Phytochemistry, Vol 28, No 10, pp 2597-2600, 1989
Printed in Great Britain

0031-9422/89 $3.00+0.00

LIPID CHARACTERIZATION IN SEEDS OF A HIGH OLEIC ACID SUNFLOWER MUTANT

RAFAEL GARCÉS, JOSÉ M. GARCÍA and MANUEL MANCHA

Instituto de la Grasa (C.S.I.C.) Apartado 1078 E-41080 Sevilla, Spain

(*Received in revised form* 27 *January* 1989)

Key Word Index—*Helianthus annuus*, Compositae, sunflower, seeds; lipids; fatty acid composition, high oleic acid mutant

Abstract—The expression of the 'high oleic' character in a sunflower mutant takes place exclusively in the developing seeds, during the synthesis of reserve lipids. The fatty acid composition of different lipid classes was similar in each genotype and depended on growth temperature. In the mutant seeds triacylglycerols contained up to 90% of oleic acid and less than 0.5% of linoleic acid. The content of oleic acid was 2.5 ± 0.7 mg/g of fresh seed all over the developing period.

INTRODUCTION

Normal cultivated sunflower seeds contain linoleic (18.2) and oleic (18:1) acids as the main components of reserve lipids. A sunflower mutant with high 18:1 content in the seed lipids was obtained by chemical mutagenesis [1]. This cultivar was quite self incompatible and segregated for both high and low 18 1 content. Using this material, a backcrossing programme was developed and the character was incorporated into normal cultivated lines [2] The fatty acid composition of normal sunflower oil is influenced by growth day/night temperature, 18.1 and 18:2 being the main components at high and low temperature, respectively [3, 4]. The high 18 1 mutant is quite stable maintaining high levels of 18:1 even when grown at low temperature (90% at 34/22° and 85% at 21/14°) while the amounts of 18:2 are very low (<1% and 7%, respectively) [5]. These concentrations of 18.2 are much lower than those reported for normal oil seeds [6, 7] However, seeds having unusual fatty acids may also have very low 18:2 contents. A few examples are *Ricinus communis* (5%), *Petroselinum sativum* (6%) [6], *Cuphea pseudovaccinum* (0.5%) [8] and *Trichilia triphyllaria* (0 2%) [9]. The unusual fatty acids are preferentially found in triacylglycerols indicating that these acids are synthesized for specific purpose [10, 11]. On the contrary, the fatty acid composition of polar lipids is consistent with the role of these lipids in the functions of membranes Polar lipids contain mainly 18·2 and linolenic (18:3) acids and very little of any unusual fatty acids [12]. Like lauric (12 0) or myristic (14·0) acids in *Cuphea* seeds, 18.1 in the high 18:1 sunflower mutant could be considered as an unusual fatty acid The low levels of 18 2 in some species of *Cuphea* and Meliaceae can be explained by the lack of 18:1 as the short or long chain fatty acids are released and used for lipid synthesis However, in the high 18:1 sunflower mutant 18:2 is not synthesized in spite of the high 18·1 content. If there is lack of oleate desaturase, or if this activity is severely controlled during seed development, needs to be determined. On the other hand, this mutant could be a valuable material to study fatty acid desaturation in plant systems.

RESULTS AND DISCUSSION

The main fatty acid synthesized by the mutant sunflower seeds was identified as (*Z*)-9-octadecenoic acid (18:1). The GC-MS of the bis-(methylthio) methyl ester derivative showed two main fragments at *m/z* 173 and 217 corresponding to a C_{18} fatty acid with a double bond at the 9,10 position. The *cis* configuration was determined by TLC on $AgNO_3$–silica gel of the methyl ester compared with authentic methyl oleate and methyl elaidate.

Expression of the high 18:1 character takes place exclusively in the seed tissues (seed tip, embryo and cotyledon) (Table 1). The fatty acid composition of the lipids of the leaves of normal and mutant high 18:1 sunflower plants was similar, being in agreement with that reported for other plants [12]. The tissues which give rise to the seed, flowers and pollen, also had a similar composition and were in agreement with those of other plants [13, 14]. The hull and the seed membrane showed some differences. 18:1 was the main fatty acid of the lipids of the hull in both genotypes, although it reached a higher value in the mutant. In the normal genotype 18:2 was the main fatty acid of seed membrane lipids whilst 18:1 was again the main one in the mutant. Nevertheless, the composition of these tissues was not too different compared with that of seed tissues. In the mutant, the seed tip, embryo and cotyledon contained very high amounts of 18:1 and very low levels of 18:2. In the cotyledon the percentage of 18 2 was as low as 0 5%. On the contrary the seed tissues of the normal genotype contained high percentages of 18:1 and 18:2. In plants containing erucic acid, this acid occurs specifically in seed tissues [15]; it was not detected in *Brassica* leaves [16] and represented less than 1% in pollen of these species [14]. Erucic is an unusual fatty acid which is associated with those tissues that are involved in seed differentiation. Similarly the high 18:1 concentration in our mutant seeds is a characteristic of seed reserve tissues.

