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Increase of the Stearic Acid Content in High-Oleic Sunflower (*Helianthus annuus*) Seeds

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We have performed an "in vivo" study of storage lipid synthesis in developing sunflower seeds, from several high-oleic genetic backgrounds, using radioactive acetate in conjunction with methyl viologen as an inhibitor of the stearoyl-ACP desaturase. As such, some backgrounds showed stronger acyl-ACP thioesterase activity on stearoyl-ACP. We have developed a saturation coefficient that quantifies stearoyl-ACP thioesterase activity among sunflower lines based on their ability to synthesize saturated fatty acids under conditions when the competing stearoyl-ACP desaturase is inhibited by methyl viologen. The saturation coefficient is defined as the ratio of sum of the stearic, araquidic, and behenic saturated fatty acid contents to the unsaturated fatty acid content. On the basis of this coefficient, we were able to select high-oleic lines that, when crossed with the high-stearic CAS-3 line, developed progeny with high-stearic content on a high-oleic background. This approach has enabled us to identify lines with a combination of alleles that synthesized oils with more stearic acid in a high-oleic background, 21% stearic and 62% oleic contents. In contrast, lines with a lower index produced progeny that contained less stearic acid, similar to those obtained previously, that were 13% stearic acid content in high-oleic background. This method could also be used for other metabolic pathways where the blockage of a principal pathway may activate a secondary pathway. However, it should be emphasized that although the stearic acid content could be augmented it was not possible to break the association or the epistatic relationship that exists between the genes that permit a high-stearic phenotype and those that determine a high-oleic background.

KEYWORDS: ***Helianthus annuus*; asteraceae; sunflower; high-stearic; fatty acid synthesis; inhibitor; methyl viologen; acyl-ACP thioesterase**

INTRODUCTION

In oil seeds, the synthesis of fatty acids up to oleic acid is carried out within organelles known as plastids. In these organelles, a collection of enzymes designated as fatty acid synthase (FAS) successively lengthens the acyl skeleton by two carbons until forming palmitoyl-ACP and stearoyl-ACP, the latter being desaturated by the stearoyl-ACP desaturase (SAD) to form oleoyl-ACP. These acyl-ACPs must be hydrolyzed by the acyl-ACP thioesterases to exit into the cytoplasm and be used in the synthesis of triacylglycerols (TAG). Among these thioesterases, there are those that display greater activity on oleoyl-ACP and others that are more specific to saturated acyl-ACPs designated FatA and FatB, respectively (*1*). Once outside the plastid, oleic acid can be desaturated to linoleic, while on the other hand the stearic acid may be elongated to long chain fatty acids (VLCFA) such as arachidic and behenic acids. From these fatty acids, complex lipids can be synthesized such as phospholipids and glycerolipids (*2*).

Sunflower mutants have been obtained in which the fatty acid composition of their oil has been modified (*3*). Among these mutants, various lines with a high-stearic or high-palmitic phenotype exist. The genetic control in these lines has been studied, demonstrating that at least two genes are necessary to control the high-stearic characteristic (*4*). Moreover, the biochemical characterization of the high-palmitic and high-stearic lines has highlighted the need for at least two modifications to obtain a high saturated phenotype. On the one hand, this involves a blockage in the main pathway at the level of FAS II in the case of the high-palmitic lines or of SAD in the case of the high-stearic lines. This must be coupled with a good acyl-ACP thioesterase activity for saturated fatty acids that permits their export from the plastid after hydrolysis (*5*, *6*). Other secondary pathways have also been activated in some oilseeds that have been modified to obtain a higher proportion of saturated fatty acids, possibly due to insufficient thioesterase activity. For example, in rapeseed the blockage of the principal pathway using antisense mRNA against the SAD increases the intraplastidial content of stearoyl-ACP, which was elongated to arachidic, behenic, and lignoceric acids in the interior of the plastid, possibly through FAS II activity (*7*). Furthermore, in the high-palmitic sunflower mutants, the palmitic acid is not exported from the plastid with sufficient velocity, and hence, through SAD desaturation and elongation by FAS II, palmitoleic and

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asclepic acids are produced (*5*). This does not occur in the high-stearic mutants of sunflower, where the accumulation of stearic acid does not provoke the appearance of any secondary pathway. This would appear to indicate that the activity of the thioesterases for stearic acid in these mutants is sufficient to export the stearoyl-ACP that accumulates within the plastid.

