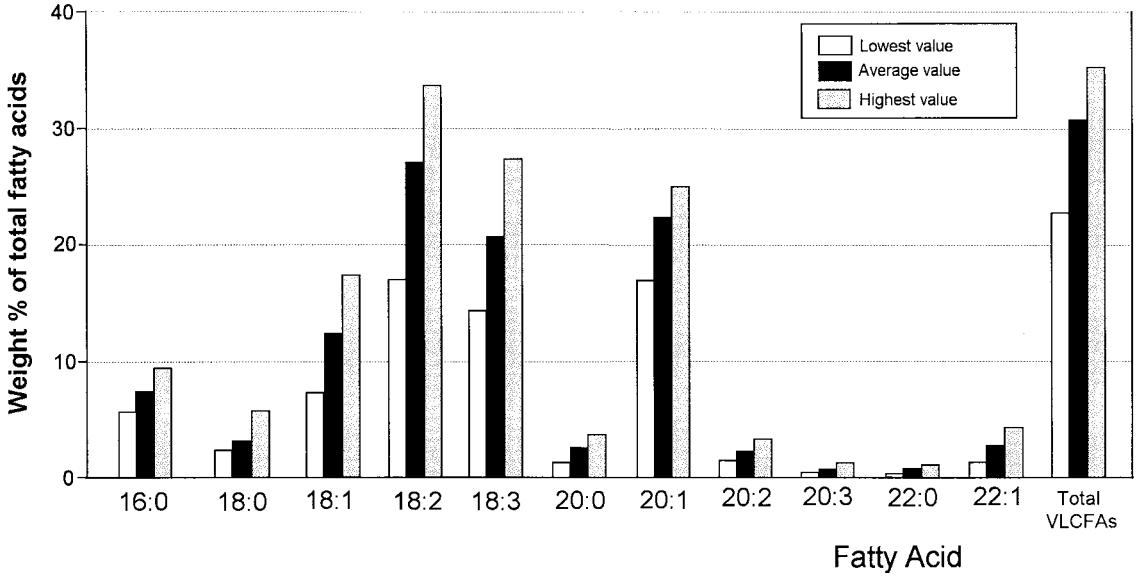

Fig. 1. The fatty-acyl composition of the seed oil of *A. thaliana* ecotypes. All measurements represent the weight percentage of the total weight of seed fatty acids. Solid bars represent the average proportions of each fatty acid for the 100 ecotypes examined. Clear bars represent the lowest proportion of each fatty acid found in the population of ecotypes. The shaded bars represent the maximal proportion.

and accumulation existed within natural *A. thaliana* populations. We were particularly interested if natural variants containing novel fatty acids, or those with a higher very-long-chain-fatty acid content, could be found among the different *A. thaliana* ecotypes. Such plants might represent a source of genes encoding enzymes with new specificities or activities. Thus, we have analyzed the fatty acid compositions of the seed oils of 100 *A. thaliana* ecotypes from a variety of geographical locations.

2. Results and discussion

The seed oil fatty-acyl composition was determined for 100 different *A. thaliana* ecotypes to examine the extent of natural variation that occurs. Fig. 1 shows a seed fatty-acyl profile that represents the average of all ecotypes analyzed. The highest and lowest levels of each fatty acid are indicated, showing the extremes of variability observed. It is apparent that, although these *A. thaliana* ecotypes originated from several different continents (Europe, Asia and North America), and from vastly different environments, their seed oils have surprisingly similar fatty-acyl compositions. All the fatty acids detected corresponded to those found in the Columbia and Landsberg erecta ecotypes, and no novel fatty acids were observed. This result is somewhat comparable to the report describing the analyses of the epicuticular wax constituents of 40 different *A. thaliana* ecotypes, in which almost no compositional variation was found (Rashotte, Jenks, Nguyen & Feldmann, 1997). Thus, both these biochemical characteristics seem to be highly conserved, in contrast to a number of other phenotypic traits analyzed amongst ecotypes of *A. thaliana* (see Ref. Rashotte et al., 1997).

