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Epistatic interaction among loci controlling the palmitic and the stearic acid levels in the seed oil of sunflower

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Abstract Two sunflower (*Helianthus annuus* L.) mutants with high concentrations of saturated fatty acids in their seed oil have been identified and studied extensively. The mutant line CAS-5 has high concentrations of palmitic acid (C16:0) (>25% compared with 7% in standard sunflower seed oil) and low-C18:0 values (3%). CAS-3 is characterized by its high levels of stearic acid (C18:0) (>22% compared with 4% in standard sunflower seed oil) and a low-C16:0 content (5%). CAS-5 also possesses elevated levels of palmitoleic acid (C16:1) (>5%), which is absent in standard sunflower seed oil. The objective of this study was to determine the relationships between the loci controlling the high-C16:0 and the high-C18:0 traits in these mutants. Plants of both mutants were reciprocally crossed. Gas chromatographic analyses of fatty acids from the seed oil of F_1, F_2, F_3 and the BC_1F_1 to CAS-5 generations indicated that the loci controlling the high-C16:0 trait exerted an epistatic effect over the loci responsible for the high-C18:0 character. As a result, the phenotypic combination containing both the high-C16:0 levels of CAS-5 and the high-C18:0 levels of CAS-3 was not possible. However, phenotypes with a saturated fatty acid content of 44% (34.5% C16:0+9.5% C18:0) were identified in the F_3 generation. These are the highest saturated (C16:0 and C18:0) levels reported so far in sunflower seed oil. When F_3 C16:0 segregating generations in both a high- and a low-C18:0 background were compared, the high-C16:1 levels were not expressed as expected in the high-C18:0 background (CAS-3 background). In this case, the C16:1 content decreased to values below 1.5%, compared with >5% in a low-C18:0 background. As the stearoyl-ACP desaturase has been reported to catalyze the desaturation from C16:0-ACP to C16:1-ACP, these results suggested that a decrease in its activity was involved in the accumulation of C18:0 in the high-C18:0 mutant CAS-3.

Key words Sunflower · *Helianthus annuus* · High palmitic acid · High stearic acid · Epistatic interaction · Inheritance · Oil quality

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Introduction

Sunflower (*Helianthus annuus* L.) mutants having either higher levels of palmitic (C16:0) or stearic acid (C18:0) in their seed oil have been induced by mutagenesis (Osorio et al. 1995). CAS-5 has more than 25% C16:0 compared with less than 8% in standard sunflower oil. In addition, CAS-5 exhibits palmitoleic acid (C16:1) in its seed oil (>4%), which is absent in significant proportions in standard sunflower oil. CAS-3 has about 25% C18:0 compared with levels below 5% in commercial cultivars. Increased levels of saturated fatty acids are important in the food industry for the development of plastic fats without harmful chemical processes such as hydrogenation or transesterification (Kritchevsky et al. 1995; Ascherio and Willet 1997).

The C16:0 concentration of CAS-5 is controlled by two alleles at each of three independent loci (*P1*, *P2*, *P3*) with partial dominance for low concentration (Pérez-Vich et al. 1999a). It was concluded that genotypes with a high-C16:0 concentration are homozygous recessive at the *P1* locus (*p1p1*) and either at the *P2* (*p2p2*) or *P3* (*p3p3*) loci.

The C18:0 concentration in CAS-3 is controlled by two alleles at each of two independent loci (*Es1*, *Es2*) with partial dominance for low concentration (Pérez-Vich et al. 1999b). The effect of the *Es1* locus on the C18:0 concentration was greater than that of the *Es2* locus.

The relationships among the loci *P1*, *P2*, and *P3* with *Es1* and *Es2*, and the effect of their combination on the phenotypic expression of the C16:0 and the C18:0 levels are unknown. In other species, there are few studies on the relationship between loci controlling different fatty acids. Nickell et al. (1991) in soybean and Ntiamoah et al. (1995) in flax crossed high- or low-palmitic- with low-linolenic-acid (C18:3) mutants, concluding in both cases that the loci controlling the two traits were independently inherited. Ladd and Knowles (1971) obtained similar conclusions in their study on the interactions of alleles at two loci regulating the stearic and the oleic acid composition in safflower.

The objective of the present research was to study the gene interaction and phenotypic expression among the loci controlling high-C16:0 (CAS-5) and high-C18:0 (CAS-3) concentrations to determine the possibility of combining the two traits.

