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Inheritance of high palmitic acid content in the seed oil of sunflower mutant CAS-5

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Abstract Sunflower genotypes with increased levels of palmitic acid (C16:0) in the seed oil could be useful for food and industrial applications. The objective of the present study was to determine the inheritance of the high C16:0 content in the sunflower mutant line CAS-5 (>25% of the total oil fatty acids). This mutant was reciprocally crossed with the lines HA-89 (5.7% C16:0) and BSD-2-691 (5.4% C16:0), the latter being the parental line from which CAS-5 was isolated. No maternal effect for the C16:0 content was observed from the analysis of F_1 seeds in any of the crosses. The inheritance study of the C16:0 content in F_1, F_2 and BC_1F_1 seeds from the crosses of CAS-5 with its parental line BSD-2-691 indicated that the segregation fitted a model of two alleles at one locus with partial dominance for the low content. The analysis of the fatty acid composition in the F_2 populations from the crosses with HA-89 revealed a segregation fitting a ratio 19:38:7 for low (<7.5%), middle (7.5–15%), and high (>25%) C16:0 content, respectively. This segregation was explained on the basis of three loci (*P1*, *P2*, *P3*) each having two alleles showing partial dominance for low content. The genotypes with a high C16:0 content were homozygous for the recessive allele *p1* and for at least one of the other two recessive alleles, *p2* or *p3*. This model was further confirmed with the analysis of the F_3 and the BC_1F_1 generations. It was concluded that both the recessive alleles *p2* and *p3* were already present in the BSD-2-691 line, the allele *p1* being the result of a mutation from *P1*. This genetic study will facilitate breeding strategies associated with the incorporation of the high C16:0 trait into agronomically acceptable sunflower hybrids.

Key words *Helianthus annuus* · Sunflower mutant · Palmitic acid · Inheritance · Fatty acid composition

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Introduction

The seed oil of standard cultivated sunflower (*Helianthus annuus* L.) is characterised by a high proportion of oleic (C18:1) and linoleic (C18:2) acid, which together account for about 90% of the total oil fatty acids. The remaining 10% correspond to the saturated fatty acids palmitic (C16:0) and stearic (C18:0) (Dorrell and Vick 1997). The relative proportion of C18:1 and C18:2 is strongly influenced by environmental conditions during seed development (Harris et al. 1978).

The use of mutagenesis permitted the development of sunflower lines with specific fatty acid profiles, such as lines with a high C16:0 content (> 25% of the total fatty acids; Ivanov et al. 1988; Osorio et al. 1995; Fernández-Martínez et al. 1997), lines with a high C18:0 content (about 10%, 15%, or >25% of the total fatty acids; Osorio et al. 1995), and a mutant line with an elevated C18:1 content (>75% of the total fatty acids; Soldatov 1976). These oils with specific fatty acid profiles are in demand because of their improved nutritional and/or technological properties (Kinney 1994). Nevertheless, their usefulness for commercial exploitation will depend on the adequate integration of the genes controlling the altered biosynthetic pathway into inbred lines with a high potential to develop agronomically acceptable hybrids. This requires previous knowledge on how the trait is inherited (Takagi and Rahman 1996).

In general, it has been shown that the fatty acid composition of the seed oil in different oilseed crops is controlled by the genotype of the embryo and is simply inherited (Ohlrogge et al. 1991). Earlier studies in sunflower demonstrated that the high C18:1 trait was controlled by a low number of loci (Miller et al. 1987; Fernández-Martínez et al. 1989). This made the development of hybrids with about 90% C18:1 and a good agronomic performance possible (Fernández-Martínez et al. 1993). The inheritance of the high C16:0 trait in sunflower has not been sufficiently studied to-date. Ivanov et al. (1988) treated it as a quantitative trait. However, studies on high C16:0 mutants of other crops demonstrated that the high C16:0 trait was a qualitative character controlled by one locus (Bubeck et al. 1989; Rahman et al. 1996) or two loci (Fehr et al. 1991) in soybean, and by one locus in flax (Ntiamoah et al. 1995).