As shown in Fig. 1, all *A. thaliana* ecotypes contained a significant proportion of VLCFAs (>20% wt/wt of total fatty acid), averaging around 31% w/w of total seed fatty acids. Each ecotype had C_{20} and C_{22} VLCFAs, but unlike in other species of the family Brassicaceae, no C_{24} VLCFAs were detected. However, there were significant variations in the accumulation of C_{20} and C_{22} VLCFAs, with an ecotype from Tadjikistan containing only 22.5% (w/w of total fatty acid), whereas an ecotype from Spain had 34.9% of VLCFAs (w/w of total fatty acid). Over-expression of the VLCFA condensing enzyme, FAE*1*, in *A. thaliana* resulted in seed oils with almost 43% VLCFAs (Millar & Kunst, 1997). Thus, no *A. thaliana* ecotype had VLCFA content approaching the level achieved in transgenic plants.

The C_{20} to C_{22} acyl chain-length ratios were similar in each ecotype [$C_{20} : C_{22} \sim 8.7$]. This ratio decreased slowly as the proportions of VLCFAs increased in seed oil, reflecting higher levels of C_{22} accumulation. A similar trend was observed when FAE*1* was over-expressed in *A. thaliana* seed (Millar & Kunst, 1997),

or when the KCS condensing enzyme from jojoba (*Simmondsia chinensis*) was over-expressed in the Canola varieties of *Brassica napus* (Lassner, Lardizabal & Metz, 1996). Thus, the variation that is observed amongst the ecotypes could simply be explained by differences in the level of expression or activity of the FAE*1* condensing enzyme.

The fact that seed fatty-acyl composition, and especially the proportion of VLCFAs, among different *A. thaliana* ecotypes shows only minor variations is surprising. Presently there is no known physiological or biochemical rationale for the presence of VLCFAs in seed oils. For example, VLCFAs are not widely distributed in seeds of higher plants, with the exception of the species of the family Brassicaceae. Furthermore, the *fae*1 mutant of *A. thaliana*, which contains less than 1% w/w of VLCFAs (Kunst et al., 1992), shows normal germination and viability under laboratory conditions, and it has no visible phenotype. The same is true of the Canola rapeseed varieties, which are also almost completely devoid of VLCFAs in their seed oil (Stefansson, Hougen & Downey, 1961). On the other hand, the strong conservation of the presence, composition and levels of VLCFAs amongst the *A. thaliana* ecotypes argues that they give the plant a selective advantage. However, this selective advantage may become apparent only under very specific growth conditions. Thus, we were interested in determining whether any correlation(s) could be established between the VLCFA levels and the environmental conditions which are characteristic for the original site of collection of each ecotype. Table 1 shows the proportions of VLCFAs in the 100 ecotypes examined, and their country of origin. According to these data, ecotypes with the lowest levels of VLCFAs (22–23% w/w of total fatty acids) originated from mountainous regions in Tadjikistan and Kashmir. In contrast, ecotypes with the highest levels of VLCFAs (34–35% w/w of total fatty acids) came from low altitude locations in southern Europe, France and Spain. This difference could simply mean that these two groups of ecotypes are genetically the most diverse. Alternatively, it may mean that seed oils from plants grown in temperate regions have a higher content of VLCFAs than seed oils from colder climates.

In conclusion, the seed oil fatty-acyl compositions of the different ecotypes of *A. thaliana* are highly conserved despite the fact that they originate from diverse geographical locations. The observed differences in fatty-acyl compositions are probably due to altered expression levels or activities of the relevant fatty acid biosynthetic enzymes. However, because no new fatty acids were detected, this collection of ecotypes does not seem to contain fatty acid biosynthetic enzymes with novel substrate specificities.

