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GENETIC CHARACTERIZATION OF SUNFLOWER MUTANTS WITH HIGH CONTENT OF SATURATED FATTY ACIDS IN SEED OIL

Begoña Pérez-Vich[1*], Rafael Garcés[2], Jose M. Fernández-Martínez[1]

[1]*Instituto de Agricultura Sostenible (CSIC), Apartado 4084, E-14080 Córdoba, Spain*
[2]*Instituto de la Grasa (CSIC), Apartado 1078, E-41080 Sevilla, Spain*

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SUMMARY

The genetic control of the high saturated fatty acid content in sunflower seed oil has been studied in the high palmitic acid (C16:0) mutant line CAS-5, and in the high stearic acid (C18:0) mutant line CAS-3. This review summarizes the pertinent results. The high saturated fatty acid content in sunflower seed oil is controlled by partially recessive alleles at two loci (*Es1, Es2*) for the high C18:0 content and at three loci (*P1, P2, P3*) for the high C16:0 content. The high C16:0 and the high C18:0 traits are not inherited independently. When their combined segregation was studied, the expected phenotypic expression of both high C16:0 and high C18:0 levels was not observed. This fact was attributed to the existence of an epistatic effect of the loci controlling the high C16:0 trait on the loci controlling the high C18:0 trait. The results obtained indicate that sunflower hybrids with a high saturated fatty acid content in their seed oil can be developed and will be in cultivation in a few years.

Key words: sunflower mutants, high saturated fatty acids, inheritance, oil quality

INTRODUCTION

The fatty acid profile of standard commercial sunflower (*Helianthus annuus* L.) seed oil is characterized by a high content of the unsaturated fatty acids, oleic (C18:1) and linoleic (C18:2), which together account for about 90% of the total oil fatty acids (Dorrell and Vick, 1997). The saturated fatty acids, palmitic (C16:0) and stearic (C18:0), are present in smaller amounts, corresponding to the remaining 10%.

New sunflower lines with specific fatty acid profiles in their seed oil are in demand because of their improved technological and/or nutritional properties (Kin-

* Corresponding author

ney, 1994). High C18:1 inbred lines of sunflower have been developed from the high C18:1 variety Pervenets (Soldatov, 1976). They were first released in the 80s (Fernández-Martínez *et al.*, 1987; Miller *et al.*, 1987), leading to a successful commercialization of high-oleic sunflower and high-oleic sunflower oil (Cole *et al.*, 1998). More recently, the saturated fatty acid content of sunflower seed oil has been modified by induced mutagenesis. Mutants having high concentrations of C16:0 (Ivanov *et al.*, 1988; Osorio *et al.*, 1995; Fernández-Martínez *et al.*, 1997), C18:0 (Osorio *et al.*, 1995), or reduced concentrations of either C16:0 or C18:0 (Vick and Miller, 1996) have been developed using chemical or physical mutagenic treatments.

In order to efficiently use the high C16:0 and the high C18:0 mutants in breeding programs aimed to develop commercial cultivars with these characteristics, genetic studies have been carried out on these mutants (Pérez-Vich *et al.*, 1999a, 1999b, 1999c). This review summarizes these works. The most outstanding results on the inheritance of the high C16:0 and the high C18:0 traits in the mutant lines CAS-5 and CAS-3, respectively, and the relationship between these two characters are presented herein.

Genetic characterization of mutants CAS-5 and CAS-3

The genetic analyses of high saturated fatty acid content in sunflower oil have been carried out on the high C16:0 mutant CAS-5 and on the high C18:0 mutant CAS-3 through crosses between these lines and (i) their original parental lines, BSD-2-691 and RDF-1-532, respectively, and (ii) the low saturated inbred line HA-89. The fatty acid composition of half-seeds of these lines is shown in Table 1.