In the case of the high-palmitic mutants, the content of this fatty acid is independent of the genetic background. Indeed, both on a standard linoleic background as well as on the high-oleic background the levels of palmitic acid are high, and they are similar in both cases (*3*). However, high-stearic mutants cannot be obtained on a high-oleic background with a stearic acid content similar to that of the standard genetic background. Only intermediate stearic content (13%) in this background could be obtained, possibly due to some association between the genes that control both phenotypes (*8*, *9*). In fact, in the sunflower, the genes of the SAD, the FatA type acyl-ACP thioesterases, and the oleate desaturase have been located nearby on the same chromosome, LG1 (*10*). If the gene responsible for one of the acyl-ACP thioesterase activities is close to that which controls the high-oleic characteristic, it could make it very difficult to recombine them and to obtain lines with both phenotypes. Indeed, biochemical characterization of the highly saturated mutants demonstrated deficient thioesterase activity against saturated fatty acids in the standard high-oleic sunflower lines (*5*). Furthermore, although a variety of these thioesterases exist, there has been a selection in favor of alleles that act weakly on saturated fatty acids during the selection of high-oleic lines in which values of up to 90% of oleic acid can be found (*11*). Thus, to maximize the possible increase in stearic content, the thioesterase alleles that present the greatest activity on saturated fatty acids in high-oleic lines must be identified, and most specifically those that act on stearic acid. This effect appears to occur in the majority of oilseeds in which the stearic content is always less on a high-oleic background (*12*). One exception to this can be seen in cotton where seeds with more than 30% of stearic acid have been obtained both on a standard as well as in a high-oleic genetic background, although they suffer certain problems in germination (*13*). Again, the thioesterases could be responsible for this behavior given that, in cotton, a considerable quantity of palmitic acid is normally accumulated in the seed oil, and thus their thioesterases may have an adequate specificity for saturated fatty acids.

In theory, it is possible to select high-oleic lines with good thioesterase activity for certain saturated fatty acids by determining thioesterase activity (*5*). However, this is a time-consuming process and the levels of activity can depend on the physiological age of the seed, as well as on the moment of the day in which the samples are obtained. Furthermore, it must be taken into account that the acyl-ACP that is the substrate of this activity must be synthesized and purified in the laboratory. However, the presence of these better thioesterases could be assayed by blocking the main pathway in their synthesis "in vivo" with an inhibitor and thereafter determining the activity of the thioesterases in such lines. Thus, it should be possible to select the best lines to be crossed with mutants in which the main SAD pathway is blocked, such as the CAS-3 high-stearic sunflower mutant line for example. It has been demonstrated that methyl viologen (MV) inhibits the conversion of stearoyl-ACP to oleoyl-ACP (*14*), which accumulates in the interior of the plastid. Thus, if a sunflower line has good thioesterase activity against stearic, the stearic acid could be exported to the cytoplasm more efficiently, and it would more readily be found in the reserve lipids than in a line with thioesterase that acts weakly on saturated fatty acids.

In this Article, we have characterized high-oleic lines on different genetic backgrounds by incubating them "in vivo" with radioactive acetate and the inhibitor methyl viologen. Lines were identified that displayed good and poor stearate accumulation under these conditions, and both types of lines were crossed with the high-stearic CAS-3 mutant. The analysis of the fatty acid composition of the storage oil in the ensuing generations enabled us to test the hypothesis that lines could be selected with a good capacity to express the high-stearic characteristic in a high-oleic background through incubation with radioactive acetate at the same time as blocking the principal pathway.

MATERIALS AND METHODS

Plant Material and Growth Conditions. In this study, we have used developing seeds from sunflower (*Helianthus annuus* L.) plants on different genetic backgrounds that produce oil with high-oleic content. As a control line, we used the high-oleic line HAOL-9 (*15*). Unless otherwise indicated, plants were cultivated in growth chambers at 25/15 °C (day/night), with a 16 h photoperiod and a photon flux density of 300 μmol m^{-2} s^{-1}. Around 1 h after sunrise on the 15th day after flowering (DAF), achenes were harvested from the external seed rings of the capitulum. Each experiment was repeated three times, a different capitulum being analyzed in each replicate.