The fatty acid composition of triacylglycerols, diacylglycerols and polar lipids of normal and mutant seeds at two growth temperatures is shown in Table 2. The composition of the different lipid classes was similar in

Table 1 Fatty acid composition of total lipids from different parts of the achene (40 DAF)* of two genotypes of sunflower, compared with that of the leaves, flower and pollen (growth temperature 34/22°)

Tissue	16 0	18 0	18 1	18 2	18 3
	G8 High 18 1 (mol %)				
Hull	12 1±1 2	11 2±0 1	55 1±7 5	18 0±2 4	3 6±1 2
Seed membrane	12 3±1 2	4 3±0 4	53 8±3 4	27 6±1 4	2 0±0 4
Seed tip	4 7±0 1	2.7±0 5	87 5±0 1	4 4±0 4	0 6±0 1
Embryo	5 3±0 2	4 7±0 5	88 6±0 8	1 4±1 1	
Cotyledon	4 0±0 1	3 7±0 7	91 3±0 8	0 5±0 1	0 4±0 1
Leaf	19 6±1 5	0.7±0 4	1 8±0 2	10 0±1 0	67 8±2 6
Pollen†	20 8±1 4	4 0±0 8	11 3±1 7	7 4±1 0	30 0±4 7
Flower‡	24 4	1 6	6 5	53 5	13 9
	Normal genotype (mol %)				
Tissue	16 0	18 0	18 1	18 2	18 3
Hull	12 3±1 3	7 9±0 6	37 0±4 8	35 2±2 2	7 5±1 2
Seed membrane	9 8±1 0	6 4±1 1	31 6±2 3	50 8±1 1	1 3±0 1
Seed tip	8 5±0 9	2 7±0 4	44 4±6 6	43 9±9 9	0 4±0 2
Embryo	8 3±0 2	3 4±0 2	49 5±6 6	38 3±6 7	0 5±0 1
Cotyledon	7 7±0 1	2 9±0 4	56 9±2 5	32 3±2 4	0 2±0 1
Leaf	20 4±3 8	0 7±0 4	1 2±0 8	6 3±1 4	71 4±7 1
Pollen†	25 7±2 4	3 0±0 8	9 0±1 0	7 8±1 1	33 2±3 7
Flower‡	21 8	3 1	9 5	45 4	20 2

*Days after flowering
†Other unusual fatty acid (20–25%) are not included
‡Combined analysis of flowers from three plants
Mean ± s d (samples from three plants)

Table 2 Fatty acid composition of lipid classes from mature (40 DAF) seeds from two sunflower genotypes grown at two temperatures

Temperature	Lipid	16 0	18 0	18 1	18 2
		G8 High 18 1 (mol %)			
21/14°	TAG	3 6±0 2	4 1±0 4	84 5±1 2	7 8±1 3
	DAG	3 3±0 1	2 0±1 0	86 2±0 6	7 8±0 6
	PL	6 9±0 5	3 5±0 2	78 9±1 7	10 8±1 3
34/22°	TAG	4 6±0 1	4 6±0 4	90 5±0 3	0 3±0 1
	DAG	5 6±0 1	3 1±0 1	89 8±0 3	1 4±0 1
	PL	8 6±0 6	4 2±0 2	84 0±0 1	3 1±0 3
		Normal genotype (mol %)			
Temperature	Lipid	16 0	18 0	18 1	18 2
21/14°	TAG	6 2±0 5	6 9±0 8	37 2±4 9	49 4±4 4
	DAG	13 1±3 1	8 4±1 5	37 4±7 8	41 0±8 5
	PL	9 7±1 5	5 9±1 4	34 2±8 6	50 0±4 9
34/22°	TAG	5 4±0 2	3 8±0 2	54 8±3 4	35 7±3 5
	DAG	8 8±1 7	5 2±1 0	51 7±2 2	34 2±0 6
	PL	10 8±1 3	5 8±0 7	39 8±2 5	43 5±2 7