Materials and methods

Plant material

The lines used in this study were the high-C18:0 mutant CAS-3, obtained after treatment of the line RDF-1–532 with ethylmethane sulphonate, and the high-C16:0 mutant CAS-5, developed from the line BSD-2–691 through mutagenic treatment with X-rays (Osorio et al. 1995). Half-seeds of CAS-3 and CAS-5 were analyzed for fatty acid composition to ensure that the plants used in the crosses had either a high-C18:0 or a high-C16:0 content.

Crossing pattern

Plants of CAS-3 and CAS-5 derived from half-seeds previously analyzed were reciprocally crossed under greenhouse conditions at the Instituto de Agricultura Sostenible (CSIC, Córdoba, Spain) in December 1994. Each head was covered with paper bags to avoid contamination with external pollen. Crossing was achieved through the emasculation of immature flower buds of the female parent followed by pollination of their stigmas with pollen from the male parent. The fatty acid composition of reciprocal F_1 half-seeds from each cross was analyzed by gas-liquid chromatography (GLC). The *t*-test for unpaired observations was used to determine significant differences between the means of reciprocal F_1s. As the results did not reveal maternal effects, the fatty acid composition of segregating generations was analyzed on single half-seeds.

Half-seeds from the F_1 and both parents were germinated in May 1995 and, after 15 days in a growth chamber, the plants were transplanted in the field. F_1 plants were self-pollinated to obtain the F_2 seed. Reciprocal crosses between the two parents were repeated to obtain reciprocal F_1 seeds in the same environment as the F_2 seed. A total of 1010 F_2 seeds were analyzed by GLC. On the basis of the F_2 results, F_3 and BC_1F_1 generations were obtained. F_3 seed was obtained by self-pollinating 20 F_2 plants coming from half-seeds representing all the different F_2 classes, and BC_1F_1 seed by pollinating emasculated CAS-5 plants with pollen from F_1 plants. The study of the F_3 generation was performed through the analysis of 60 half-seeds from each segregating F_2 plant, and of about 12 half-seeds from each non-segregating F_2 plant. For the BC_1F_1 to CAS-5, 329 half-seeds were analyzed.

The C16:0- and C18:0-contents of F_2, BC_1F_1 and F_3 seeds were assigned to phenotypic classes based on the values found for the parentals grown under the same environmental conditions. The observed proportions within each phenotypic class were compared to those expected on the basis of three loci for C16:0 (F_2 genetic ratio 19:38:7, Pérez-Vich et al. 1999a) or two loci for C18:0 (F_2 genetic ratio 1:14:1, Pérez-Vich et al. 1999b). Goodness-of-fit to tested ratios was measured by the chi-square test. The population size where a given genotype is expected to appear was calculated according to Sanchez-Monge and Jouve (1981).

Fatty acid analyses

Fatty acid methyl esters were obtained as described by Garcés and Mancha (1993) and analyzed on a Perkin-Elmer Autosystem gas-liquid chromatograph (Perkin-Elmer Corporation, Norwalk, Conn, USA) with a 2-m-long column packed with 3% SP-2310/2% SP-2300 on Chromosorb WAW (Supelco Inc., Bellefonte, Pa., USA). The oven, injector and flame ionization detector were held at 190, 275 and 2500 C, respectively.

Results

Relationships between the high-C16:0 and the high-C18:0 traits

The fatty acid composition of the seed oil of CAS-3, CAS-5, their reciprocal F_1s, and a sunflower control line with standard fatty acid profile (HA-89), all grown in the same environment, is presented in Table 1. CAS-5 had a high-C16:0 (32%), a high-C16:1 (7.6%), and a low-C18:0 content (1.7%), while CAS-3 had a low-C16:0 (7.7%), no C16:1, and a high-C18:0 content (18%). This C18:0 content was lower than the average value that CAS-3 shows (Osorio et al. 1995), but was in the range described for this mutant under different environmental conditions (Pérez-Vich et al. 1998). Moreover, when CAS-3 half-seeds of this study were self-pollinated, the average C18:0 values of the next generation were about 25%. Therefore, the C18:0 value of 18% found in CAS-3 when the F_1 and the F_2 generations were evaluated was attributed to environmental effects.