Incubation with Sodium [2-^{14}C]Acetate. Achenes were collected from the capitulum immediately prior to incubating them with [2-^{14}C]-acetate, and 5−6 seeds (achenes without hull and seed coat) were used for each assay. The fresh weight was recorded, the seeds were sliced into three equal-sized parts, and they were placed into a 10 mL glass tube. The incubation with radioactive acetate was carried out (*16*), with some modifications. The seeds were incubated in 300 μL of 50 mM MES buffer pH 6.0 (2-[*N*-morpholino] ethanesulphonic acid; Sigma-Aldrich, Steinheim, Germany) in glass tubes, and the assay was initiated by adding 185 kBq of sodium [2-^{14}C]acetate in 0.3 mM MES buffer pH 6.0 in a final volume of 500 μL (specific activity 2.11 GBq/mmol; Amersham-Pharmacia, Buckinghamshire, UK). The seeds were incubated with agitation at 20 °C for 4 h, and the assays were terminated by immersing the tubes in a water bath at 80 °C before the seeds were washed three times in water. The inhibitor methyl viologen (Sigma-Aldrich, Steinheim, Germany) was dissolved in 50 mM MES buffer pH 6.0 and was added just before the sodium [2-^{14}C]acetate solution.

Lipid Analysis. After the seeds were ground with a pestle and sand in a screw-cap glass tube (10 × 13 mm), the lipids were extracted (*17*). The total lipids were dissolved in 1 mL of hexane:isopropanol (7:2; by vol.), and the radioactivity incorporated to the lipid fraction was measured in a scintillation counter (Rackbeta II; LKB, Sweden). The total lipid fraction was separated by TLC (Merck, Darmstadt, Germany), the plates were developed with hexane:ether:formic acid (75:25:1; by vol.), and lipids separated into TAG, diacylglycerol, free fatty acid, wax, and polar lipid fractions. Fatty acid methyl esters were prepared by treating the lipid sample with 3 mL of methanol:toluene:H_2SO_4 (88:10:2; by vol.) for 1 h at 80 °C (*18*), and, after cooling, fatty acid methyl esters were extracted twice with 2 mL of heptane. To isolate the fatty acids on the basis of chain length, methyl esters were separated by reversed-phase TLC on silica gel plates previously coated with 2.5% vaseline oil (Panreac, Barcelona, Spain) in hexane, using acetonitrile:hexane (90:10; by vol.) as the solvent. Palmitic and oleic acid methyl esters have the same R_f in these plates, and they were scraped off and separated on $AgNO_3$-TLC plates (1:9 by wt in acetonitrile) using hexane:ether (85:15; by vol.) as the mobile phase. The different radioactive lipid classes and fatty acid methyl esters were detected and quantified on the TLC plates using a two-dimensional autoradiography scanner (Instant Imager, Packard, Canberra), and they were identified by comparison with known standards.

The fatty acid composition of various sunflower lines was determined by GC of the methyl esters with an Agilent 6890 gas chromatograph using an SP-2380 column of fused silica (30 m length; 0.25 mm i.d.; 0.20 μm film thickness: Supelco, Bellefonte, PA). Hydrogen was used

Table 1. Fatty Acid Composition of Seeds from the Different High-Oleic Sunflower Lines Employed in This Study[a]

	fatty acid composition (mol %)			
line	palmitic	stearic	oleic	linoleic
HAOL-9	3.7 ± 0.2	3.5 ± 1.2	89.1 ± 1.2	2.1 ± 0.3
BK-65	3.5 ± 0.4	8.0 ± 2.9	82.9 ± 4.9	3.0 ± 0.8
BK-73	3.5 ± 0.2	7.6 ± 0.9	83.6 ± 1.0	2.9 ± 0.4
BL-41	3.4 ± 0.4	7.1 ± 1.3	84.7 ± 2.5	2.8 ± 1.0
BL-45	3.9 ± 0.1	11.7 ± 1.1	76.7 ± 0.3	4.4 ± 1.1
BP-72	3.0 ± 0.2	5.7 ± 1.0	87.2 ± 1.4	2.8 ± 0.7
BP-80	4.4 ± 0.6	3.5 ± 0.4	79.9 ± 6.7	10.4 ± 5.7

[a] The data are the mean of at least 12 determinations, with their respective standard deviations.

as the carrier gas at 28 cm s^{-1}, and while the detector and injector temperatures were 200 °C, the oven temperature was 170 °C. Different methyl esters were identified by comparison with known standards (Sigma, S. Louis, MI).