The objective of the present study was to determine the inheritance of the high C16:0 mutant CAS-5, with a view to designing efficient strategies to incorporate this trait into commercial hybrids.

Materials and methods

Plant material

The lines used in this study were the high C16:0 mutant line CAS-5, obtained after mutagenic treatment with X-rays (Osorio et al. 1995), its parental line BSD-2-91, and the line HA-89, widely used for the development of commercial hybrids (Fernández-Martínez et al. 1993). The lines BSD-2-691 and HA-89 are characterised by a standard sunflower low saturated fatty composition of the seed oil.

Genetic study

Half-seeds of CAS-5, HA-89 and BSD-2-691 were individually analyzed for fatty acid composition (Conte et al. 1989) to ensure that the plants used in the genetic study bred true for either high or low C16:0 content. The mutant line CAS-5 was reciprocally crossed with its parental line BSD-2-691 in a greenhouse in December 1995. Paper bags were used 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. F_1 half-seeds were analysed by gas-liquid chromatography (GLC). Since the results did not reveal maternal effects for the C16:0 content, the fatty acid composition analysis of segregating generations was performed on single seeds. A total of 15 F_1 plants were transplanted into the field in the Spring of 1996. F_1 plants were selfed and backcrossed to both parents, and reciprocal crosses between the two parents were also made. About 50 seeds from each of two backcrosses, and about 150 F_2 seeds were analysed by GLC.

Plants derived from half-seeds of the mutant line CAS-5 were reciprocally crossed with plants of the line HA-89 in September 1994. The plants were grown in a mesh cage. The fatty acid composition of F_1 half-seeds from each cross was analysed by GLC. The parents and a total of 20 F_1 plants from both reciprocal crosses were grown in the field in the Spring of 1995. F_1 plants were selfed and backcrossed to both parents, and reciprocal crosses between the two parents were made again. About 100 BC_1F_1 half-seeds from four backcrosses, and about 150 F_2 half-seeds from four F_1 plants were analysed by GLC. A total of 25 F_2 half-seeds, representing all the classes for the C16:0 content, were selected and transplanted into the field in the Spring of 1996. A screening on 12 F_3 seeds from each of the 25 F_2 plants was performed to identify the presence or absence of segregation for a high C16:0 content. Up to 90 additional F_3 seeds were analysed from those populations showing segregation for C16:0 content. Twenty four F_3 seeds were also analysed from non-segregating F_3 populations derived from F_2 seeds with a C16:0 content close to the limit between the two classes.

Means were calculated for all characters in the parental and F_1 generations and compared using the *t*-test. The C16:0 content of BC_1F_1, F_2 and F_3 seeds was assigned to phenotypic classes based on the appearance of discontinuities in its frequency distribution. The proportions of seeds observed in each phenotypic class were compared to those expected on the basis of appropriate genetic hypotheses. Goodness of fit to tested ratios was measured by the chi-square statistic.

Fatty acid analyses

Fatty acid methyl esters were obtained as described by Garcés and Mancha (1993) and analysed on a Perkin-Elmer Autosystem gas-liquid chromatograph (Perkin-Elmer Corporation, Norwalk, USA) with a 2-m-long column packed with 3% SP-2310/2% SP-2300 on Chromosorb WAW (Supelco Incorporated, Bellefonte, USA). The oven, injector and flame ionisation detector were held at 190, 275 and 250°C, respectively.