Table 1 Fatty acid composition of the seed oil (% of the total oil fatty acids)±standard deviation of CAS-3, CAS-5, their reciprocal F_1s, and a low saturated control (HA-89)

Material	*n*[a]	C16:0	C16:1	C18:0	C18:1	C18:2
Control (HA-89)	11	7.2±0.3		4.0±0.5	18.9±2.6	69.9±2.8
CAS-3	45	7.7±0.6 a[b]		18.0±1.3 a	14.4±1.6 a	59.8±1.9 a
F_1 (CAS-3×CAS-5)	14	9.7±0.6 b	0.4± 0.1 a	7.6±2.5 b	27.8±3.0 b	54.5±5.4 b
F_1 (CAS-5×CAS-3)	5	10.3±0.6 b	0.5± 0.1 a	9.3±2.2 b	21.0±5.3 c	58.9±7.4 a
CAS-5	11	32.0±2.4 c	7.6± 1.5 b	1.7±0.4 c	9.0±0.6 d	47.7±1.7 c

[a] Number of half-seeds analyzed
[b] Means followed by the same letter within each column are not significantly different (based on *t*-tests, *P*=0.05)

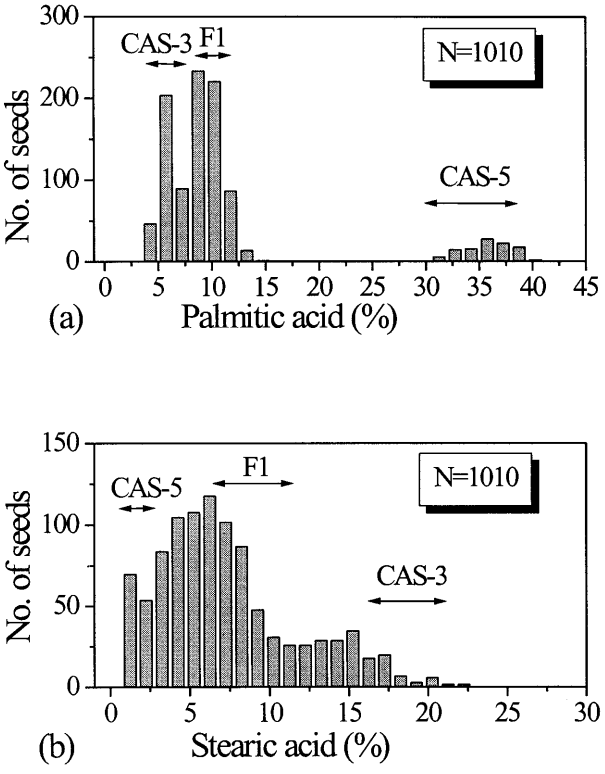

Fig. 1 (**a**) Frequency distribution of the palmitic acid (C16:0) content of F_2 pooled data from the cross between CAS-3 and CAS-5. (**b**) Frequency distribution of the stearic acid (C18:0) content of F_2 pooled data from the cross between CAS-3 and CAS-5. *Arrows* labelled with F_1, CAS-3 and CAS-5 indicate the C16:0 or C18:0 range observed for these seeds in the same environmental conditions as the F_2 generation

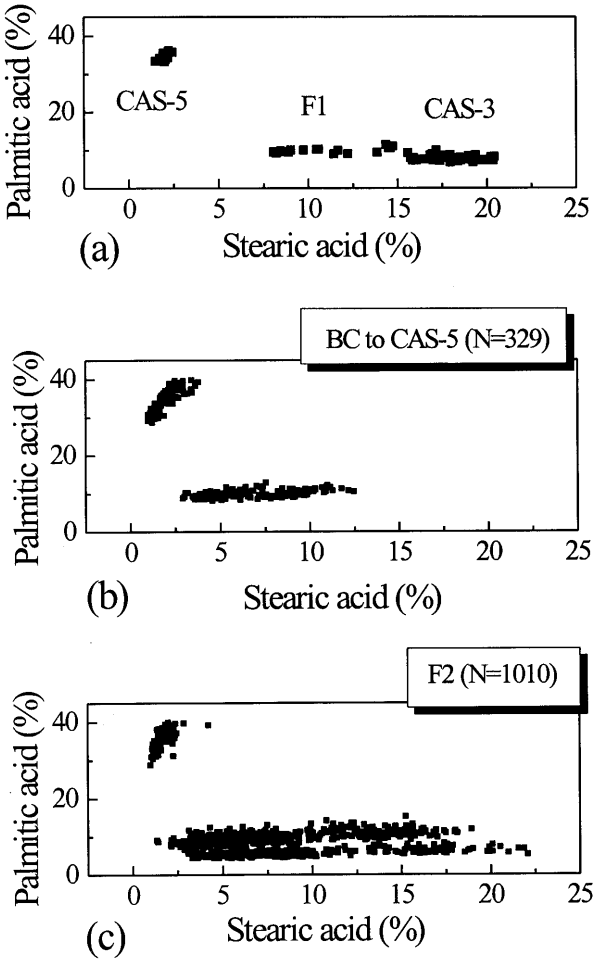

Fig. 2 (**a**) Scatter plot of stearic acid (C18:0) vs palmitic acid (C16:0) in the oil from seeds of the parental lines CAS-3 and CAS-5 and their reciprocal F_1s. (**b**) Scatter plot of C18:0 vs C16:0 in the oil from BC_1F_1 to CAS-5 half-seeds of crosses between CAS-3 and CAS-5. (**c**) Scatter plot of C18:0 vs C16:0 in the oil from F_2 half-seeds of crosses between CAS-3 and CAS-5

