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WILD *Helianthus annuus*, A POTENTIAL SOURCE OF REDUCED PALMITIC AND STEARIC FATTY ACIDS IN SUNFLOWER OIL

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SUMMARY

The current trend in human diets is to decrease consumption of the saturated palmitic and stearic fatty acids. Healthy diets restricting not only total fat, but the saturated portion of that fat, would decrease blood serum cholesterol and the risk of coronary heart diseases. Edible vegetable oils are the principal source of fats in many diets. Sunflower oil, which is fifth in production among edible vegetable oils in the world, contains 65 g kg^{-1} saturated palmitic and 45 g kg^{-1} saturated stearic acids. These concentrations are high compared to rapeseed oil with 40 g kg^{-1} palmitic and 20 g kg^{-1} stearic acids. A reduction of saturated fats in traditional sunflower oil would lead to a healthier edible oil. The objective of this preliminary study was to search the vast genetic diversity available from wild *Helianthus annuus*, the closest relative of the cultivated sunflower, for potential sources of reduced saturated fatty acids; less than 70 g kg^{-1} combined palmitic and stearic fatty acids. Achenes of eighty-two populations of wild *H. annuus* were collected from the central Great Plains of the USA. Composited 20-achene samples from each population were analyzed for saturated fatty acids using organic base-catalyzed transesterification of fatty acid methyl esters and capillary gas chromatography. The average palmitic acid concentration ranged from 39 to 65 g kg^{-1} for the populations. Average stearic acid concentrations ranged from 19 to 37 g kg^{-1}. Achene oil of one population of wild *H. annuus* from Holmquist, South Dakota, USA had a palmitic acid concentration averaging 39 g kg^{-1}, while stearic acid averaged 19 g kg^{-1}. The combined 58 g kg^{-1} palmitic and stearic acids is almost 50% lower than the present level of these fatty acids in sunflower oil. The level of saturated fatty acids observed in the population remained low when plants were grown in the greenhouse under uniform conditions. In the greenhouse, palmitic acid concentration of this population averaged 40 g kg^{-1}, while stearic acid averaged 19 g kg^{-1}. Crosses between this population and an inbred cultivated line produced F_1 plants with an achene oil that averaged 39 g kg^{-1} palmitic and 21 g kg^{-1} stearic acid. In comparison, the inbred cultivated parent averaged 61 g kg^{-1} palmitic and 51 g kg^{-1} stearic acid. F_2 plants produced achene oil that averaged 45 g kg^{-1} palmitic and 23 g kg^{-1} stearic acid, for a total of 68 g kg^{-1}. When

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F_1 plants were backcrossed to the cultivated inbred, BC_1F_1 plants produced an achene oil that averaged 45 g kg^{-1} palmitic and 26 g kg^{-1} stearic acid for a total of 71 g kg^{-1}. In comparison, the inbred cultivated parent averaged 65 g kg^{-1} palmitic and 42 g kg^{-1} stearic acid, for a total of 107 g kg^{-1}. Preliminary information indicates that palmitic and stearic fatty acids in sunflower oil can be reduced by introducing genes from a wild annual population into cultivated sunflower. Further research will be needed to determine the inheritance of these fatty acids. Other agronomic traits will also have to be monitored during introgression of the fatty acids genes.

Key words: **palmitic acid, stearic acid, saturated fatty acids, *H. annuus*, wild species**

INTRODUCTION

In recent years consumers have become concerned about the consumption of saturated fats in their diets. High levels of saturated fat consumption may contribute to increased blood serum cholesterol which in turn increases the risk of coronary heart disease (Mensink *et al.*, 1994; Willett, 1994). Prompted by nutritional recommendations to consume fats lower in saturates and food manufacturers' interest in reducing the use of hydrogenated oil, food processors are interested in sunflower oil with specific fatty acid profiles (Fitch-Haumann, 1994). Vegetable oils are the principle source of fats in many diets. Compared with many edible vegetable oils, the saturated fatty acid concentration of 120 g kg^{-1} in sunflower (*Helianthus annuus* L.*)* oil is considered moderate, with the principal saturated fatty acids being palmitic (65 g kg^{-1}) and stearic (45 g kg^{-1}) acids. Canola (*Brassica napus* L.) oil with only 60 g kg^{-1} is considered low in saturated fats. A reduction of saturated fatty acids in sunflower oil to the 60 to 80 g kg^{-1} level would enhance the acceptability of sunflower oil.