Mean ± s d (seeds from three plants)
TAG = Triacylglycerols DAG = Diacylglycerols, PL = polar lipids

each genotype, but it was very different between them In normal seeds the composition of the different lipid classes was highly dependent on growth temperature. At low temperature (22/14°) the main component was 18 2 while at high temperature (34/22°) it was 18.1 In mutant seeds 18 1 represented more than 78% in all cases and the growth temperature dramatically affected the content of 18:2. At low temperature triacylglycerols and diacylglycerols contained 7.8% of this acid and polar lipids contained 10 8%, at the high temperature triacylglycerols, diacylglycerols and polar lipids contained only 0 3, 1 4 and 3 1%, respectively The similarity in the fatty acid

composition of the different lipid classes was in agreement with that reported for other oil seeds containing usual fatty acids in normal proportions [17]. In contrast, in *Brassica* seeds erucic acid was concentrated in triacylglycerols and diacylglycerols while polar lipids contain only small amounts [10]. As in this case, triacylglycerols from the mutant seeds were expected to contain more 18:1 and consequently less 18:2 than polar lipids. However, only small differences were observed. It has been postulated that 18:2 is needed for normal membrane fluidity and some membrane bound enzymatic activities [18]. The reported fatty acid composition of total membrane and individual complex lipids [12] showed that the percentage of 18:1 ranged from 1 to 20% and only occasionally reached 40%. Depending on the nature of the membrane, the main component was palmitic acid (16:0) or, in most cases, 18:2 or 18:3. Only in olive fruits, in which the lipids are especially rich in 18:1, the polar lipids [19] and the individual complex lipids except monogalactosyldiacylglycerol [20] contained 18:1 as the main component. The results of Table 2 suggested that in the mutant seeds there was an 'overflow' of oleic acid into all lipid classes. Apparently, the fatty acid composition of polar lipids was appropriate for their normal function in mutant seeds.

The amount of the different fatty acids in the total lipids during seed development are represented in Fig. 1. In this experiment the growth temperature was selected to reduce the 18:2 content. In both genotypes, the amount of saturated acids (16:0 and 18:0) increased slightly with seed development. In normal seeds, 18:1 and 18:2 contents increased regularly. Nevertheless, in the high 18:1 mutant the content of 18:2 remained constant, being 2.5 ± 0.7 mg/g of seed fr. wt throughout the developing period. 18:1 was almost the only fatty acid responsible for lipid accumulation in the mutant seed.

The fatty acid composition of total lipids and different lipid classes from developing seeds obtained from normal and mutant sunflower plants grown at 34/22° are shown in Table 3. In normal seeds the different lipids had a similar composition throughout the developing period, while in mutant seeds this composition changed. The fatty acid composition of lipids from very young seeds was similar in both genotypes and even the mutant seeds contained more 18:2 than normal ones at this developmental step. However, when active triacylglycerol synthesis started, *ca* 12 days after flowering (DAF), the 18:2 content was reduced dramatically in all lipids from the mutant seeds. Triacylglycerols contained up to 90% of 18:1 but only 0.3% of 18:2. Diacylglycerols and polar lipids contained similar percentages of 18:1 and slightly higher percentages of 18:2. All these results support the hypothesis that the acyl-CoA pool is in equilibrium with diacylglycerols and polar lipids and acts as acyl donor for triacylglycerols synthesis [21, 22].

EXPERIMENTAL

Sunflowers used in this work were the normal varieties HA-89 and RHA-274 and the high 18·1 mutant G8. High 18 1 seeds obtained after crossing 'pervenets' x RHA-274 were a generous gift of J. M. Fernández-Martínez (CIDA, Córdoba, Spain). This material was self-pollinated several times in our laboratory and G8 seeds selected. Plants were grown in a controlled-environment cabinet with 14 hr photoperiod and 10 hr night, at 21/14°

Table 3. Fatty acid composition of lipid classes from developing seeds of two sunflower genotypes grown at 34/22°