The average C16:0 and C18:0 contents of the F_1 half-seeds (10.0% and 8.4%, respectively) were significantly different from both parents (Table 1) and lower than the mid-parent values (19.9% and 9.9%, respectively), indicating a partial dominance of the low over the high levels for both fatty acids. No significant reciprocal differences in the F_1 half-seeds for either the C16:0 or the C18:0 levels were observed (Table 1), suggesting the absence of maternal effects. These results are in agreement with those previously obtained in genetic studies on each of the mutants separately (Pérez-Vich et al. 1999a, b) and also with those reported by Ivanov et al. (1988) for a different sunflower mutant with a high-C16:0 content. Additionally, the F_1 seeds had appreciable amounts of C16:1 (0.45%) (Table 1).

A first evaluation of the distribution of the fatty acid composition in the F_2 seeds was performed by grouping separately the segregations of the high-C16:0 and the high-C18:0 traits. In the six F_2 populations analyzed, the C16:0 content followed a trimodal distribution (Fig. 1a). The three C16:0 classes identified (low: intermediate: high) fitted satisfactorily a 19:38:7 ratio (Table 2), which is in agreement with the genetic model proposed for the inheritance of C16:0 in the mutant CAS-5 (Pérez-Vich et al. 1999a). However, the C18:0 content of the F_2 seeds followed a continuous distribution (Fig. 1b). A previous study reported the presence of two genes controlling the high-C18:0 content in CAS-3 (Pérez-Vich et al. 1999b). According to the class limits defined on the basis of the C18:0 content of both parents grown in the same environment, the proportion of individuals in the low-C18:0 class was significantly higher in all the F_2 families analyzed than that expected for this trait segregating independently (ratio 1:14:1; Pérez-Vich et al. 1999b). Consequently, the observed proportions in the high and intermediate classes were lower than expected (Table 2).

When both fatty acids were evaluated simultaneously in the F_2 seeds, a complete absence of intermediate or high-C18:0 in combination with a high-C16:0 content was observed (Fig. 2c). According to the size of the analyzed population (1010 F_2 seeds), 34.8 seeds for the high-C16:0/intermediate-C18:0 class and 2.3 seeds in the high-C16:0/high-C18:0 class were expected for both traits segregating independently (P=0.05). The lack of

Table 2 Frequency distributions for C16:0 and C18:0 in F_2 and BC_1F_1 to CAS-5 half-seeds of crosses between CAS-3 and CAS-5, and chi-square analyses

Generation	No of F_2 seeds in C16:0 or C18:0 classes[a]						Ratio tested and Chi-square value (P)	
	C16:0			C18:0			C16:0	C18:0
	L	I	H	L	I	H	19:38:7	1:14:1
F_2 generation								
F2–1	23	31	7	14	41	6	2.08 (0.35)	31.53 (<0.01)
F2–4	19	33	9	14	45	2	1.11 (0.57)	29.55 (<0.01)
F2–5	130	272	44	57	364	25	0.71 (0.70)	32.41 (<0.01)
F2–6	15	44	10	14	49	6	2.52 (0.28)	24.70 (<0.01)
F2–7	67	143	26	36	193	7	0.20 (0.90)	35.28 (<0.01)
F2–10	36	86	15	26	102	9	0.81 (0.66)	38.03 (<0.01)
Pooled	290	609	111	161	794	55	0.47 (0.79)	161.48 (<0.01)
Heterogeneity							6.96 (0.75>*P*>0.50)	29.90 (0.001)
BC_1F_1 to CAS-5 generation							5:3	1:3
BC5–1		116	62	74	104		0.60 (0.44)	379.04 (<0.01)
BC5–2		86	65	75	76		1.80 (0.18)	485.82 (<0.01)

[a] The limits between the different classes for each fatty acid are explained in the text, L=Low; I=Intermediate; H=High

