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INHERITANCE OF REDUCED SATURATED FATTY ACID CONTENT IN SUNFLOWER OIL

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SUMMARY

In recent years, consumers have become concerned with reducing the saturated fat content of their diet. Studies have indicated that high levels of saturated fat consumption are correlated with increased risk of coronary heart disease. The total saturated fat content of oil from current sunflower hybrids averages about 130 g kg^{-1}. To identify sunflower germplasm with reduced saturated fatty acid composition, a total of 884 cultivated sunflower accessions from the USDA-ARS North Central Regional Plant Introduction Station, Ames, Iowa, were screened for fatty acid composition by gas chromatography. PI 250542, a cultivar collected in Egypt by Paul Knowles and deposited into the National Plant Germplasm System in 1958, was identified as an accession with reduced saturated fatty acid content. The fatty acid composition of 26 half-seeds of PI 250542 was determined, and the seeds with lowest saturated fatty acids were grown in the greenhouse. Pollen from a single plant was used to pollinate NMS HA 89, and the F_1 seed was grown in the field and self-pollinated. After three generations of selection by half-seed analysis, two lines with a low saturated fatty acid trait were selected. Line RS1 has a striped black and dark gray achene, whereas line RS2 has a light gray achene which often bleaches to white when grown in the field. The total saturated fatty acid composition of RS1 including C16 to C24 fatty acids was 77 g kg^{-1}, and for RS2 was 76 g kg^{-1} when grown at Fargo, North Dakota, in 2000. To determine inheritance of the reduced saturated fatty acid trait, RS1 and RS2 were pollinated by HA 821, a high saturated fatty acid line, and grown in the greenhouse. The resulting F_1 seeds were slightly higher in saturated fatty acids than the RS1 or RS2 parents, but far lower than the HA 821 parent, suggesting that the reduced saturated fatty acid trait was partially dominant.

Key words: **inheritance, palmitic, reduced saturated fatty acids, stearic, sunflower**

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INTRODUCTION

During the 1990s, consumers in the United States began to show interest in reducing the saturated fat content of their diet. Studies have shown that high levels of saturated fat consumption are correlated with increased risk of coronary heart disease. While the 120 to 130 g kg^{-1} saturated fatty acid content of sunflower oil is considered low compared with most vegetable oils, canola oil with about 70 g kg^{-1} saturated fatty acids remains a major competitor to sunflower oil in the marketplace. Manufacturers of canola oil products exploit the low saturated fatty acid content in their advertising strategies. For sunflower oil to compete with canola oil and other vegetable oils with low saturated fatty acid content, it is desirable to decrease the saturated fat level of sunflower oil. This paper reports the development of two germplasm lines, RS1 and RS2, which are low in saturated fatty acids. The lines were selected from a cultivated sunflower line, PI 250542, collected in Egypt by Paul Knowles and deposited into the USDA-ARS National Plant Germplasm System in 1958.

MATERIALS AND METHODS

Plant material

Bulk samples of 884 cultivated sunflower accessions from the USDA-ARS North Central Regional Plant Introduction Station, Ames, Iowa, were screened for fatty acid composition by gas chromatography (Vick *et al.*, 1998). PI 250542, a cultivar collected in Egypt by Paul Knowles and deposited into the National Plant Germplasm System in 1958, was identified as an accession with reduced saturated fatty acid content. The fatty acid composition of 26 half-seeds of PI 250542, in which the meristem half of the seed is saved for planting and the other half is analyzed for fatty acids, was determined by gas chromatography. The seed with lowest saturated fatty acids was grown in the greenhouse in 1994 and used to pollinate NMS HA 89, and the F_1 seed was grown in the field and self-pollinated. One F_2 seed with reduced palmitic acid was selected by half-seed analysis of 40 F_2 seeds, and identified as RP13 (RP=reduced palmitic). The RP13 plant was grown in the greenhouse and backcrossed with pollen from HA 89. The BC_1F_1 seed from this cross was planted in the field and the BC_1F_2 seed was screened for low palmitic and low stearic acids. BC_1F_2 seeds with low saturated fatty acid composition were grown in the greenhouse, and the self-pollinated BC_1F_3 seeds were screened by half-seed analysis for both low palmitic and stearic acid content. Two seeds of different colors were chosen for further enhancement, and these became the origin of the two reduced-saturated fatty acid genetic stocks, RS1 and RS2 (RS=reduced saturated). RS1 and RS2 were planted alternately in the greenhouse and field and self-pollinated, continually being screened for both low palmitic and low stearic acid content through the BC_1F_8 generation.