Results and discussion

Crosses between BSD-2-691 and CAS-5

Table 1 shows the fatty acid composition of the seed oil in the mutant line CAS-5, its parental line BSD-2-691, and their reciprocal F_1s. The reciprocal F_1s did not differ significantly for C16:0 content, indicating that the levels of this fatty acid in developing embryos are controlled by the genotype of the embryo and are not affected by the genotype of the maternal parent. The average C16:0 content of the reciprocal F_1s (8.5% and 8.9%) was significantly different from that of both parents BSD-2-691 (5.4%) and CAS-5 (33.2%), and also from the midparent value (19.3%), indicating a partial dominance of low over high C16:0 levels (Table 1 and Fig. 1). These results are in agreement with those reported by Ivanov et al. (1988) for the genetic analysis of the high C16:0 sunflower mutant 275HP.

The C16:0 content of individual half-seeds from the F_2 population showed a trimodal distribution (Fig. 1), with half-seeds having a low (<7.5%), an intermediate (7.5% to 15%) or a high (>25%) C16:0 content. The observed data satisfactorily fitted a 1:2:1 ratio for the three classes (Table 2), which indicated the segregation of alleles at a single locus. This one-gene inheritance was supported by the good fit of the backcross populations to a 1:1 genetic ratio (Fig. 1 and Table 2).

Table 1 Fatty acid composition of the seed oil (% of the total fatty acids) of BSD-2-691, CAS-5, and their reciprocal F_1s. Fatty acids are expressed as a mean value and a standard deviation

Material	n[a]	C16:0	C16:1	C18:0	C18:1	C18:2
BSD-2-691	2	5.4 ± 0.5 a[b]		7.6 ± 0.8 a	16.9 ± 3.3 a	70.0 ± 3.5 a
F_1 (BSD-2-691 × CAS-5)	6	8.5 ± 0.7 b	0.5 ± 0.1 a	7.5 ± 1.0 a	17.4 ± 1.9 a	66.1 ± 2.2 b
F_1 (CAS-5 × BSD-2–691)	2	8.9 ± 0.4 b	0.5 ± 0.1 a	7.3 ± 1.5 a	18.3 ± 3.9 a	65.0 ± 5.0 b
CAS-5[c]	10	33.2 ± 0.9 c	6.3 ± 0.5 b	2.1 ± 0.3 b	8.4 ± 0.6 b	48.0 ± 1.3 c

[a] Number of single-plants analysed. Within each one, about 12 half-seeds were analysed
[b] Values followed by the same letter are not significantly different at the 0.05 probability level based on *t*- tests (except for C16:0 content, at the 0.01 probability level)
[c] This line also showed an average C16:2 content of 1.9 ± 0.2

Table 2 Number of seeds having different C16:0 contents in the analysis of F_2 and BC_1F_1 populations from the crosses between BSD-2-691 and CAS-5

F_2 or BC_1F_1	Number of seeds with % C16:0			Chi-square (*P*) 1:2:1	Chi-square (*P*) 1:1
	<7.5	7.5–15	>25		
F_2 (BSD-2-691 × CAS-5)	38	71	34	0.23 (0.89)	
BC_1F_1 to BSD-2-691	25	23			0.08 (0.77)
BC_1F_1 to CAS-5		28	20		1.33 (0.25)