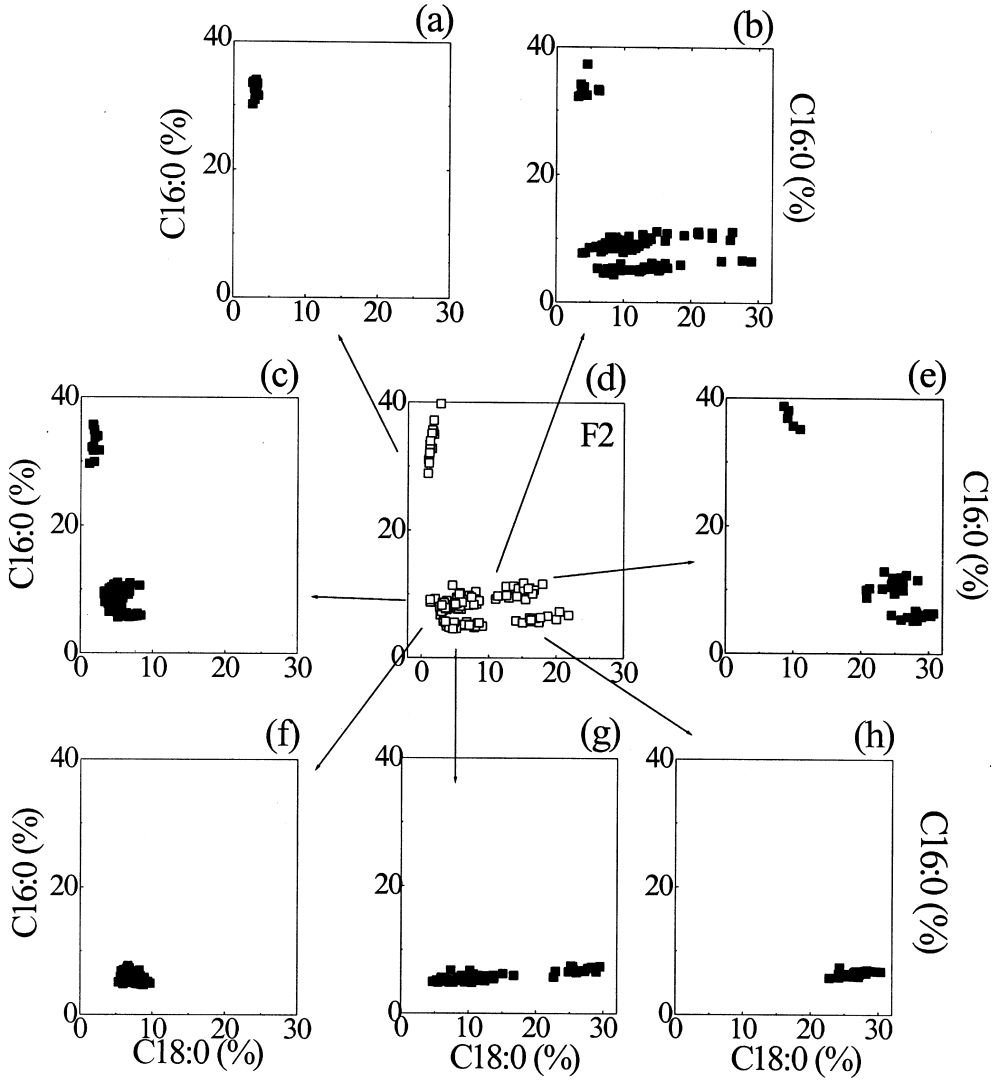

Fig. 3a–h Scatter plots of stearic acid (C18:0) vs palmitic acid (C16:0) in the oil from the F_3 seeds arising from different F_2 classes from crosses between CAS-3 and CAS-5. (**a**) F_3 seeds from the F_2 class high-C16:0/low-C18:0. (**b**) F_3 seeds from the F_2 class intermediate C16:0/intermediate C18:0. (**c**) F_3 seeds from the F_2 class intermediate C16:0/low-C18:0. (**d**) F_2 generation. (**e**) F_3 seeds from the F_2 class intermediate C16:0/high-C18:0. (**f**) F_3 seeds from the F_2 class low-C16:0/low-C18:0. (**g**) F_3 seeds from the F_2 class low-C16:0/intermediate C18:0. (**h**) F_3 seeds from the F_2 class low-C16:0/high-C18:0

intermediate- or high-C18:0 classes in combination with a high-C16:0 content explains the observed distortion in the segregation of C18:0 when the levels of C16:0 were not considered (see above).

This was further observed in the BC_1F_1 to CAS-5 in which seeds with an intermediate-C18:0 and a high-C16:0 content were not present (Fig. 2b). According to the population size (329 BC_1F_1 seeds), more than 11 seeds with this phenotype were expected (P=0.05). In this generation, the evaluation of both fatty acids separately revealed a good fit to the expected genetic ratios for C16:0 but not for C18:0, confirming the observations in the F_2 generation (Table 2).