Fatty acid composition analysis

A small portion of pulverized seeds (10 to 20 mg) was transferred to a disposable filter column (Fisher**) and eluted with 3.5 ml of diethyl ether. The oil in the diethyl ether solution was converted to methyl esters according to the procedure of Metcalfe and Wang (1981) by the addition of 200 μl of tetramethylammonium hydroxide (10% in methanol), followed by vortexing. After 30 min, water was gently added to the reaction mixture, and the upper diethyl ether layer was transferred to a glass vial and capped. The sample was injected into a Hewlett-Packard 5890 gas chromatograph containing a DB-23 capillary column (25 m x 0.25 mm, J&W Scientific), which was held at 190°C for 5 min, then programmed to 220°C at 10°C/min, held at 220°C for 1 min, then programmed to 240°C at 20°C/min, and finally held at 240°C for 0.5 min, for a total run time of 10.5 min.

Inheritance studies

RS1 and RS2 were crossed in the greenhouse with HA 821 as the male parent. HA 821 is a traditional high linoleic acid sunflower germplasm line with a total saturated fatty acid content of about 130 g kg^{-1}. F_1 and F_2 seeds of these crosses were produced simultaneously in the same greenhouse, and analyzed by gas chromatography for fatty acid composition by half-seed analysis.

RESULTS

RS1

RS1 has a striped black and dark gray seed, and the plants occasionally demonstrate bicephalism, with the two heads sometimes completely separated and sometimes fused. The total saturated fatty acid composition of RS1, including C16 to C24 fatty acids, was 77 g kg^{-1} when grown in the field in Fargo, North Dakota, in 2000. This was considerably lower than several control hybrids or inbred lines in the same plot whose seed oils averaged 107-145 g kg^{-1} total saturated fatty acids. The inheritance studies reported here were conducted on sunflower plants grown in the greenhouse. Typically, the content of saturated fatty acids in the seed oil is lower in greenhouse-grown plants than in field-grown plants. Thus, when RS1 was grown in the greenhouse, the total saturated fatty acid composition was only 66.3 g kg^{-1} (Table 1).

For the inheritance study, we chose to cross RS1 with HA 821, which has a considerably higher saturated fatty acid content of 115 g kg^{-1} when grown in the greenhouse. Both the F_1 and F_2 seed from the RS1 x HA 821 cross contained seed oil fatty acid compositions that resembled the RS1 female parent more than the HA 821 male parent. The F_1 seed oil contained 72.7 g kg^{-1} of total saturated fatty acids

** Names of products are included for the benefit of the reader and do not imply endorsement or preferential treatment by the United States Department of Agriculture.