Table 1: Fatty acid composition of the seed oil (% of the total fatty acids) of the mutant lines CAS-5 and CAS-3, their original parental lines, BSD-2-691 and RDF-1-532, respectively, and the low saturated inbred line HA-89

Material	C16:0	C16:1	C18:0	C18:1	C18:2
CAS-5	33.2± 0.9	6.3± 0.5	2.1± 0.3	8.4± 0.6	48.0± 1.3
BSD-2-691	5.4± 0.5		7.6± 0.8	16.9± 3.3	70.0± 3.5
CAS-3	7.3± 0.4		25.0± 1.8	15.0± 2.6	52.6± 2.7
RDF-1-532	8.6± 0.6		8.0± 0.9	23.1± 3.7	60.2± 3.9
HA-89	5.7± 0.5		4.6± 0.3	26.4± 6.5	63.2± 6.4

Type of gene action and effects of the maternal or embryo genotypes

The high C16:0 and C18:0 traits are partially recessive. C16:0 or C18:0 F_1 values of crosses between low saturated (HA-89, RDF-1-532, BSD-2-691) and high saturated (CAS-3 and CAS-5) lines were intermediate between the parents, but showing a shift towards the low saturated parent (Table 2). Almost all seed oil fatty acid induced mutations have been reported to be recessive or partially recessive (review in Velasco *et al.*, 1999). However, there are some exceptions in which dominant mutations have been observed, as for example the high C18:1 character in sunflower (Fernández-Martínez *et al.*, 1989).

The high C16:0 trait is controlled by the genotype of the embryo (not affected by the genotype of the maternal parent). Significant differences between reciprocal F_1s for the C16:0 content were not observed in crosses involving the high C16:0 mutant CAS-5 (Table 2). In contrast, a slight maternal effect in the expression of the high C18:0 character was detected in crosses between HA-89 and CAS-3 (Table 2). It was concluded that the genetic control of the high C18:0 trait was mainly gametophytic, although a partial maternal effect could be present in some crosses involving the high C18:0 mutant line CAS-3. In general, it has been shown that the seed oil fatty acid concentration is controlled by the genotype of the embryo, although significant maternal effects have been observed in some oilseed mutants (Velasco *et al.*, 1999).

Table 2: Fatty acid composition of the seed oil (% of the total oil fatty acids) ± standard deviation of the reciprocal F_1s of crosses involving the mutant lines CAS-5 and CAS-3, their original parental lines BSD-2-691 and RDF-1-532, respectively, and the low saturated inbred line HA-89

Material	C16:0	C16:1	C18:0	C18:1	C18:2
F_1 (BSD-2-691 x CAS-5)	8.5± 0.7 a [a]	0.5± 0.1 a	7.5± 1.0 a	17.4± 1.9 a	66.1± 2.2 a
F_1 (CAS-5 x BSD-2-691)	8.9± 0.4 a	0.5± 0.1 a	7.3± 1.5 a	18.3± 3.9 a	65.0± 5.0 a
F_1 (HA-89 x CAS-5)	8.7± 0.9 a	0.4± 0.1 a	4.7± 0.8 a	19.4± 5.6 a	66.8± 5.8 a
F_1 (CAS-5 x HA-89)	8.4± 0.6 a	0.3± 0.1 a	6.2± 1.4 b	18.6± 2.9 a	66.4± 3.6 a
F_1 (RDF-1-532 x CAS-3)	6.8± 0.7 a		10.5± 1.3 a	22.7± 6.9 a	59.9± 6.4 a
F_1 (CAS-3 x RDF-1-532)	7.8± 1.1 b		10.5± 1.3 a	22.5± 7.8 a	59.1± 7.4 a
F_1 (HA-89 x CAS-3)	6.8± 0.9 a		8.0± 0.9 a	36.1± 8.9 a	49.2± 8.8 a
F_1 (CAS-3 x HA-89)	6.4± 0.6 a		9.7± 1.0 b	28.3± 5.9 a	55.6± 5.2 b
F_1 (CAS-3 x CAS-5)	9.7± 0.6 a	0.4± 0.1 a	7.6± 2.5 a	27.8± 3.0 a	54.5± 5.4 a
F_1 (CAS-5 x CAS-3)	10.3± 0.6 a	0.5± 0.1 a	9.3± 2.2 a	21.0± 5.3 b	58.9± 7.4 b