The progenies of 20 F_2 half-seeds representing all the observed F_2 classes were studied (Fig. 3). The F_3 families derived from F_2 half-seeds with low-C18:0 levels (<4%) and a low-C16:0 content (<7.5%) bred true for low values of both fatty acids (Fig. 3f). Similarly, the F_3 progenies from F_2 seeds with low-C16:0 values and a C18:0 content above 16% or a low-C18:0 and a C16:0 content above 25% bred true for high-C18:0 (Fig. 3h) or high-C16:0 values (Fig. 3a), respectively. The F_3 high-C18:0 seeds (Fig. 3h) recovered the normal values for this fatty acid observed in the mutant CAS-3 (Osorio et al. 1995). In those F_3 populations where only one of the fatty acids was segregating while the other had a low content, the observed data fit satisfactorily the previously reported genetic hypotheses for the C16:0 (Table 3; Fig. 3c), and for the C18:0 classes (Table 3; Fig. 3g). Conversely, when both fatty acids segregated the observed ratios followed a similar pattern as in the F_2 generation (Table 3; Fig. 3b).

The F_3 families from F_2 half-seeds with a high-C18:0 (>16%) and an intermediate-C16:0 content (from 7.5% to 15%) segregated for high-, intermediate-, and low-C16:0 contents in a high-C18:0 background (Fig. 3e; Table 3). The C18:0 values of these F_3 families remained consistently high (>20%) for the genotypes with a low or intermediate C16:0, but those having high-C16:0 concentrations showed a reduction of C18:0 content to levels below 10% (Fig. 3e).

Analysis of the distribution of the C16:1 content in C16:0 F_3 segregating populations from crosses between CAS-3 and CAS-5 in both a low- and a high-C18:0 background

The high-C16:0 mutant CAS-5 also exhibited increased C16:1 levels (7.6%) (Table 1). This fatty acid is absent in the seed oil of CAS-3 and standard sunflower lines (Table 1). The F_1 seeds from crosses between CAS-3 and CAS-5, which had intermediate-C16:0 contents (7.5%–15%), showed appreciable amounts of C16:1 (0.45%) (Table 1), as observed in the F_1 generation from crosses between the mutant CAS-5 and the standard low-saturated inbred line HA-89 (Pérez-Vich et al. 1999a).

The levels of C16:1 were associated with the C16:0 content as suggested by the very high correlation between these fatty acids (r=0.96) in F_3 C16:0 segregating families in a low-C18:0 background from crosses between CAS-3 and CAS-5 (Fig. 3c; Fig. 4a). Moreover, the distribution of the C16:1 content in these families was trimodal (Fig. 4a) with three classes: low (0%), intermediate (0.2–0.6%), and high (>5%). The number of seeds in each class was 20:62:12. This observed ratio satisfactorily fitted the ratio 19:38:7 (χ^2=3.2; P=0.2) described for the F_2 segregation of the high-C16:0 trait in

Table 3 Frequency distributions for C16:0 and C18:0 in F_3 seeds coming from different F_2 classes of crosses between CAS-3 and CAS-5, and chi-square analyses

F_3 family and F_2 class of origin	Fatty acid content (%) from F_2 half-seed of origin			No of F_3 seeds in C16:0 or C18:0 classes						Ratio tested and Chi-square value (P)	
	C16:0	C16:1	C18:0	C16:0[a]			C18:0[b]			C16:0	C18:0
				L	I	H	L	I	H		
Intermediate C16:0/ low-C18:0										19:38:7	
F3–1[c]	8.4	0.5	4.0	23	59	12				1.32 (0.51)	
F3–2	8.2	0.7	3.1	7	14	2				0.11 (0.94)	
Low-C16:0/ intermediate C18:0											1:14:1
F3–5[c]	5.0	–	6.5				6	52	8		5.17 (0.08)
Intermediate C16:0/ intermediate C18:0										19:38:7	1:14:1
F3–7[c]	9.0	0.5	5.5	13	27	7	16	26	5	0.71 (0.70)	65.1 (<0.01)
F3–8	9.1	0.4	6.6	32	53	10	20	68	7	0.73 (0.69)	36.2 (<0.01)
Intermediate C16:0/ high-C18:0										19:38:7	
F3–11	10.0	–	16.0	6	21	4				1.58 (0.44)	
F3–14[c]	13.0	–	18.8	16	26	6				0.54 (0.76)	
F3–16	11.5	–	16.5	14	25	9				3.11 (0.21)	