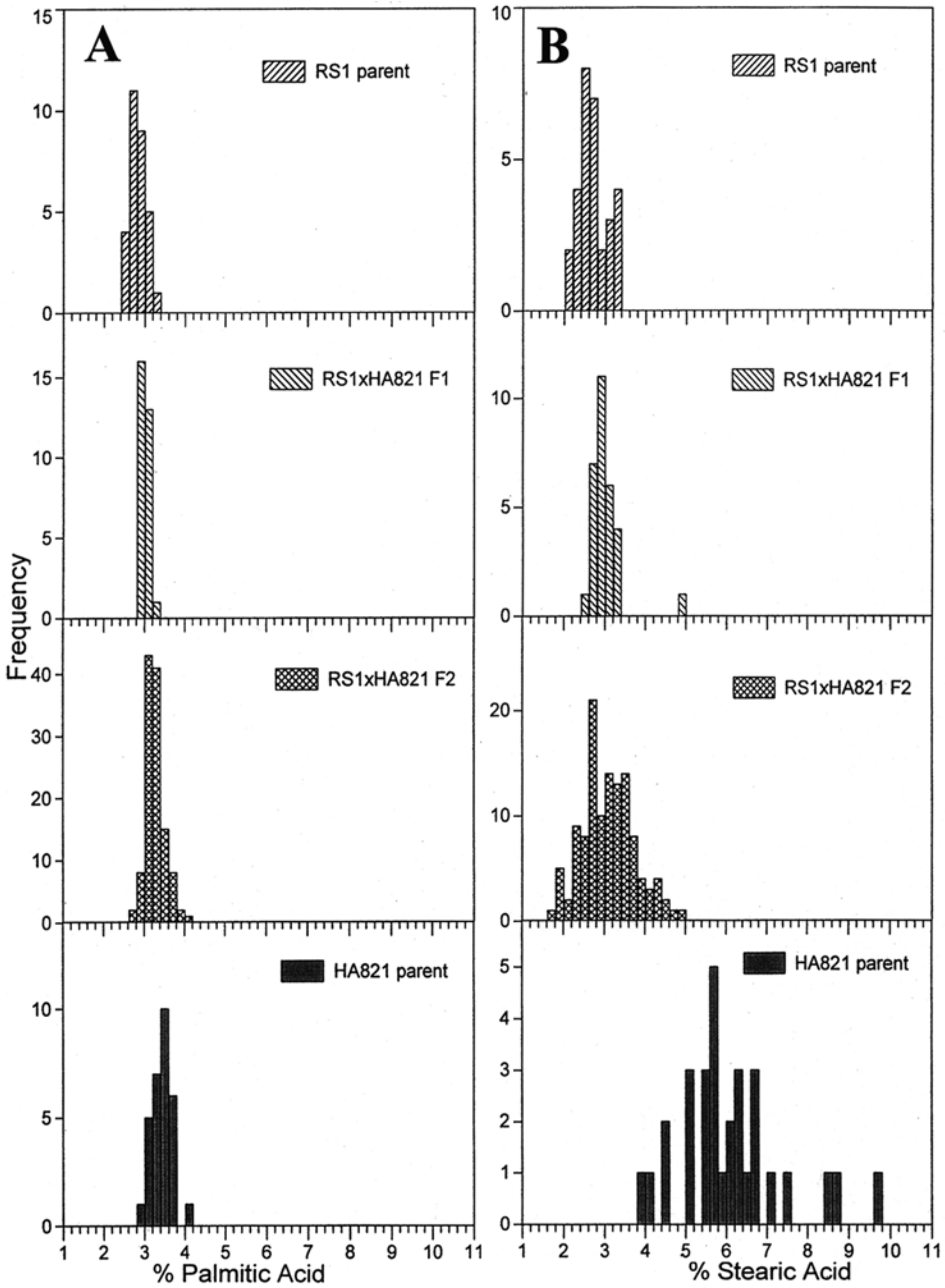

Figure 1: Frequency distribution of A) palmitic acid and B) stearic acid in the parents of cross RS1×HA 821, and the F_1 and F_2 progeny

and the F_2 seed contained 76.4 g kg^{-1}. The results suggested that the low saturated fatty acid trait of RS1 was partially dominant.