Calculation of Saturation Coefficient. The coefficient of saturation was calculated from data obtained by the two-dimensional autoradiography TLC scanner; see above. The coefficient was calculated by dividing the percentage of radioactivity found in stearic acid plus VLCFAs by that found in unsaturated fatty acids in triacylglycerols.

Sexual Crosses. Taking into consideration that there is no maternal effect in inheriting the high-stearic characteristic and that the reciprocal crosses are equivalent (*4*), the crosses were carried out by exposing the female high-stearic mutant to pollen from the high-oleic plants. The F_1 seeds generated by the cross are high-oleic because this characteristic is dominant (*19*).

RESULTS AND DISCUSSION

Selection of the Lines. Our initial aim was to determine whether it is possible to identify lines with alleles whose main acyl-ACP thioesterase acts predominantly on the stearoyl-ACP after incubating developing sunflower seeds with radioactive acetate as the substrate and methyl viologen (MV) to inhibit the SAD (*14*). A series of high-oleic sunflower lines were analyzed in this way. The fatty acid composition of these lines is shown in **Table 1**. These plants were grown and the seeds were incubated with radioactive acetate at 15 DAF in the presence of distinct concentrations of MV (0−20 mM). On blocking desaturation of stearic acid in the presence of MV, this fatty acid accumulates within the plastid, and, depending on the thioesterases present in each line, the stearoyl-ACP may be more or less efficiently hydrolyzed to stearic acid. Once outside the plastid, stearic acid could be used in the synthesis of TAGs, which represent more than 95% of the lipids synthesized in developing sunflower seeds. Part of this stearic acid will also be elongated to the very long chain fatty acids (VLCFA) arachidic and behenic. As such, the content of radiolabelled stearic acid within the TAG fraction reflects the capacity of the thioesterase system to hydrolyze this fatty acid. To quantify this capacity, we defined the saturation coefficient as the relationship between the radiolabel in TAGs that contain the products of this blockage, including both the stearic acid and the increment in the VLCFAs synthesized from it, and the radioactivity present in the products of unsaturation (**Figure 1**). We found that the relative incorporation into TAG was maximal in the presence of 10 mM MV (*14*). Moreover, in the presence of 10 mM MV, two well-defined and distinct behaviors were observed in the high-oleic lines assayed: one with a coefficient greater than 20 and others with a coefficient of less than 10 (**Figure 1**). Hence, 10 mM MV was chosen for use in this assay to test our hypothesis and thus to select the lines with the thioesterases most active on stearic acid. From these data, we selected two plants with a high coefficient that according to our hypothesis must contain thioesterases highly effective with stearoyl-ACP. Likewise, two plants with a low coefficient were also selected that probably do not have good thioesterase activity with stearoyl-ACP. Hence, we chose the high-oleic BP-72 and BK-73 lines that had a coefficient greater than 20 at a concentration of 10 mM MV, and the BP-80 and BL-41 lines that had a low coefficient. At all of the concentrations of MV assayed, the high-oleic control line HAOL-9 had one of the lowest coefficients observed. This data are in accordance with previous results (*8*), where a poor stearic content was obtained in a high-oleic background on crossing the high-oleic line (HA-OL-9) with the mutant high-stearic CAS-3.