[a] L=Low class (C16:0<7.5%); I=Intermediate class (7.5%<C16:0<15%); H=High class (C16:0>25%)
[b] L=Low class (C18:0<6%); I=Intermediate class (6%<C18:0<22%); H=High class (C18:0>22%)
[c] Represented in Fig. 3

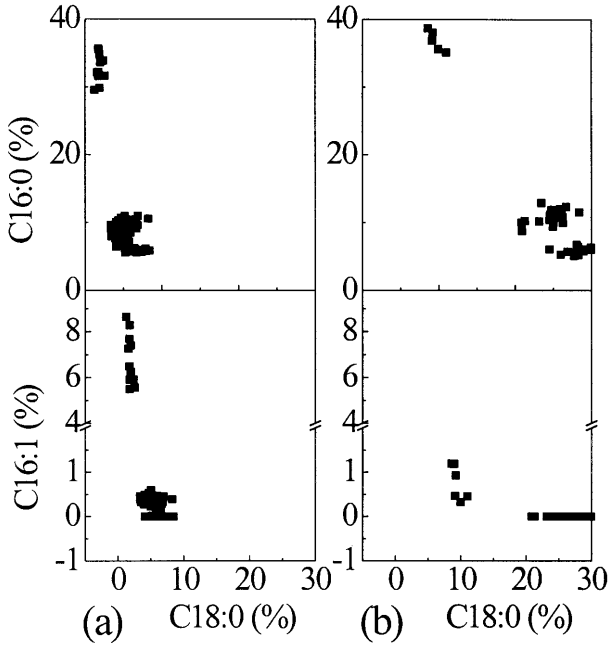

Fig. 4 (**a**) Scatter plot of stearic acid (C18:0) vs palmitic acid (C16:0) and of C18:0 vs palmitoleic acid (C16:1) in the oil from the F_3 seeds segregating for C16:0 in a low-C18:0 background (families of Fig. 3c). (**b**) Scatter plot of C18:0 vs C16:0 and of C18:0 vs C16:1 in the oil from the F_3 seeds segregating for C16:0 in a high-C18:0 background (families of Fig. 3e)

CAS-5 (Pérez-Vich et al. 1999a). In these segregating families, the high-C16:0 values (>25%) were associated with a high-C16:1 content (>5%) (Fig. 4a), as in the mutant line CAS-5. Similarly, the intermediate C16:0 levels (7.5%–15%) were related to an intermediate-C16:1 content (0.2%–0.6%) (Fig. 4a), and the low-C16:0 (<7.5%) seeds had no C16:1 (Fig. 4a).

The analysis of the F_3 generation from crosses between CAS-3 and CAS-5 also indicated the possibility of studying the distribution of the C16:0 and the C16:1 contents in families segregating for C16:0 in a high-C18:0 background. The distribution of the C16:0 levels in such a background has already been shown (Fig. 3e), and that of the C16:1 content was bimodal (Fig. 4b) with two classes (0% and 0.3% to 1.2%). In such a segregation, the F_3 seeds with low- or intermediate-C16:0 and high-C18:0 contents had no C16:1 (Fig. 4b), while the F_3 seeds with high-C16:0 levels (>25%) had C16:1 values below 1.5% (Fig 4b) compared with the C16:1 content of above 4% expected in the seeds with high-C16:0 (>25%) levels, as in the mutant CAS-5. Therefore, C16:1 was not expressed at the expected levels in a high-C18:0 background.

Discussion

The absence of phenotypes having high-C16:0/high-C18:0 or high-C16:0/intermediate C18:0 in the segregating generations F_2 and BC_1F_1 to CAS-5 from the crosses between CAS-3 and CAS-5 indicated that the high levels of both fatty acids were not independently inherited. The results revealed that this was a consequence of a suppressing or masking effect of the high-C16:0 over the high-C18:0 trait. This effect, observed in the F_2 and BC_1F_1 to CAS-5, was confirmed in the F_3 families derived from the F_2 class with an intermediate C16:0/high-C18:0 (Fig. 3e). These F_3 families were expected to be stable for high-C18:0 (genotype *es1es1es2es2*) and to segregate for the C16:0 content. However, the seeds with the highest C16:0 levels (>25%) (genotype *p1p1p2p2__es1es1es2es2* or *p1p1__p3p3es1es1es2es2*) did not express the expected high-C18:0 phenotypic values, showing a C18:0 content below 10%. Therefore, the results suggest that the loci controlling the high-C16:0 trait exert an epistatic effect over the loci responsible for the high-C18:0 content. These loci were not linked, since recombinant genotypes were obtained, although the epistatic effect masked the phenotypic expression of some of these recombinant genotypes.