[a] Means followed by the same letter within each column, and for each reciprocal cross, are not significantly different (based on *t*-tests, $P = 0.05$)

Genetic study of crosses with the original parental lines of the mutants

The distribution of the C16:0 or the C18:0 content in the F_2 generation of crosses between the high saturated mutants CAS-3 and CAS-5 and their original parental lines, RDF-1-532 and BSD-2-691, respectively, was trimodal, showing in both cases three phenotypic classes, low, intermediate and high (Figure 1a for C16:0 and Figure 1b for C18:0). The number of seeds, 40:65:32 (crosses between CAS-3 and RDF-1-532), and 38:71:34 (crosses between CAS-5 and BSD-2-691), observed in each of the three identified F_2 classes, satisfactorily fitted the 1:2:1 ratio indicating a one-gene inheritance for the high C16:0 or the high C18:0 traits (Pérez-Vich *et al.*, 1999a, 1999b). The evaluation of the BC_1F_1 for both parents confirmed these results, as all the backcrosses fitted the 1:1 ratio (Pérez-Vich *et al.*, 1999a, 1999b) for the two classes identified (Figure 1).

Genetic study of crosses with an unrelated low saturated line

Crosses of the mutant lines CAS-5 and CAS-3 with the low saturated inbred line HA-89 showed a more complex inheritance of the high C16:0 and C18:0 traits. The C16:0 content in the F_2 generation from crosses between HA-89 and CAS-5 showed a trimodal distribution, satisfactorily fitting the 19:38:7 genetic ratio for the low (C16:0 < 7.5%), intermediate (from 7.5% to 15%), and high (C16:0 > 25%) C16:0 classes identified, respectively (Pérez-Vich *et al.*, 1999a). The segregation of two backcrosses to HA-89 and two backcrosses to CAS-5 was also studied. The results obtained satisfactorily fitted the ratio of 5 low to 3 intermediate C16:0 phenotypes in the backcrosses to HA-89 and 5 intermediate to 3 high C16:0 phenotypes in the backcrosses to CAS-5 (Pérez-Vich *et al.*, 1999a).

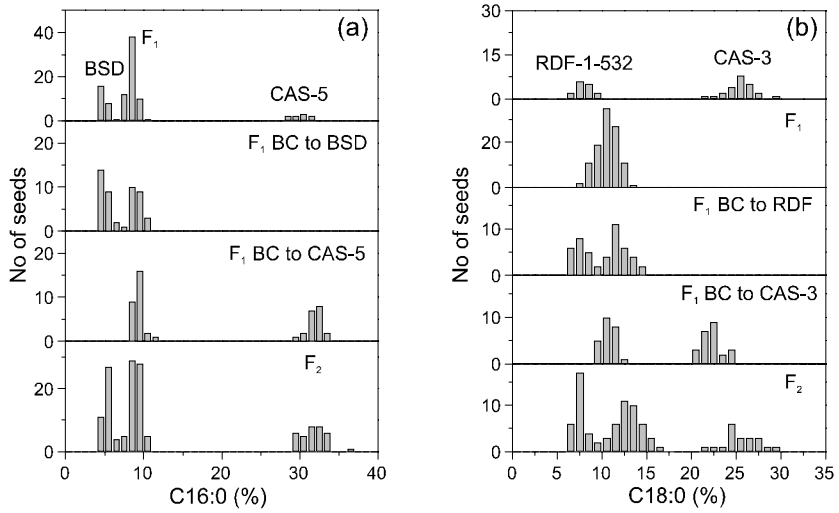

Figure 1: (a) Distribution of palmitic acid (C16:0) content in individual seeds of the parental lines BSD-2-691 and CAS-5, and their F_1, F_2 and BC_1 populations.
(b) Distribution of stearic acid (C18:0) content in individual seeds of the parental lines RDF-1-532 and CAS-3, and their F_1, F_2 and BC_1 populations.