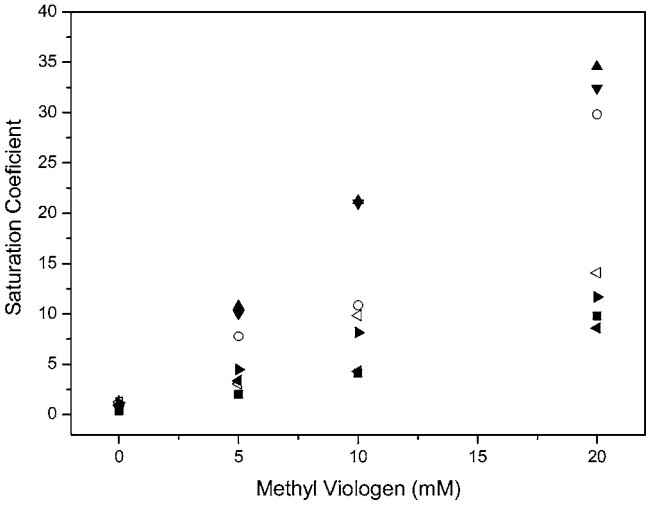

Figure 1. Coefficient of saturation in triacylglycerols expressed as the percentage of radioactivity in stearic acid plus VLCFAs divided by that found in unsaturated fatty acids as a function of the concentration of the inhibitor methyl viologen (MV). The high-oleic sunflower lines assayed at 15 DAF were: BL-41 (left-facing open triangle), BL-45 (right-facing closed triangle), BK-65 (○), BP-72 (▼), BK-73 (▲), BP-80 (left-facing closed triangle), and the control high-oleic HAOL-9 (■). The data are the mean of three different plants with at least two repetitions of each plant.

Table 2. Fatty Acid Composition of Seeds from High-Oleic Plant Donors of Pollen (BP-80, BP-72, BK-73, and BL-41), Mutant CAS-3 Plants That Are Receptors of the Pollen, and F_1 Generation Obtained after the Crosses[a]

	fatty acid composition (mol %)					
	palmitic	stearic	oleic	linoleic	arachidic	behenic
			Parental			
CAS-3	6.8 ± 0.4	24.9 ± 0.7	14.0 ± 3.3	51.6 ± 3.0	1.4 ± 0.2	1.4 ± 0.2
BL-41	4.7 ± 0.5	8.9 ± 1.4	81.8 ± 2.1	1.9 ± 0.6	0.8 ± 0.1	1.9 ± 0.3
BK-73	4.5 ± 0.4	8.9 ± 2.0	81.6 ± 2.5	2.4 ± 1.1	0.8 ± 0.2	1.8 ± 0.3
BP-72	4.2 ± 0.3	4.6 ± 0.7	88.1 ± 1.5	1.6 ± 0.8	0.4 ± 0.1	1.2 ± 0.2
BP-80	5.7 ± 0.7	2.6 ± 0.5	86.0 ± 2.0	4.0 ± 1.2	0.5 ± 0.1	1.3 ± 0.2
			F_1			
BL-41 × CAS-3	4.5 ± 0.3	13.0 ± 1.6	77.7 ± 2.0	1.5 ± 0.3	1.0 ± 0.1	2.2 ± 0.1
BK-73 × CAS-3	4.3 ± 0.8	10.9 ± 1.8	80.2 ± 2.7	2.0 ± 1.0	0.9 ± 0.1	1.8 ± 0.3
BP-72 × CAS-3	4.8 ± 0.5	12.2 ± 1.1	77.3 ± 2.1	2.3 ± 1.1	1.0 ± 0.1	2.3 ± 0.4
BP-80 × CAS-3	4.6 ± 0.7	6.7 ± 1.6	82.2 ± 5.5	4.4 ± 4.3	0.7 ± 0.2	1.4 ± 0.3

[a] The data are the mean of at least 12 determinations, with their respective standard deviations.

Crosses. The selected high-oleic lines were crossed with the high-stearic line CAS-3 and the fatty acids in F_1 seeds analyzed. The stearic content of the F_1 half seeds was significantly different from that of the parental CAS-3 line (**Table 2**) or that in the high-oleic parental line ($P < 0.05$). Indeed, the stearic