Current knowledge on fatty acid synthesis in developing seeds (reviewed in Harwood 1996) suggests a preliminary hypothesis to explain the nature of the association between the high-C16:0 and -C18:0 traits. The C16:0-acyl carrier protein (ACP) is a substrate for the enzymes of the fatty acid synthetase complex to be elongated to C18:0-ACP. One of the intra-plastidial enzymes of this complex is the β-ketoacyl-ACP synthetase II (KAS II) which in the main is responsible for the accumulation of C16:0 in the mutant CAS-5 (Martínez-Force et al. 1999). As a consequence of the modified activity of KAS II in CAS-5, there is an accumulation of C16:0-ACP inside the plastid. Also, a higher acyl-ACP thioesterase activity, releasing C16:0 from the C16:0-ACP, was found in this study in the mutant line CAS-5. If this thioesterase activity did not operate properly with the C18:0-ACP then the C18:0 could not be exported efficiently out of the plastid and a high accumulation of C18:0 would not occur.

The considerable decrease of the C16:1 levels in genotypes with high-C16:0 content in a high-C18:0 background (Fig. 4b), compared with the levels of C16:1 in high-C16:0 seeds in a low-C18:0 background (Fig. 4a), can also be interpreted taking into account some biochemical considerations. The enzyme stearoyl-ACP desaturase, which is responsible for the dessaturation from C18:0-ACP to C18:1-ACP, has been reported to catalyze the dessaturation from C16:0-ACP to C16:1-ACP in developing seeds (McKeon and Stumpf 1982; Gibson 1993). The reduction of the C16:1 levels in a high-C18:0 background gives indirect evidence about a possible decrease of the activity of the stearoyl-ACP desaturase in such a background and, in consequence, in the mutant CAS-3. Thus, with the recombination of CAS-3 and CAS-5, the presence of the alleles responsible for the high-C18:0 content not only determine a high accumulation of C18:0, but also a less efficient de-saturation from C16:0 to C16:1. A decreased rate of C18:0-ACP de-saturation due to a modification in the activity of the enzyme

stearoyl-ACP de-saturase has also been suggested to explain the increased C18:0 content in soybean mutants (Ohlorogge et al. 1991), transgenic *Brassica napus* (Knutzon et al. 1992), or in *Arabidopsis* mutants (Lightner et al. 1994).

Previous reports on the genetic relationships between mutant alleles controlling the synthesis of different fatty acids focused on the study of the relationships between the high- or low-C16:0 and the low-C18:3 contents in soybean (Nickell et al. 1991) and flax (Ntiamoah et al. 1995). In both studies, the authors concluded that the traits were independently inherited, with no epistatic interaction or genetic linkage between the loci controlling them, so that a combination of the characters was possible. Similarly, Ladd and Knowles (1971) by crossing high- or intermediate-C18:1 safflower cultivars (genotypes *ololStSt* and *ol*^{1}*ol*^{1}*StSt*, respectively) with a high-C18:0 safflower cultivar (genotype *OlOlstst*) obtained recombinants expressing both characters (genotypes *ololstst* and *ol*^{1}*ol*^{1}*stst*). In *Arabidopsis* too, it was possible to obtain novel seed-lipid phenotypes in combinations of a mutant deficient in C18 elongation (lacking C20:1) with four different mutants with modified fatty acid composition in its seed oil (high-C18:1, high-C18:2, high-C16:0, and high-C18:0, respectively). For all the combinations, the traits were found to segregate independently (James and Dooner 1991).

An additional objective of the present research was to recombine the high-C16:0 and the high-C18:0 traits to develop a sunflower oil with a novel fatty acid profile. Some F_3 half-seeds were identified with 34.5% of C16:0 and 9.6% of C18:0 (Fig. 3e), when F_2 plants derived from F_2 half-seeds with intermediate-C16:0 and high-C18:0 levels were self-pollinated. In addition, these seeds had a lower-C16:1 content than that found in CAS-5 (Fig. 4b). The average saturated fatty acid (C16:0 + C18:0) content in these F_3 seeds was of about 44%. This level represents a further increment with respect to the original mutants CAS-3 and CAS-5 (C16:0 + C18:0<35%). Biochemical and molecular studies are necessary to elucidate the validity of the preliminary hypothesis drawn in this study to explain the observed relationships between CAS-3 and CAS-5. A detailed knowledge of the basis of such relationships will enable the development of alternative strategies to overcome the limitations found in this study to recombine the high saturated fatty acid levels in both mutants.

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