Table 1: Average saturated fatty acid compositions of RS1, HA 821, and the F_1 and F_2 generations of RS1×HA 821 grown in the greenhouse

	Palmitic acid g kg^{-1}	Stearic acid g kg^{-1}	Total saturated fatty acids† g kg^{-1}
HA 821	34.4 ± 2.6	60.7 ± 12.9	115 ± 16.7
RS1	28.1 ± 1.8	26.7 ± 3.5	66.3 ± 4.6
RS1×HA 821 (F_1)	30.0 ± 0.9	30.2 ± 4.2	72.7 ± 6.0
RS1×HA 821 (F_2)	32.7 ± 2.3	30.9 ± 6.4	76.3 ± 8.0

C16 to C24 saturated fatty acids

The compositions of the two major saturated fatty acids, palmitic and stearic, were analyzed separately in the seed oil of the RS1 and HA 821 parents, the F_1 seed, and the F_2 seed. The results showed that a reduction in stearic acid content from 60.7 g kg^{-1} to 30.2 g kg^{-1} was largely responsible for the decrease in total saturated fatty acid content in the F_1 seed oil compared with HA 821 (Figure 1). Palmitic acid was reduced only slightly. A similar significant reduction in stearic acid was maintained in the F_2 seed, which averaged 30.9 g kg^{-1} of stearic acid. Again, there was only a small reduction in palmitic acid content in the F_2 seed. However, the frequency of distribution of stearic acid was much broader in the F_2 seed than in the F_1 seed, but no clear segregation classes were evident. The distribution resembled a continuous normal distribution curve, suggesting that the reduced stearic acid trait is not controlled by a single gene.

RS2

RS2 has a light gray seed which often bleaches to white when grown in the field. RS2 plants are frequently bicephalic, with the two heads sometimes completely separated and sometimes fused. The C16 to C24 saturated fatty acid composition of RS2 was 76 g kg^{-1} when grown in the field at Fargo, North Dakota, during 2000, significantly lower than control inbred lines and hybrids which averaged 107-145 g kg^{-1} total saturated fatty acid. When RS2 was grown in the greenhouse, the total saturated fatty acid composition was 64.3 g kg^{-1} (Table 2).

Table 2: Average saturated fatty acid compositions of RS2, HA 821, and the F_1 and F_2 generations of RS2×HA 821 grown in the greenhouse

	Palmitic acid g kg^{-1}	Stearic acid g kg^{-1}	Total saturated fatty acids† g kg^{-1}
HA 821	34.4 ± 2.6	60.7 ± 12.9	115 ± 16.7
RS2	34.2 ± 4.1	21.1 ± 4.8	64.3 ± 5.5
RS2×HA 821 (F_1)	29.3 ± 1.3	28.1 ± 3.7	67.3 ± 5.4
RS2×HA 821 (F_2)	34.2 ± 3.2	24.5 ± 9.2	69.3 ± 10.3

C16 to C24 saturated fatty acids

For the inheritance study, RS2 was crossed with HA 821, which had a saturated fatty acid content of 115 g kg^{-1} when grown in the greenhouse. Both the F_1