			G8 High 18:1 mol%				Normal genotype mol %			
DAF	Lipid	%	16 0	18 0	18:1	18:2	16:0	18:0	18·1	18:2
6	Total	100	17 4	7 8	33.6	41.1	20.7	8.6	45.0	25.6
	TAG	51.3	18.4	11.1	36.3	34.1	18.8	12.1	28.3	40.8
	DAG	12.3	18.0	14.4	46.7	20.7	16.4	9.4	32.0	42.2
	PL	26 7	17 9	8.0	34.2	39 6	13 3	9.0	31.8	45.8
12	Total	100	8.3	11.6	72.1	6.2	14.3	8.3	47.5	29.9
	TAG	87 1	9.1	12 8	72.5	4.5	17.7	11.0	43.6	27.5
	DAG	1 7	10.9	9.8	65.7	11.9	15.9	13.2	34.4	36.5
	PL	10.5	11.0	8.3	71.4	8 0	14.9	7.4	33.7	43.9
20	Total	100	4.9	8.1	86.4	0.6	6.1	4.9	62.5	26 4
	TAG	94 3	5 0	8.4	86.2	0.4	6.6	5 4	67.1	20.8
	DAG	1 9	6 4	4 3	87 3	1 9	9 6	5.2	55.0	30.1
	PL	2 7	8 2	5 4	83 7	2.6	9 8	4 2	43.9	42.0
30	Total	100	4 3	5.4	89 8	0 5	5 9	3.2	54.9	35.9
	TAG	93.3	4 4	5.4	89 7	0 3	5 8	3.6	56.3	34 3
	DAG	2 4	6.2	3 9	88.2	1.7	8.7	4.3	43.9	42.9
	PL	3.3	8.3	3 8	86.4	1.4	11.2	5 2	43 5	40.1

Mean of data from three plants.
s.d. similar to Table 2
Abbreviations of lipid classes as Table 2.

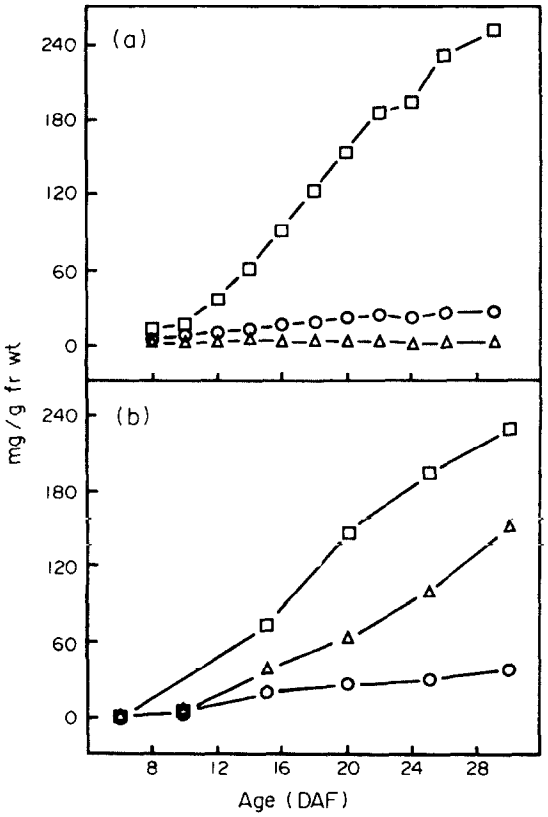

Fig 1 Evolution of the content of 18 1 (□), 18 2 (△) and saturated fatty acids (○) in total lipids from developing seeds of high 18 1 sunflower seeds (A) and the normal genotype (B) Results are expressed as mg of fatty acid / g seed fr wt

or 34/22°, light intensity 300 $\mu E/m^2$ sec. Achenes were harvested at the indicated DAF. Seed tissues were sectioned with a razor blade Samples were ground in test tubes using sand and a glass rod, and the total lipids extracted into petrol–*iso*-PrOH [23] Triacylglycerols, diacylglycerols and polar lipids were isolated by TLC on silica gel plates developed with petrol–Et_2O–HCO_2H (75 25 1) Lipids were converted into Me esters [24] and the fatty acid composition determined by GC Total lipid content and the proportions of lipid classes were determined by GC of the corresponding Me esters after adding 17.0 as int. standard Identification of 18 1 was done by GC-MS analysis of the bis-(methylthio) Me ester [25] using EI ionization at 70 eV and TLC of Me esters on $AgNO_3$–silica gel

Acknowledgements—Thanks are due to M C Ruiz for skilful technical assistance and to J L Rios for GC/MS analysis This work was supported by a grant from Junta de Andalucía, Spain

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