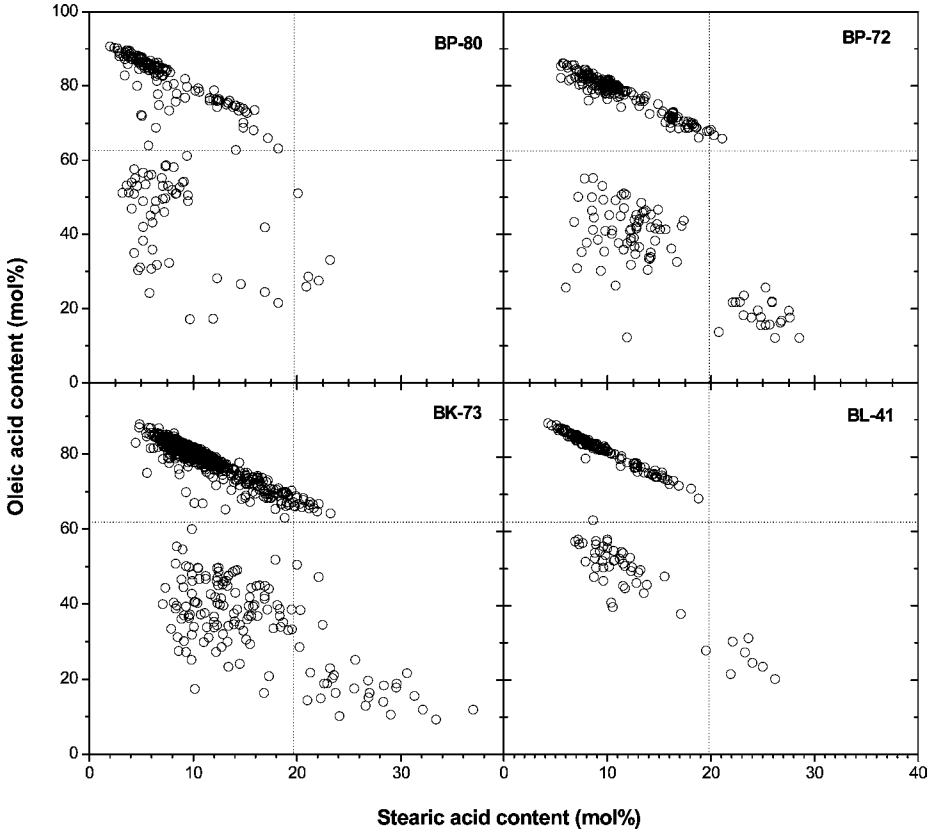

Figure 2. Representation of the stearic acid content versus the oleic acid content in seeds from F_2 plants grown from the F_1 seeds of the cross between CAS-3 and the four parental high-oleic lines.

content fell between the values of the two parental lines, although the fact that this value laid closer to the low stearic parental line indicated the partial dominance of the low stearic character. Similarly, the content of oleic acid in the F_1 generation with respect to their respective high-oleic parental lines was significantly different ($P < 0.05$; **Table 2**). The data obtained were similar to previous results described (*8*) when CAS-3 plants were crossed with the high-oleic mutant HAOL-9. In the study with HA-OL9, only small differences were described between the oleic content of the half seeds from the F_1 plants and the parental HAOL-9, all of which confirms that the crosses had been produced and that the high-oleic character had been inherited with absolute dominance over the low-oleic content, as proposed previously (*8*, *19*).

The F_1 seeds were grown and the plants were self-fertilized to obtain the F_2 generation (**Figure 2**), which was seen to behave distinctly. In the F_2 seeds from the BP-80 and BL-41 lines, none of the half seeds contained more than 20% stearic on a high-oleic background (>62% of oleic), although such levels could be found in the F_2 segregations of the crosses with the BP-72 and BK-73 lines. In previous studies, no seeds were found with more than 20% of stearic in a high-oleic background (*8*). It is also important to note that in the segregation of the BP-72 and BK-73 crosses, high-stearic and high-linoleic (low-oleic) seeds could be found with approximately 30% stearic acid, implying that these lines must have an allele that favors the expression of the high-stearic characteristic. All of the F_2 populations analyzed corresponded to a segregation 15:1 (low-intermediate: high). As such, the segregation observed was distributed in a manner similar to that previously described (*8*) despite the fact that seeds with a higher stearic content were found in a high-oleic background. Hence, these results support the linkage of the genes that control the high-oleic and high-stearic characteristics. This would suggest that the higher stearic lines obtained on a high-oleic and a normal background might be due to the improved activity of the thioesterases on stearic acid due to the expression of a gene other than that proposed (*8*). If various genes encode acyl-ACP thioesterases and the linkage or pleiotropic effect might persist, the genes selected by this method must be distinct to the linked genes.