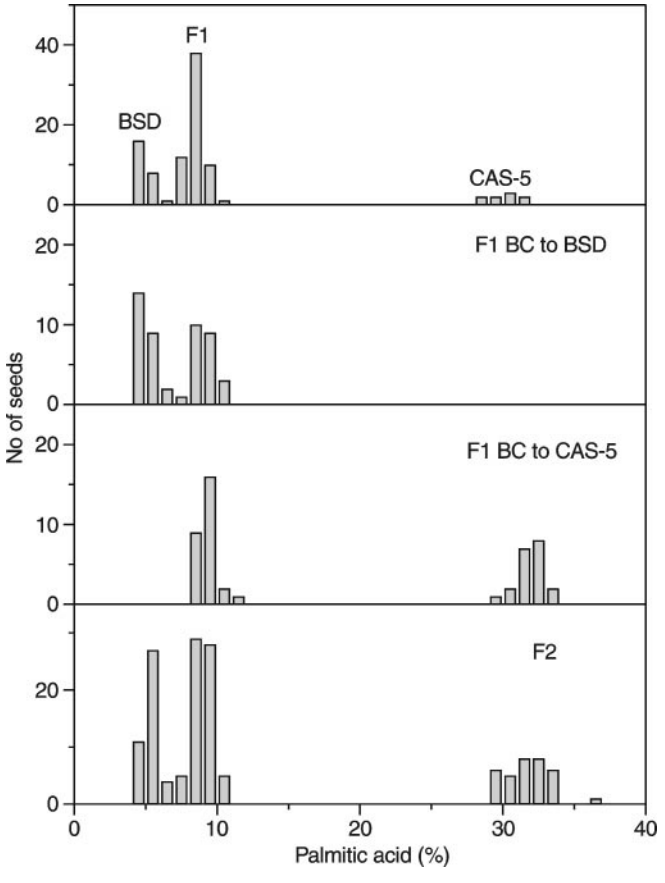

Fig. 1 Frequency distributions of palmitic acid content in individual seeds of BSD-2-691, CAS-5, and their F_1, F_2 and BC_1 populations

Crosses between HA-89 and CAS-5

The analysis of F_1 half-seeds from reciprocal crosses between the mutant line CAS-5 and the standard low C16:0 line HA-89 (Table 3) confirmed the absence of maternal effects in the expression of the C16:0 content and the partial dominance of low over high C16:0 levels, as concluded from the crosses with BSD-2-691. Figure 2 shows the C16:0 content in CAS-5, HA-89 and the F_1 population.

The analysis of F_2 seeds indicated that the C16:0 content segregated into three phenotypic classes: the normal low C16:0 class (<7.5%), the intermediate class (from 7.5% to 15%) and the high C16:0 class (>25%) (Fig. 2). In the four F_2 populations analysed, two from each reciprocal cross, the distribution of C16:0 content fitted a ratio of 19:38:7 for the normal, intermediate and high class, respectively (Table 4). This segregation suggests the presence of three independent loci, which we have called *P1*, *P2* and *P3*, for the control of the high C16:0 trait in the sunflower mutant CAS-5. The genetic model proposed to interpret the 19:38:7 segregation is based on a different behaviour of the allele *p1* as compared with the alleles *p2* and *p3*. Table 5 shows the possible allelic configurations in the F_2 generation and their phenotypic expression. The genotypes with high levels of C16:0 would be homozygous for the recessive allele *p1* and for at least one of the other two recessive alleles *p2* or *p3*. Intermediate levels would be a result of homozygosis for the allele *p1* but not for any of the others, or else

Table 3 Fatty acid composition of the seed oil (% of the total fatty acids) of HA-89, CAS-5, and their reciprocal F_1s. Fatty acids are expressed as a mean value and a standard deviation

Material	n[a]	C16:0	C16:1	C18:0	C18:1	C18:2
HA-89	10	5.7 ± 0.5 a[b]		4.6 ± 0.3 a	26.4 ± 6.5 a	63.2 ± 6.4 a
F_1 (HA-89 × CAS-5)	6	8.7 ± 0.9 b	0.4 ± 0.1 a	4.7 ± 0.8 a	19.4 ± 5.6 b	66.8 ± 5.8 b
F_1 (CAS-5 × HA-89)	2	8.4 ± 0.6 b	0.3 ± 0.1 a	6.2 ± 1.4 b	18.6 ± 2.9 b	66.4 ± 3.6 b
CAS-5[c]	9	30.0 ± 1.1 c	5.0 ± 0.4 b	3.4 ± 0.8 c	8.0 ± 0.3 c	51.6 ± 1.5 c

[a] Number of single-plants analysed. Within each one, about 12 half-seeds were analysed
[b] Values followed by the same letter are not significantly different at the 0.05 probability level, based on *t*- tests
[c] This line also showed an average C16:2 content of 1.8 ± 0.1