3. Experimental

3.1. Plant material

Seeds of 100 different ecotypes of *A. thaliana* were obtained from the Nottingham Arabidopsis Stock Centre. All the ecotypes at the Stock Centre were propagated under controlled environmental conditions (22°C, 20 h photoperiod) in a greenhouse. The stock numbers for each ecotype are shown in brackets; Je54 (N924), Mt-0 (N1380), Bs-1 (N996), S96 (N914), Sn(5)-1 (N930), Bla-14 (N988), Wei-0 (N3110), Es-0 (N1144), Cl-0 (N1082), Kas-1 (N903), Co-1 (N1084), En-2 (N1138), Enkheim-T (N921), Edi-0 (N1122), Ei-2 (N1124), Bla-12 (N986), Gy-0 (N1216), El-0 (N1134), Tsu-1 (N1640), Be-0 (N964), Ko-2 (N1288), Hau-0 (N1220), Bch-1 (N956), Le-0 (N1308), Di-0 (N1106), Db-0 (N1100), Ang-0 (N948), Petergof (N926), Yo-0 (N1622), Da-0 (N1098), Shahdara (N929), Ost-0 (N1430), Ct-1 (N1094), Aa-0 (N900), Chi-0 (N1072), Abd-0 (N932), Te-0 (N1550), Bd-0 (N962), Condara (N916), Pa-1 (N1438), Mv-0 (N1386), Ag-0 (N901), Mr-0 (N1372), Pog-0 (N1476), Mh-0 (N904), Lu-1 (N1352), Hodja-Obi-Garm (N922), Rsch-0 (N1490), Kin-0 (N1272), Ca-0 (N1060), Bla-1 (N970), Cen-0 (N1066), Je-0 (N1246), Bu-0 (N1006), Ll-0 (N1338), Pi-0 (N1454), Sei-0 (N1504), Oy-1 (N1643), Hi-0 (N1226), Dr-0 (N1114), Bla-6 (N980), Cal-0 (N1062), Ema-1 (N1637), Lc-0 (N1306), Da(1)-12 (N917), Gre-0 (N1210), Gr-1 (N1198), Kn-0 (N1286), Dra-0 (N1116), Tsu-0 (N1564), Pla-0 (N1458), Nok-1 (N1400), Kä-0 (N1266), Can-0 (N1064), Ws-0 (N1602), Ge-1 (N1188), Kas-1 (N1264), Dijon G (N910), An-1 (N944), Se-0 (N1502), Ita-0 (N1244), Eil-0 (N1132), Mir-0 (N1378), Sah-0 (N1500), Oy-0 (N1436), Per-1 (N1444), Sorbo (N931), Rubezhnoe-1 (N927), Est-0 (N1148), Wil-1 (N1594), H55 (N923), Col-4 (N933), Cvi-0 (N902), Bay-0 (N954), Lip-0 (N1336), St-0 (N1534), Ts-1 (N1552), Ty-0 (N1572), Ms-0 (N905), Bu-6 (N1016).

3.2. Fatty-acyl analysis

For the determination of the seed fatty acid composition, fatty acid methyl esters were prepared according to Browse, McCourt & Somerville (1986) by transmethylation in 1 N methanolic-HCl (Supelco) at 80°C, extracted in hexane and analyzed by gas–liquid chromatography (Kunst et al., 1992). Each sample consisted of at least 30 seeds. The conditions used allowed the detection of VLCFAs of at least 24 carbon units in length.