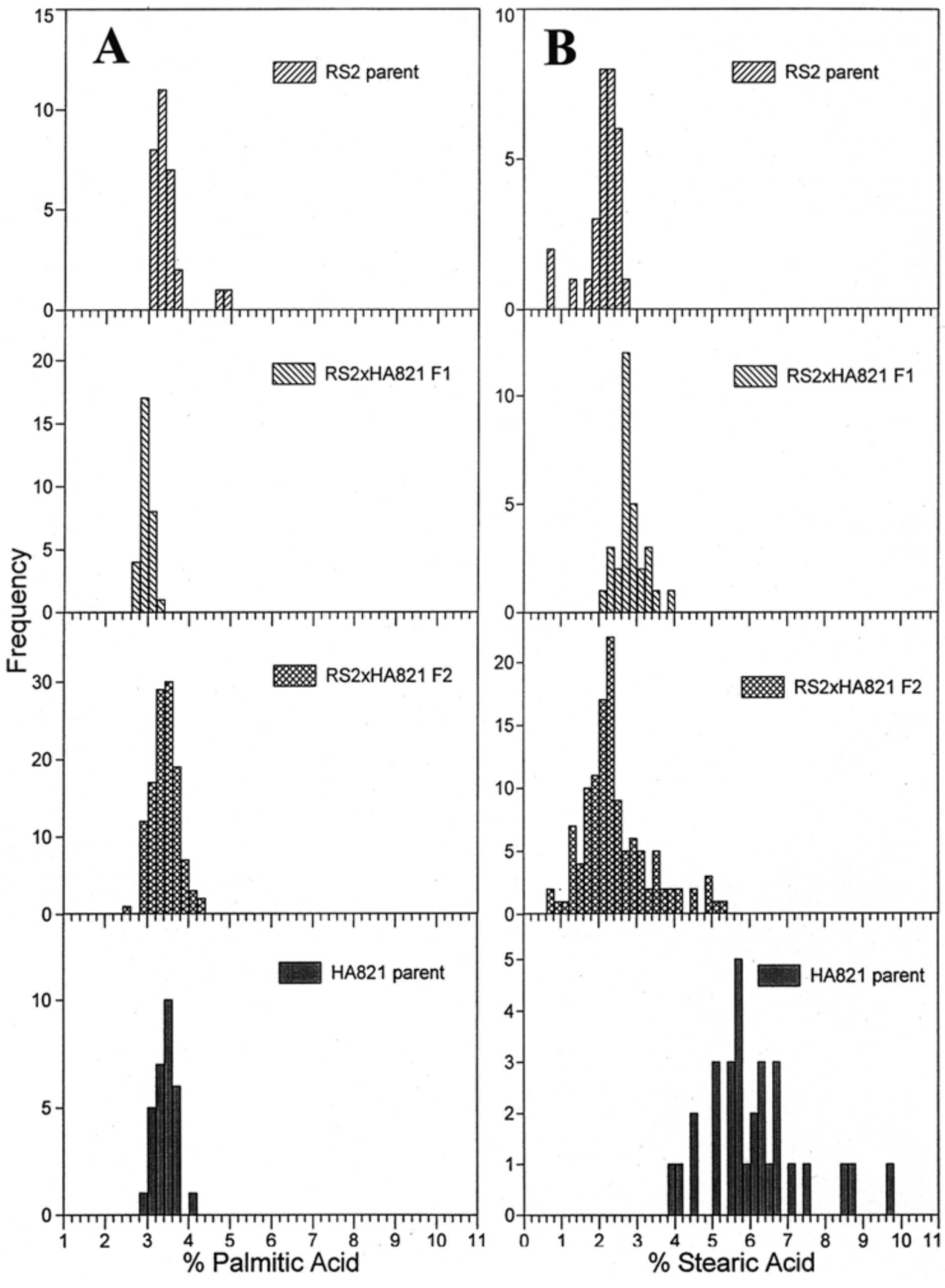

Figure 2: Frequency distribution of A) palmitic acid and B) stearic acid in the parents of cross RS2×HA 821, and the F_1 and F_2 progeny

and F_2 seed from the RS2 × HA 821 cross possessed oils similar in fatty acid composition to the RS2 female parent. The F_1 seed oil contained 67.3 g kg^{-1} of total saturated fatty acids and the F_2 seed contained 70.5 g kg^{-1}, suggesting that the low saturated fatty acid trait of RS1 was partially dominant.

When palmitic and stearic acid compositions were analyzed in the seed oil of the RS2 and HA 821 parents, as well as the F_1 and F_2 seed, the results again showed that a reduction in stearic acid content was largely responsible for the decrease in total saturated fatty acid content in the F_1 seed oil compared with HA 821 (Figure 2). Stearic acid was reduced from an average of 60.7 g kg^{-1} in HA 821 to 28.1 g kg^{-1} in the F_1 seed. Palmitic acid was reduced only slightly. A similar significant reduction in stearic acid occurred in the F_2 seed, which averaged 25.2 g kg^{-1} of stearic acid. Again, there was only a small reduction in palmitic acid content in the F_2 seed. As observed with RS1, the frequency of distribution of stearic acid was much broader in the F_2 seed than in the F_1 seed, yet no clear segregation classes were apparent. The distribution resembled a continuous normal distribution curve, suggesting that the reduced stearic acid trait is not controlled by a single gene in RS2.