In summary, from the data obtained from the F_2 generation, we can conclude that more segregants displayed a high-stearic phenotype on a high-oleic background from the BK-73 and BP-72 lines. In contrast, neither the BP-80 nor the BL-41 line produced segregants within high-stearic characteristics on a similar high-oleic background.

To test whether these crosses could really serve to select better high-stearic lines on a high-oleic background, seeds from the F_2 progeny of the BK-73 and BP-72 plants with a high-stearic character (>20%) and a high-oleic background (>62.0%) were selected. These seeds were germinated, self-fertilized, and the seeds from the resulting F_3 plants were analyzed (**Figure 3**). F_3 plants that had a high-stearic content and that still segregated with a high-oleic content were identified (**Figure 3**). Taking into account that the high-stearic character is controlled by at least two genes and the high-oleic phenotype by a minimum of three, it is not surprising that the two characteristics analyzed were still not fixed in the seeds. Indeed, the stearic content had improved significantly in the high-oleic background with respect to the previous generation. In all of the F_3 segregants, there were many seeds with a stearic content superior to 20% in a high-oleic background, when in the earlier studies, seeds of this type could not be found (*8*). Nevertheless, although the parental CAS-3 stearic content was not observed in any of the high-oleic backgrounds, many F_3 seeds with an average of 24% stearic content were observed in a high-oleic background.