These results were interpreted as the existence of two additional loci as compared with the one locus detected in the crosses between BSD-2-691 and CAS-5. The three loci were designated *P1*, *P2*, and *P3*. The genetic model proposed to explain the inheritance of the high C16:0 trait in the crosses between CAS-5 and HA-89 was based on a different behaviour of the allele *p1* as compared with *p2* and *p3*, in such a way that the genotypes with a high C16:0 phenotype were homozygous for the recessive allele *p1* and for at least one of the other two recessive alleles, *p2* or *p3* (Table 3). In consequence, the alleles *p2* and *p3* lacked any phenotypic expression in the absence of *p1* and showed completely interchangeable phenotypic effects.

Similarly, the crosses between HA-89 and CAS-3 showed a more complex segregation of the high C18:0 trait than that observed in the crosses between RDF-1-532 and CAS-3. The F_2 and BC_1F_1 of both parent populations derived from reciprocal crosses between HA-89 and CAS-3 produced seeds with C18:0 phenotypes low (equal to the parent with the least C18:0 content), intermediate (intermediate to the C18:0 content of the parents), and high (equal to the parent with the greatest C18:0 content). The observed segregation ratio for these classes in the F_2 populations was 1:14:1 (Pérez-Vich *et al.*, 1999b). Segregation in the BC_1F_1s of HA-89 and to CAS-3 generations produced seeds in the low and intermediate, and in the intermediate and high C18:0 classes, respectively. The numbers in each class fitted the 1:3 ratio in the BC_1F_1 of HA-89 and the 3:1 ratio in the BC_1F_1 of CAS-3 (Pérez-Vich *et al.*, 1999b).

Table 3: Possible genotypes, expected frequencies, and expected phenotypes for C16:0 content in the F_2 segregation from crosses between HA-89 and CAS-5

F_2 genotypes	Expected F_2 frequency	Expected phenotype
P1P1 __ __ __ __ [a] __ __ *P2P2 P3P3*	19/64	Normal C16:0 content (<7.5%)
P1p1 p2 __ __ __ *P1p1* __ __ *p3* __ *p1p1 P2 p2 P3P3* *p1p1 P2P2 P3p3* *p1p1 P2p2 P3p3*	38/64	Intermediate C16:0 content (7.5-15%)
p1p1 p2p2 __ __ *p1p1* __ __ *p3p3*	7/64	High C16:0 content (>25%)

[a] The symbol "__ __" indicates that any allelic configuration may occur in that locus.

These results suggested the existence of two major genes responsible for the high C18:0 content in CAS-3. The two loci were named *Es1* and *Es2*. The proposed genotypes (C18:0 content) of the lines CAS-3, RDF-1-532 and HA-89 are shown in Table 4. This model implies that the influence on the C18:0 content of the *es2* allele would be lower than that of the *es1* allele, as demonstrated by the inheritance pattern of the crosses between RDF-1-532 and CAS-3, and between HA-89 and CAS-3, and by the C18:0 levels of the parents involved in each cross.

Table 4: Proposed genotypes of the lines CAS-3, RDF-1-532, and HA-89 for the C18:0 content

Line	Genotype (C18:0 content)	C18:0 phenotype (% of the total oil fatty acids)
CAS-3	*es1es1 es2es2*	22-26%
RDF-1-532	*Es1Es1 es2es2*	8-11%
HA-89	*Es1Es1 Es2Es2*	3-6%

The different genetic ratios obtained in the segregating generations from the crosses of the mutant lines CAS-3 and CAS-5 with their original parental lines (one-gene inheritance for both the high C18:0 and the high C16:0 traits) or with HA-89 (two or three-genes inheritance for the high C18:0 or the high C16:0 content,

respectively) suggested that the original parental lines RDF-1-532 and BSD-2-691 already carried some of the recessive alleles involved in the control of the high C18:0 or the high C16:0 traits (Pérez-Vich *et al.*, 1999a, 1999b). These results indicated that the genetic background of the original parental line may play an important role in obtaining new seed oil fatty acid profiles by induced mutagenesis.