DISCUSSION

The manipulation of the saturated fatty acid content in vegetable oils is an important consideration for competitiveness in the marketplace and for future development of new products. Consumers in the United States are knowledgeable about the health risks associated with diets high in saturated fat, and show a purchasing preference for low saturated fat products. In contrast, a significantly increased saturated fat composition of a vegetable oil could be advantageous for certain specialty products, such as margarine, without the need for hydrogenation.

Two reports have addressed the inheritance of saturated fatty acid content in mutant sunflower lines. Miller and Vick (1999) reported on the inheritance of reduced palmitic and stearic acid in sunflower lines treated with two mutagens, *N*-nitroso-*N*-methylurea and ethyl methanesulfonate. They concluded that the low palmitic acid content in line RHA 274 LP-1 was controlled by a single allele *fap1* with additive gene action. Two lines were identified with reduced stearic acid content. In one line, HA 821 LS-1, the low stearic acid trait was regulated by one gene, *fas1*, with additive gene action. In RHA 274 LS-2 the reduced stearic acid content was controlled by two genes, *fas2* and *fasx*, with additive gene action. None of the mutant lines produced in this study were reduced in both palmitic and stearic acids.

Perez-Vich *et al.* (2000) identified two sunflower mutant lines with a high composition of saturated fatty acids. CAS-5 was a mutant line with a palmitic acid concentration greater than 250 g kg^{-1}, while CAS-3 was a mutant line with stearic acid concentration greater than 220 g kg^{-1}. From their inheritance studies, they concluded that the loci controlling the high-palmitic acid trait exerted an epistatic effect over the loci responsible for the high-stearic acid character. Thus, it was not possible by crossing to produce a high-palmitic and high-stearic sunflower line.

In our studies, sunflower lines RS1 and RS2 were significantly reduced in both palmitic and stearic acids when comparisons were made with control inbred lines and hybrids under field conditions. The inheritance experiments reported here were conducted in the greenhouse. We found that the seed oil in greenhouse-grown sunflowers was significantly lower in saturated fatty acid content than in field-grown sunflowers. In our experiment, the palmitic acid composition of greenhouse-grown HA 821 was quite low compared with the field-grown HA 821. Thus, the difference in seed oil palmitic acid content between RS1/RS2 and HA 821 was barely noticeable when the plants were grown in the greenhouse. Therefore, the inheritance of the reduced palmitic acid trait was not clearly evident. In contrast, stearic acid did not show this variability. A cross of either RS1 or RS2 with HA 821 resulted in progeny with reduced stearic acid content, similar to RS1 or RS2, suggesting that the reduced stearic acid trait of RS1 and RS2 was partially dominant. A future inheritance study will be conducted in the field, and is expected to clarify the inheritance of the reduced palmitic acid trait.

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HERENCIA DEL CONTENIDO REDUCIDO DE ACIDOS GRASOS SATURADOS EN EL ACEITE DE GIRASOL