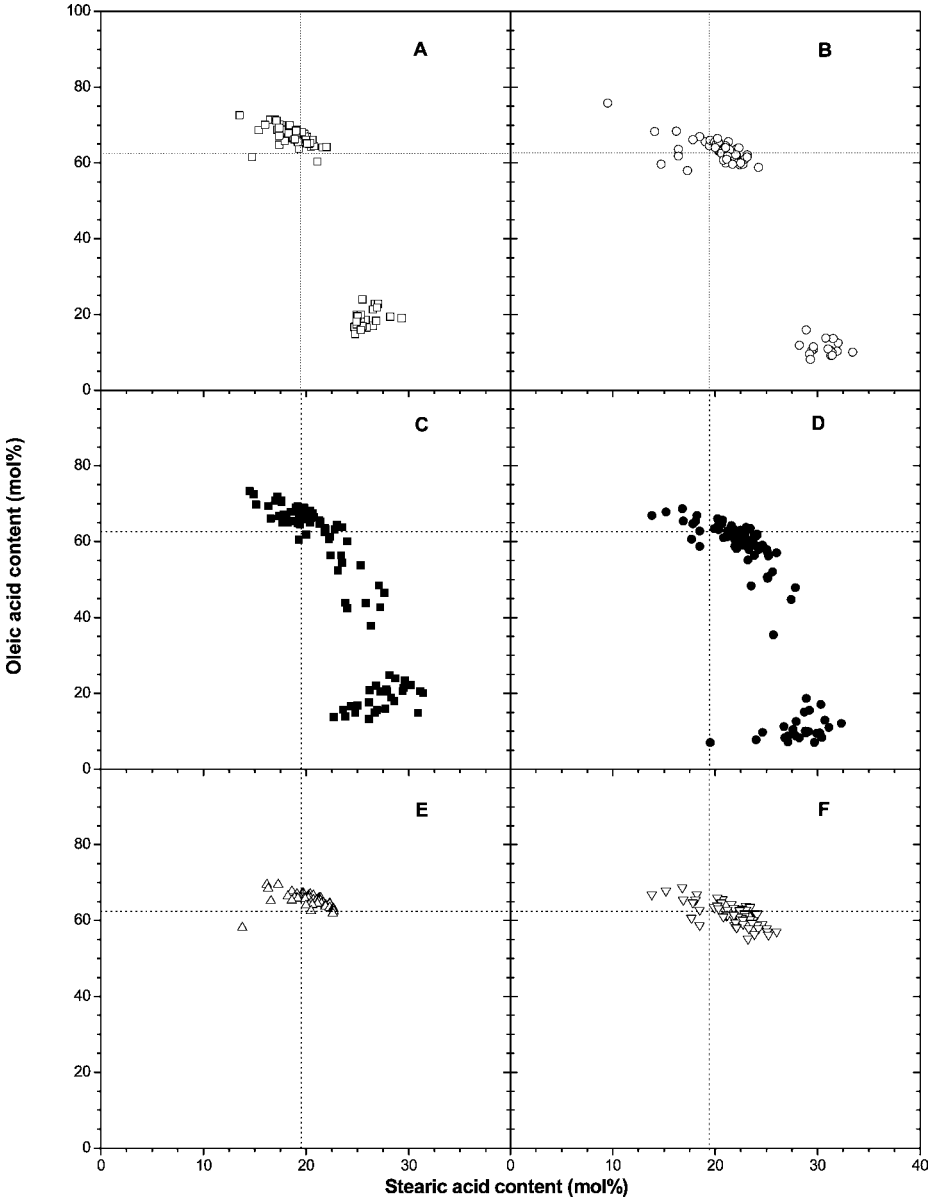

Figure 3. Representation of the stearic acid content versus the oleic acid content in the F_3 seeds obtained from F_2 segregant plants of the crosses of CAS-3 × BP-72 and of CAS-3 × BK-73. The results shown are of seeds from F_2 plants selected with high stearic and oleic content. A (BP-72-1), B (BP-72-2), C (BK-73-6), D (BK-73-2), E (BP-72-4), and F (BK-73-5).

Table 3. Analysis of the Fatty Acid Composition of Two Lines Selected from the Crosses of the BP-72 and BK-73 Lines with the High-Stearic Mutant CAS-3, in Comparison with the Data of the High-Stearic Parental CAS-3 Grown in the Same Conditions[a]

	fatty acid composition (mol %)					
	palmitic	stearic	oleic	linoleic	arachidic	behenic
			Parental			
CAS-3	7.5 ± 0.4	27.2 ± 2.2	13.0 ± 2.3	48.8 ± 4.0	1.7 ± 0.1	1.8 ± 0.3
			F_7			
F7-(BK-73 × CAS-3)	6.0 ± 0.4	21.4 ± 1.6	63.4 ± 3.8	4.6 ± 3.3	1.8 ± 0.2	2.8 ± 0.3
F7-(BP-72 × CAS-3)	6.6 ± 2.0	21.4 ± 1.3	61.9 ± 3.5	6.0 ± 2.9	1.5 ± 0.1	2.3 ± 0.4

[a] The data are the mean of at least 12 determinations, with their respective standard deviations.

Furthermore, high-stearic F_3 seeds in high-linoleic (low-oleic) background have greater stearic content than that found in the parental CAS-3 line. The results obtained from two F_3 segregant plants that appear to have fixed the high-stearic and high-oleic character are shown in **Figure 3** (panels E and F), although, as mentioned previously, they did not reach the value of 24% found in the high-stearic parent.

The selection of the best lines has been continued up to the F_7 generation, and the fatty acid composition of these lines as compared to that of the parental high-stearic lines is shown in **Table 3**. The fact that we have not been able to reach the stearic acid values of the parental CAS-3 line suggests that, over and above the potentially improved thioesterases identified and introduced in this study, a linkage must exist between the genes

that control the high-stearic and high-oleic characters, as proposed previously (*8*). Alternatively, a pleiotropic effect that impedes the full expression of the high-stearic character in a high-oleic background may occur.

Hence, we can conclude that the proposed selection method involving incubations with radioactive acetate in the presence of 10 mM MV in 15 DAF seeds permits high-oleic lines expressing acyl-ACP thioesterase alleles with greater activity on saturated substrates to be identified. Through crosses of the selected high-stearic parental lines, lines with a greater stearic acid content in a high-oleic background could be obtained, although we must emphasize that the linkage or pleitropic effect postulated (*8*) cannot be disregarded. Indeed, the improvement observed must be due to other genes that are probably situated on other chromosomes, possibly encoding thioesterases.

ABBREVIATIONS USED

SAD, stearoyl-ACP desaturase; VLCFA, very long chain fatty acids; TAG, triacylglycerols; MV, methyl viologen; FAS, fatty acid synthase; KAS II, β-ketoacyl-ACP synthase II; TE, acyl-ACP thioesterase; DAF, days after flowering.

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