Table 4 Number of seeds having a different C16:0 content in the analysis of F_2 and BC_1F_1 populations from the crosses between HA-89 and CAS-5

F_2 or BC_1F_1	Number of seeds with % C16:0			Chi-square (*P*) 19:38:7	Chi-square (*P*) 5:3
	< 7.5	7.5–15	> 25		
F_2 (HA-89 × CAS-5)	38	87	18	0.87 (0.65)	
F_2 (HA-89 × CAS-5)	50	79	15	1.76 (0.40)	
F_2 (CAS-5 × HA-89)	42	79	23	3.84 (0.14)	
F_2 (CAS-5 × HA-89)	52	76	16	3.06 (0.22)	
Pooled	182	321	72	3.16 (0.20)	
Heterogeneity				6.4 ($0.50 > P > 0.25$)	
BC_1F_1 to HA-89	54	41			1.30 (0.25)
BC_1F_1 to HA-89	56	39			0.51 (0.47)
Pooled	110	80			1.72 (0.19)
Heterogeneity					0.09 (0.75)
BC_1F_1 to CAS-5		68	27		3.34 (0.07)
BC_1F_1 to CAS-5		62	30		0.94 (0.33)
Pooled		130	57		4.18 (0.04)
Heterogeneity					0.10 (0.75)

heterozygosis for the allele *p1* together with the presence of at least one dose of the recessive alleles *p2* or *p3*. Any other allelic distribution would result in standard low C16:0 levels. Therefore, the presence of the allele *p1* is indispensable to obtain intermediate or high levels of C16:0. In contrast, only one of the other alleles is essential, and their effects seem to be completely interchangeable.

Two backcrosses to the parent line HA-89 and two backcrosses to the parent line CAS-5 were analysed (Fig. 2). According to this model, a ratio of 5 low to 3 intermediate C16:0 phenotypes is expected in the first case, while a ratio of 5 intermediate to 3 high C16:0 phenotypes is expected in the second case. The results obtained in the four backcrosses fitted satisfactorily with the theoretical ratios (Table 4), supporting the proposed model.

A progeny test was performed for the C16:0 content in order to further confirm the genetic model proposed. A total of 25 F_2 half-seeds from the three observed phenotypic classes were selected and F_2 plants were developed, the F_3 populations being analysed. Table 6 shows the results obtained. All F_3 progenies derived from F_2 half-seeds with a C16:0 content lower than 7.5% showed no segregation for this fatty acid, the C16:0 content of all the F_3 seeds being below 7.5%. Similarly, the F_3 seeds of the F_2 plants coming from F_2 half-seeds with a C16:0 content higher than 25% bred true for values of this fatty acid greater than 25%. In contrast, all the F_3 progenies from F_2 half-seeds from the intermediate class (C16:0 content between 7.5% and 15%) showed segregation for high C16:0 values (>25%), the C16:0 content ranging from <7.5% to >25%. A total of 15 F_3 progenies from this class were analysed for their C16:0 content. Five of them segregated with a 1:2:1 ratio, one was adjusted to a 1:8:7 ratio, five fitted a 7:8:1 ratio, and four were adjusted to a 19:38:7 ratio. These ratios indicate segregation for one, two, two, and three loci, respectively. The segregation 7:8:1 (two loci) would correspond to a genotype heterozygous for the *P1* locus and for one of the other two loci, the third locus being in a homozygous dominant state. The segregation 1:8:7 (two loci) would correspond to a genotype heterozygous for the *P2* and the *P3* loci, the *P1* locus being in homozygous recessive state. These results confirmed the three-loci model proposed to explain the segregation observed in the F_2 generation of crosses between HA-89 and CAS-5.