Genetic relationships between CAS-3 and CAS-5

The relationship between the high C16:0 and the high C18:0 traits was studied in crosses between CAS-3 and CAS-5. After the analysis of the F_1, F_2, F_3 and BC_1F_1 generations of CAS-5, it was concluded that the loci controlling the high C16:0 content exerted an epistatic effect on the loci responsible for the high C18:0 character (Pérez-Vich *et al.*, 1999c). As a result, the phenotypic combination containing 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 + C18:0) levels reported so far for sunflower seed oil.

The information provided by these studies will facilitate the successful incorporation of the *P1*, *P2*, *P3*, *Es1*, and *Es2* genes into commercial sunflower hybrids. Sunflower hybrids with high C18:0 content, high C16:0 levels, or with a partial combination of both traits (high C16:0 + intermediate C18:0) will be foreseeable in cultivation in a few years. These hybrids with a high content of saturated fatty acids will have an important impact on the food industry because their oil will permit the production of semi-solid fats without the need of health-detrimental processes such as hydrogenation or transesterification (Álvarez-Ortega *et al.*, 1997). Future work will be conducted to transfer the mutated alleles into varieties with a good agronomic performance and to determine the influence of the mutated alleles on the agronomic performance of the high-yielding varieties, which will allow the selection of the best background for the development of high saturated sunflower hybrids.

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CARACTERIZACION GENETICA DE MUTANTES DE GIRASOL CON ALTO CONTENIDO EN ACIDOS GRASOS SATURADOS

RESUMEN

El control genético del alto contenido de ácidos grasos saturados en el aceite de la semilla de girasol se ha estudiado en la línea mutante CAS-5, con elevados niveles de ácido palmítico (C16:0), y en la línea mutante CAS-3, con alto contenido en ácido esteárico (C18:0). Esta revisión presenta los resultados obtenidos mas relevantes. El alto contenido de ácidos grasos saturados en el aceite de la semilla de girasol está controlado por alelos parcialmente recesivos en dos loci (*Es1, Es2*) para el alto contenido en C18:0 o en tres loci (*P1, P2, P3*) para los elevados niveles de C16:0. Los elevados niveles de C16:0 y de C18:0 no se heredan independientemente. La combinación de las líneas mutantes CAS-3 y CAS-5 no presentó la expresión fenotípica esperada de contenidos altos en ambos ácidos grasos. Esta observación se atribuyó a la existencia de un efecto epistático de los loci que controlan el carácter C16:0 sobre los loci responsables del carácter alto C18:0. Los resultados obtenidos indican que en pocos años se pueden desarrollar y entrar en cultivo híbridos de girasol con alto contenido de ácidos grasos saturados en su aceite.

CARACTÉRISATION GÉNÉTIQUE DES MUTANTS DE TOURNESOL À HAUT CONTENU D'ACIDES GRAS SATURÉS DANS L'HUILE

RÉSUMÉ

Le contrôle génétique du haut contenu d'acides gras saturés dans l'huile de la graine de tournesol a été observé dans la ligne mutante CAS-5, qui possède un haut contenu d'acide palmitique (C16:0) et dans la ligne mutante CAS-3 qui possède un haut contenu d'acide stéarique (C18:0). Cet article résume les résultats les plus significatifs. Un haut niveau d'acides gras saturés dans l'huile de la semence de tournesol se trouve sous contrôle d'allèles partiellement récessifs en deux locus (*ES1, ES2)* pour un haut contenu C18:0 ou en trois locus (*P1, P2, P3)* pour un haut niveau C16:0. Les hauts contenus C16:0 et C18:0 ne se succèdent pas indépendamment. Quand leur ségrégation combinée a été examinée, l'expression phénotypique attendue de hauts niveaux C16:0 et C18:0 n'a pas été constatée. Ce fait s'explique par la présence de l'effet d'épistasie des locus qui contrôlent le haut contenu C16:0 sur les locus qui contrôlent le haut niveau C18:0. Les résultats obtenus montrent qu'il est possible de créer des hybrides de tournesol à haut contenu d'acides gras saturés dans l'huile. Leur introduction dans la production est attendue d'ici quelques années.