Acknowledgements

We thank Dr. Mary Anderson of the Arabidopsis

Table 1
Analysis of the VLCFA accumulation in *Arabidopsis* ecotypes[a]

VLCFA (w/w of total FA)	Number of ecotypes	Origin of ecotypes (representative countries)
22	2	Tadjikistan (N922, N929)
23	4	USSR (N1444), Kashmir (N1264), Tadjikistan (N931, N916)
24	0	
25	0	
26	3	Japan (N1640), Spain (N988), USSR (N910)
27	7	Finland (N1144), Tadjikistan (N921), Japan (N1564), UK (N933), Sweden (N1534), Cape Verde Islands (N902), Germany (N1098)
28	15	Ukraine (N927), Spain (N986), Italy (N1094), Switzerland (N3110), Czechoslovakia (N923, N930), Germany (N1114, N1082, N1306), Netherlands (N1400, N1226), USSR (N1072, N1594, N1602, N1148)
29	14	Norway (N1436), Morocco (N1244), Austria (N1454), Kashmir (N903), Poland (N904, N1336), Denmark (N1220), USA (N1210, N1622), Sweden (N1430), Italy (N1504, N1372), Czechoslovakia (N924), Switzerland (N996)
30	22	Germany (N954, N1138, N964, N956, N1100, N962, N900, N1132), Finland (N1550), Canada (N1476), Scotland (N1122), Czechoslovakia (N917, N1116), Netherlands (N1308), Libya (N1380), Belgium (N948), Italy (N1378, N1438), USA (N1272), Sweden (N1352), Canary Islands (N1064), USSR (N1286)
31	18	Germany (N1246, N1006), France (N901), Norway (N1643), Russia (N905), Netherlands (N914), USSR (N1490), Switzerland (N1188), USA (N1386), UK (N1637, N1062, N1572), Austria (N1266), Spain (N1458, N1502, N1500, N1552), Belgium (N944)
32	5	Portugal (N1084), Germany (N1124), France (N1066), Russia (N926), Austria (N1198)
33	7	Germany (N1134, N1060, N1016), UK (N932), Denmark (N1288), Spain (N1338, N980)
34	2	France (N1216, N1106)
35	1	Spain (N970)

[a] The Nottingham Arabidopsis Stock Centre stock number for each ecotype is given in parentheses.

Resource Centre, Nottingham, UK, for providing the seed of all the ecotypes.

References

Arondel, V., Lemieux, B., Hwang, I., Gibson, S., Goodman, H. M., & Somerville, C. R. (1992). *Science*, *258*, 1353.

Browse, J., McCourt, P., & Somerville, C. R. (1986). *Anal. Biochem*, *152*, 141.

Browse, J. A., & Somerville, C. R. (1994). In E. M. Meyerowitz, & C. R. Somerville, *Arabidopsis* (pp. 881–912). New York: Cold Spring Harbor Laboratory Press.

James, D. W., & Dooner, H. K. (1990). *Theor. Appl. Genet*, *80*, 241.

James, D. W., Lim, E., Keller, J., Plooy, I., Ralston, E., & Dooner, H. K. (1995). *Plant Cell*, *7*, 309.

Katavic, V., Reed, D. W., Taylor, D. C., Giblin, E. M., Barton, D. L., Zou, J., MacKenzie, S. L., Covello, P. S., & Kunst, L. (1995). *Plant Physiol*, *108*, 399.

Kunst, L., Taylor, D. C., & Underhill, E. W. (1992). *Plant Physiol. Biochem*, *30*, 425.

Lassner, M. W., Lardizabal, K., & Metz, J. G. (1996). *Plant Cell*, *8*, 281.

Lemieux, B., Miquel, M., Somerville, C., & Browse, J. (1990). *Theor. Appl. Genet*, *80*, 234.

Millar, A. A., & Kunst, L. (1997). *Plant J*, *12*, 121.

Miquel, M., & Browse, J. (1992). *J. Biol. Chem*, *267*, 1502.

Murphy, D. J. (1996). *Trends in Biotech*, *14*, 206.

Okuley, J., Lightner, J., Feldmann, K., Yadav, N., Lark, E., & Browse, J. (1994). *Plant Cell*, *6*, 147.

Rashotte, A. M., Jenks, M. A., Nguyen, T. D., & Feldmann, K. A. (1997). *Phytochemistry*, *45*, 251.

Stefansson, B. R., Hougen, F. W., & Downey, R. K. (1961). *Can. J. Plant Sci*, *41*, 218.