The genus *Helianthus* contains 51 species, 37 perennial and 14 annual (Schilling and Heiser, 1981). Wild *Helianthus* species have been used to improve the economic and agronomic characteristics of cultivated sunflower (Seiler, 1992; Seiler and Rieseberg, 1997). Considerable emphasis has been placed on oil concentration and fatty acid composition of the oil. Interest has centered on the enhancement of the linoleic or oleic fatty acids, and the reduction of saturated palmitic and stearic fatty acids. Wild sunflower species provide a resource for improving fatty acid composition in cultivated sunflower (Dorrell and Whelan, 1978; Thompson *et al.*, 1981; Seiler, 1985, 1994). Potential sources with lower saturated palmitic and stearic acids from the wild species have been identified (Seiler, 1994, 1998), but their stability and transfer into cultivated sunflower have not been documented.

This study evaluated populations of wild annual *H. annuus,* the closest relative of cultivated sunflower, for lower palmitic and stearic acids, to determine their stability, and explored the possibility of introgressing the lower saturated fatty acid genes into cultivated sunflower.

MATERIALS AND METHODS

Achenes of eighty-two populations of *H. annuus* were collected from the central Great Plains of the USA (Seiler, 1994). Achenes were stored at 5°C and low humidity (<40%) until analyzed. Each sample represented an isolated open-pollinated population, usually having achenes collected from at least 25 plants.

Fatty acid composition was determined using a 20-achene sample of the *H. annuus* populations. For F_1, F_2, and BC_1F_1 interspecific hybrids, 10-achene samples were used for analysis. A small portion of pulverized sample (10 to 20 mg) was transferred to a disposable filter column and eluted with 3.5 ml of diethyl ether. The oil in the diethyl ether solution was converted to methyl esters using an organic base-catalyzed transesterification of the triacylglycerols by the addition of 200 μl of tetramethylammonium hydroxide (10% in methanol), followed by vortexing (Metcalfe and Wang, 1981). The sample was injected into a Hewlett-Packard 5890[1] gas chromatograph containing a DB-23 capillary column (25 m × 0.25 mm, J&W Scientific[**], which was held at 190°C for 5 minutes, then programmed to 220°C at 10°C per minute, held at 220°C for one minute, then programmed to 240°C at 20°C per minute, and finally held at 240°C for 0.5 minutes, for a total time of 10.5 minutes. The detector was a flame ionization detector (FID). The fatty acid standard used contained the following acids which typically occur in sunflower oil: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), and arachidic (20:0). Fatty acid concentrations were determined in two samples per population.

The stability of the reduced saturated fatty acid trait for a selected population of wild *H. annuus (*ANN-2229*)* was confirmed by growing the progeny of the original population in a common environment in the greenhouse to see if the low levels of saturated fatty acids observed in the population from its native habitat were expressed in the progeny. Interspecific F_1 hybrids were produced in the field using a nuclear male sterile analogue of NMS HA89 as the female parent and the wild *H. annuus* population (ANN-2229) as the male parent.

Pollen from the F_1 plants was used to backcross to NMS HA89 to produce field grown BC_1F_1 progeny. Nuclear male fertile analogue of HA89 was used as the check for normal concentrations of fatty acids for all generations.