The different genetic ratios obtained in the segregating generations from crosses HA-89 × CAS-5 and BSD-2–691 × CAS-5 can only be explained if the low C16:0

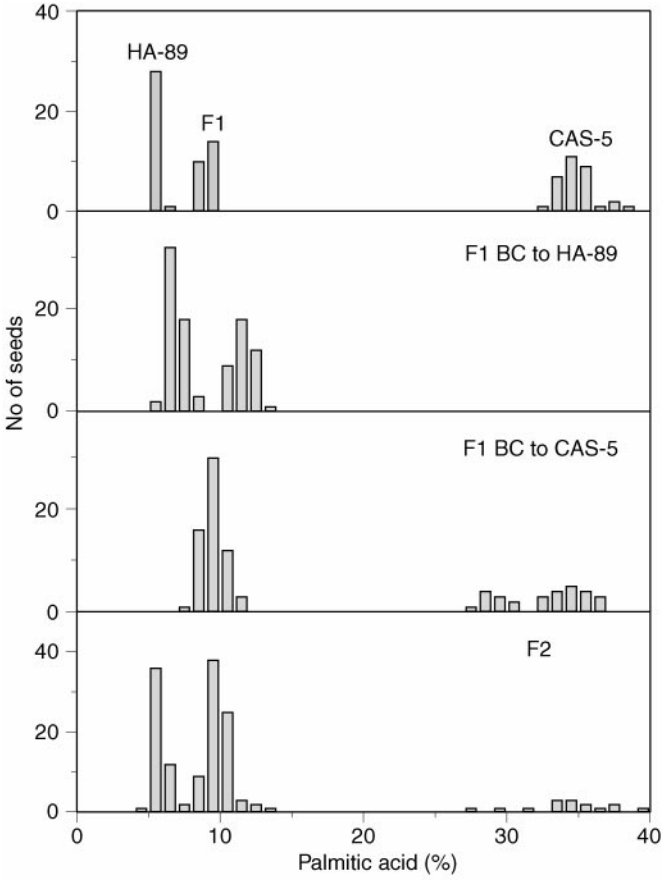

Fig. 2 Frequency distributions of palmitic acid content in individual seeds of HA-89, CAS-5, and their F_1, F_2 and BC_1 populations

Table 5 The C16:0 phenotypic pattern, and the possible genotypes and their frequency in the F_2 generation after crosses between HA-89 and the mutant line CAS-5, based on a model which predicts a 19:38:7 F_2 ratio

C16:0 phenotypic pattern	Proposed F_2 genotypes			Genotypic frequency
Normal (<7.5%)	*P1P1*	*P2P2*	*P3P3*	1
	P1P1	*P2P2*	*P3p3*	2
	P1P1	*P2P2*	*p3p3*	1
	P1P1	*P2p2*	*P3P3*	2
	P1P1	*P2p2*	*P3p3*	4
	P1P1	*P2p2*	*p3p3*	2
	P1P1	*p2p2*	*P3P3*	1
	P1P1	*p2p2*	*P3p3*	2
	P1P1	*p2p2*	*p3p3*	1
	P1p1	*P2P2*	*P3P3*	2
	p1p1	*P2P2*	*P3P3*	1
			Total:	19
Intermediate (7.5–15%)	*P1p1*	*P2p2*	*P3P3*	4
	P1p1	*P2p2*	*P3p3*	8
	P1p1	*P2p2*	*p3p3*	4
	P1p1	*p2p2*	*P3P3*	2
	P1p1	*p2p2*	*P3p3*	4
	P1p1	*p2p2*	*p3p3*	2
	P1p1	*P2P2*	*P3p3*	4
	P1p1	*P2P2*	*p3p3*	2
	p1p1	*P2p2*	*P3P3*	2
	p1p1	*P2P2*	*P3p3*	2
	p1p1	*P2p2*	*P3p3*	4
			Total:	38
High (>25%)	*p1p1*	*p2p2*	*P3P3*	1
	p1p1	*p2p2*	*P3p3*	2
	p1p1	*p2p2*	*p3p3*	1
	p1p1	*P2P2*	*p3p3*	1
	p1p1	*P2p2*	*p3p3*	2
			Total:	7