RESUMEN

En los años recientes, los consumidores comenzaron cuidar de la reduccion del conteniido de acidos grasos saturados en su alimentacion. Las investigaciones indicaban que el consumo de altas dosis de grasas saturadas es ligado con el riesgo agrandado de aparicion de enfermedades del corazon. El contenido total de grasas saturadas en el aceite de hibridos del girasol, que por el momento predominan en la produccion, es de 130 g kg^{-1}. Para identificar el germplasma del girasol, que posee el contenido reducido de acidos grasos saturados, 884 muestras del girasol de USDA-ARS e la Estacion Regional para la introduccion de plantas, Ames, Iova, fueron investigadas con respecto al contenido de acidos grasos por medio de la hematografia de gas. Para PI 250542, la muestra que Paul Knowles llevo en 1958 de Egipto y depuso en el Sistema Nacional para el germplasma vegetal, se ha constatado que posee el contenido reducido de acidos grasos saturados. El contenido de acidos grasos en la muestra PI 250542 fue constatado en 26 mitades de semillas, y esas con el minimo contenido de acidos grasos saturados fueron plantadas en el invernaculo. El polen de una planta era utilizado para la polinizacion de la linea NMS HA 89, y las plantas de la generacion F_1 eran cultivadas en el campo y autopolinizadas. Despues de tres generaciones de seleccion con la utilizacion del analisis de mitades de semillas, han sido formadas dos lineas que poseian la caracteristica del contenido reducido de acidos grasos saturados. La linea RS1 tiene los aquenios negros-gris oscuros, mientras la linea RS2 tiene los aquenios gris claros que muchas veces palidecen al color blanco durante el cultivo en el campo. El contenido total de acidos grasos saturados en la linea RS1, inclusive la gama de acidos grasos de C16 a C24, era de 77 g kg^{-1}, y en la linea RS2 76 g kg^{-1}, en las condiciones del cultivo en Fargo, North Dacota, durante 2000. Para determinar la herencia de la propiedad del contenido reducido de acidos grasos saturados, las lineas RS1 y RS2 han sido polinizadas con el polen de la linea HA 821, que posee el alto contenido de acidos grasos saturados, y pues cultivadas en el invernaculo. Las semillas F_1 asi obtenidas tenian un poco mas alto contenido de acidos grasos saturados que los padres RS1 y RS2, pero tambien mucho mas bajo contenido que los padres HA 821, lo que indica que la propiedad del bajo contenido de acidos grasos saturados se hereda como parcialmente predominante.

TRANSMISSION D'UN CONTENU RÉDUIT D'ACIDES GRAS SATURÉS DANS L'HUILE DE TOURNESOL

RÉSUMÉ

Ces dernières années, les consommateurs ont commencé à souhaiter une diminution des acides gras saturés dans leur alimentation. Des recherches ont démontré que la consommation de doses élevées de gras saturés était liée à une augmentation du risque de maladies cardiaques. Le contenu total des gras saturés dans l'huile des hybrides de tournesol qui domine actuellement le marché est d'environ 130 g kg^{-1}. Dans le but d'identifier le germeplasme du tournesol ayant un contenu réduit d'acides gras saturés, la composition en acides gras saturés d'un total de 884 échantillons de tournesol de culture de la station régionale du centre nord pour l'introduction des plantes USDA-ARS,

Ames, Iowa a été examiné par chromatographie en atmosphère gazeuse. On a constaté que le cultivar PI 250542 apporté d'Égypte en 1958 et déposé au Système national pour le germeplasme végétal par Paul Knowles possédait un contenu réduit d'acides gras saturés. La composition en acides gras de 26 demi-graines de l'échantillon PI 250542 a été déterminée et les graines qui contenaient le moins d'acides gras saturés ont été semées en serre. Le pollen d'une plante a servi à la pollinisation d'une ligne NMS HA 89, et les plantes F_1 ont été cultivées dans un champ et autofécondées. Après trois générations de sélection par analyse de demi-graines, deux lignes possédant comme caractéristique un contenu réduit en acides gras saturés ont été créées. La ligne RS1 a des akènes à rayures grises et blanches et la ligne RS 2, des akènes gris clair qui pâlissent souvent jusqu'à devenir blancs quand elle est cultivée dans les champs. Le contenu total d'acides gras saturés de la ligne RS1, incluant des acides gras de C16 à C24 était de 77 g kg^{-1} et celui de la ligne RS2, 76 g kg^{-1} dans les conditions de culture de Fargo, Dakota du Nord en l'an 2000. Pour que soit déterminée la transmission du trait de contenu réduit en acides gras saturés, les lignes RS1 et RS2 ont été pollinisées par la ligne HA 821 qui possède un haut contenu d'acides gras saturés pour être ensuite cultivées en serre. Le contenu en acides gras saturés a été légèrement plus élevé dans les graines F_1 que dans celles des parents RS1 et RS2, mais beaucoup plus bas que dans le parent HA821, ce qui suggère que le trait de contenu réduit en acides gras saturés était partiellement dominant.