RESULTS AND DISCUSSION

The wild *H. annuus* populations from North Dakota had the lowest average stearic acid concentration with 23 g kg^{-1}, while the highest concentration observed was in populations from Kansas with 35 g kg^{-1}. North Dakota also had the lowest

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average palmitic acid concentration with 41 g kg^{-1}, while the highest observed was from Kansas with 56 g kg^{-1}.

A single population of wild *H. annuus* (ANN-2229, PI 586886) from Holmquist, South Dakota, USA, had the lowest total saturated fatty acid concentration of 58 g kg^{-1} with a palmitic acid concentration of 39 g kg^{-1}, and a stearic acid concentration with 19 g kg^{-1}. This concentration is 50% lower than that generally observed in oil of cultivated sunflower. This population was chosen to determine the stability of the reduced saturated fatty acid trait to see if the genes controlling these acids were dominant, and if they could be transferred into cultivated sunflower.

Progeny of the ANN-2229 population were grown in the greenhouse at 22-25°C and 16 hours of daylight. The plants were sib-pollinated. The saturated fatty acids in achene oil of the population were very similar to the levels observed in the original population (Table 1).

Table 1: Comparison of palmitic and stearic fatty acids from plants of wild *H. annuus* (ANN-2229) from the original population and plants grown in a common environment in the greenhouse

Environment	Palmitic acid	Stearic acid	Total
	g kg^{-1}	g kg^{-1}	g kg^{-1}
ANN-2229 (Original)	47	18	65
ANN-2229 (Greenhouse)	48	16	64
Cultivated NMS HA89 (GH)	65	44	109

A standard cultivated line, fertile NMS HA89, was also grown as a control in the greenhouse and had a palmitic acid concentration of 65 g kg^{-1} and a stearic acid concentration of 44 g kg^{-1} for a total of 109 g kg^{-1}. The low levels of saturated fatty acids observed in the original population appear to be stable, indicating that the reduced levels of palmitic and stearic acids have a genetic base and the potential to be introgressed into cultivated sunflower.

The F_1 achenes produced in the field had an average palmitic acid concentration of 39 g kg^{-1} and a stearic acid concentration of 21 g kg^{-1} in the oil. These values were the average of 10 F_1 plants.

The cultivated inbred line NMS HA 89 used to produce F_1 hybrids averaged 61 g kg^{-1} palmitic and 51 g kg^{-1} stearic fatty acids. F_1 plants were self-pollinated in the field to produce F_2 achenes. Achene oil of field grown F_2 plants averaged 41 g kg^{-1} palmitic acid and 18 g kg^{-1} stearic acid totaling 59 g kg^{-1} of saturated fatty acids. The averages were based on 10 individual plants.

F_1 plants were backcrossed in the field with cultivated NMS HA89 as the female to produce BC_1F_1 achenes. Achene oil of the BC_1F_1 plants averaged 38 g kg^{-1} palmitic acid and 19 g kg^{-1} stearic acid. These values were based on 10 plants from one backcross family. In comparison, the cultivated NMS HA 89 line averaged 65 g kg^{-1} of palmitic acid and 42 g kg^{-1} of stearic acid.

CONCLUSIONS

Preliminary results indicate that palmitic and stearic acid levels in sunflower oil can be lowered by the introgression of genes from a population of the closest wild relative of the cultivated crop. The genes appear to be relatively stable after transfer. Further research will be needed to determine the inheritance of the genes controlling palmitic and stearic fatty acids. Acceptable agronomic traits will also have to be bred into the lines during the introduction of the genes into cultivated sunflower.