parental line BSD-2-691 already carried the recessive alleles *p2* and *p3* involved in the control of the high C16:0 content. In this case, crosses with the CAS-5 mutant would only show phenotypic segregation for the *P1* locus. Moreover, if the *p2* and *p3* alleles were already present in the BSD-2-691 line, it would only need the induction of a genetic change in the wild dominant allele *P1*, resulting in the mutant recessive allele *p1*, instead of the highly unlikely double mutation of the two wild alleles *P1* and *P2* or *P1* and *P3* if this were not the case. This hypothesis was consistent with the analysis of the isolation process of the mutant CAS-5, which indicated that it was obtained after a single recessive mutation, being detected in the M_2 generation with a ratio of 3 standard to 1 high C16:0 M_2 seeds (Osorio et al. 1995).

These results indicate that the genetic background of the original parental line may be one of the main factors in obtaining new phenotypes for the fatty acid composition of the seed oil after a mutagenic treatment. Similar results indicating a single-gene control of an altered fatty acid content in crosses between mutant lines and their original parental lines have been reported in soybean for the C16:0 (Erickson et al. 1988; Rahman et al. 1996), C18:0 (Rahman et al. 1997), C18:1 (Takagi and Rahman 1996) and C18:3 (Wilcox and Cavins 1985; Rahman and Takagi 1997) content, and in flax for the C18:3 content (Green 1986).

Because of the low number of genes involved in the genetic control of the high C16:0 trait in the mutant CAS-5, a successful transference of this character into breeding lines can be performed in a few generations. Furthermore, the absence of maternal effects will allow an efficient use of the half-seed technique, which will speed up the selection process. The information provided by this research will facilitate the development of commercial hybrids producing an oil rich in palmitic acid, thus satisfying the increasing demand for vegetable oils with high saturated acid levels for food and industrial products.

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Table 6 Number of seeds having a different C16:0 content in the analysis of 25 F_3 populations from the cross HA-89 × CAS-5

F_3 population	% C16:0 in F_2 half-seed	Average % C16:0 in F_3 seed	No. F_3 seeds with % C16:0			Chi-square (*P*) 1:2:1	Chi-square (*P*) 1:8:7	Chi-square (*P*) 7:8:1	Chi-square (*P*) 19:38:7
			< 7.5%	7.5–15	> 25%				
F3-1	4.8	5.5	12						
F3-2	5.0	6.0	12						
F3-3	5.8	5.2	12						
F3-4	6.6	6.2	12						
F3-5	7.4	6.3	24						
F3-6	7.7	11.2	24	65	19	4.94 (0.08)			
F3-7	8.0	13.4	13	35	13	1.33 (0.51)			
F3-8	8.4	10.4	33	60	13				0.38 (0.83)
F3-9	8.9	15.0	12	31	15	0.59 (0.74)			
F3-10	9.1	9.2	41	57	6			0.96 (0.62)	
F3-11	9.2	15.6	6	37	17		6.24 (0.04)		
F3-12	9.3	11.6	18	28	11	1.74 (0.42)			
F3-13	9.6	9.8	52	76	5			3.33 (0.19)	
F3-14	10.3	9.1	46	60	3			2.60 (0.27)	
F3-15	10.4	10.9	28	66	13				0.69 (0.71)
F3-16	10.7	12.5	32	58	15				1.40 (0.50)
F3-17	10.9	10.8	32	62	14				0.47 (0.79)
F3-18	11.0	13.9	14	33	12	0.97 (0.62)			
F3-19	11.2	10.8	44	56	6			0.35 (0.84)	
F3-20	13.2	10.7	37	66	5			5.34 (0.07)	
F3-21	28.0	28.6			24				
F3-22	33.1	31.5			12				
F3-23	36.7	33.6			12				
F3-24	36.9	36.6			12				
F3-25	37.9	34.2			12				

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