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***Helianthus annuus* SILVESTRE COMO UNA FUENTE POTENCIAL DE CONTENIDO DISMINUIDO DEL ÁCIDO PALMÍTICO Y ESTEÁRICO EN EL ACEITE DE GIRASOL**

RESUMEN

Actualmente en la alimentación humana está presente la tendencia hacia un reducido consumo del ácido palmítico y esteárico saturados. Con una alimentación sana, en la cual está limitado no sólo el contenido total de grasas, sino también el contenido de la parte saturada de dicha grasa, se disminuiría el contenido de colesterol en el suero sanguíneo y el riesgo de las enfermedades cardíacas coronarias. Los aceites comestibles de origen vegetal, son la principal fuente de grasas en muchas dietas. El aceite de girasol, que entre los aceites vegetales comestibles, ocupa el quinto lugar según las cantidades producidas en el mundo, contiene 65 g kg^{-1} del ácido palmítico saturado y 45 g kg^{-1} del ácido esteárico saturado. Estas concentraciones son altas en comparación con el aceite de colza, que contiene 40 g kg^{-1} de ácido palmítico, y 20

g kg^{-1} del ácido esteárico. Reduciendo el contenido de grasas saturadas en el aceite de girasol estándar, se obtendría aceite comestible más sano. El objetivo de esta investigación preliminar era, en la enorme divergencia genética que existe en el girasol silvestre (*Helianthus annuus*), el más cercano familiar del girasol cultivado, de buscar fuentes potenciales del reducido contenido de ácidos grasos, es decir, las fuentes con menos de 70 g kg^{-1} del ácido palmítico y esteárico juntas. Los aquenios de 82 poblaciones de girasol silvestre, se recolectaron en la región de Great Plains en lso E.E.U.U. Las muestras compuestas de 20 aquenios de cada población, fueron analizados para el contenido de los ácidos grasos saturados, mediante transesterificación de metilo ester de ácidos grasos catalizados con las bases orgánicas y cromatografía capilar de gases. La concentración promedia del ácido palmítico en las poblaciones investigadas varía entre 39 y 65 g kg^{-1}. La concentración promedia del ácido esteárico, era entre 19 y 37 g kg^{-1}. En una población de girasol silvestre, del lugar llamado Holmquist en Dakota del Sur (E.E.U.U.), la concentración promedia del ácido palmítico en el aceite de aquenios, era 39 g kg^{-1}, y el contenido promedio del ácido esteárico, era 19 g kg^{-1}. La suma concentración del ácido palmítico y del esteárico de 58 g kg^{-1} es más baja por 50% del contenido actual de esos ácidos grasos en el aceite de girasol. El contenido de los ácidos grasos saturados en esta población se ha quedado bajo, también cuando se cultivaban las plantas en el invernadero, en las condiciones uniformes. En el invernadero, la concentración promedia del ácido palmítico en esa población, era 40 g kg^{-1} y del esteárico, 19 g kg^{-1}. Con cruzamientos entre esta población y una línea consanguínea del girasol cultivado, se obtuvieron las plantas F_1 con el aceite de aquenios que contiene, en promedio, 39 g kg^{-1} de ácido palmítico, y 21 g kg^{-1} del ácido esteárico. Por comparación, la línea consanguínea utilizada como padre, en un promedio tiene 61 g kg^{-1} de ácido palmítico y 51 g kg^{-1} del ácido esteárico. Las plantas F_2 tenían el aceite de aquenios, con el promedio de 45 g kg^{-1} de ácido palmítico y 23 g kg^{-1} del ácido esteárico, lo que en suma, asciende a 68 g kg^{-1}. Una vez efectuados los cruzamientos reversibles entre las plantas de F_1 y dicha línea consanguínea, las plantas BC_1F_1 dieron aceite de aquenio, que en un promedio tiene 45 g kg^{-1} de ácido palmítico y 26 g kg^{-1} de ácido esteárico, o sea, en total, 71 g kg^{-1}, mientras que la línea consanguínea utilizada como padre, tiene en un promedio, 65 g kg^{-1} de ácido palmítico y 42 g kg^{-1} de ácido esteárico, o sea, en total, 107 g kg^{-1}. Las informaciones preliminares sugieren que el contenido del ácido palmítico y el esteárico en el aceite de girasol, puede reducirse, introduciendo los genes de las poblaciones silvestres anuales en el girasol cultivado. Se necesitan investigaciones adicionales, para determinar la forma de herencia de esos ácidos grasos. También tendrán que seguirse las demás propiedades agronómicas, durante la introgresión del gen para estos ácidos grasos.

ESPECE SAUVAGE *Helianthus annuus*, COMME SOURCE POTENTIELLE DE RÉDUCTION DE GRAS ACIDES PALMITIQUES AND STÉARIQUES DANS L'HUILE DE TOURNESOL

RESUME

Actuellement dans le régime de nourriture humaine, une tendance est présente vers la réduction de consomation d'acides saturés, palmitiques et stéariques. La nourriture saine limite, non seulement le total de matière grasse, mais le contenu saturé de matière grasse afin de diminuer le contenu de cholestérol dans le sérum sanguin et le danger de maladies coronaires, cardiaques. Les huiles comestibles d'origine végétale sont la source principale de régimes diététiques. L'huile de tournesol, qui est au cinquième rang dans la production d'huiles comestible, contient 65g kg^{-1} d'acide saturé palmitique et

45g kg^{-1} d'acide saturé stéarique. Ces concentrations sont assez élevées en comparaison de l'huile de colza qui contient 40g kg^{-1}d'acide palmitique et 20g kg^{-1} d'acide stéarique. Par la réduction de contenu d'acide gras saturé dans l'huile standard de tournesol, une huile comestible saine pourrait être obtenue. Le but de cette étude préliminaire pourrait servir de trouver les sources potentielles de contenu faible d'acides gras, c'est-à-dire les sources d'acides palmitiques et stéariques moins de 70g kg^{-1}au total dans l'énorme divergence génétique d'espèces sauvages de Helianthus annuus, le parent le plus proche du tournesol cultivé. Les achènes de 82 populations de tournesol sauvage sont recueillis dans la région de Great Plains aux Etats Unis. Les échantillons composés de 20 achènes de chaque population sont analysés pour déterminer le contenu d'acides gras saturés au moyen de trans-estérification d'acides gras du méthyle-ester, catalysés par les bases organiques et par la chromatographie capillaire gazeuse. La concentration moyenne d'acide palmitique de la population examinée était entre 39 et 65 g kg^{-1}. La concentration moyenne d'acide stéarique était entre 19 et 37 g kg^{-1}. Chez une population du tournesol sauvage de Holmquist en Dakota de Sud aux Etats Unis, une concentration moyenne d'acide palmitique dans l'huile d'achène, était enregistrée de 39 g kg^{-1}, tandis que le contenu d'acide stéarique était de 19 g kg^{-1}. La somme totale de concentrations d'acides palmitiques et stéariques de 58 g kg^{-1}, est inférieure de 50% du contenu actuel de tous les acides gras dans l'huile de tournesol. Le contenu d'acides gras saturés de cette population est resté faible même si les plantes sont cultivées dans les conditions uniformes d'orangerie. Dans les conditions d'orangerie, la concentration moyenne d'acide palmitique de cette population était de 40 g kg^{-1}, tandis que la valeur d'acides stéarique était de 19 g kg^{-1}. Par le croisement entre cette population et une ligne de tournesol cultivé, les plantes de F_1 sont obtenues d'une huile d'achène ayant, en moyenne, les acides palmitiques de 39 g kg^{-1} et acides stéariques de 21 g kg^{-1}. Comparant la ligne cultivée, utilisée comme parent qui contient, en moyenne, d'acides palmitiques de 61 g kg^{-1} et acides stéariques de 51 g kg^{-1}avec les plantes de F^1qui possèdent l'huile d'achène, en moyenne, d'un contenu d'acides palmitiques de 45 g kg^{-1} et acides stéariques de 23 g kg^{-1}, dont la somme totale est de 68 g kg^{-1}. Quand les croisements réciproques sont réalisés entre les plantes de F_1 et les lignes mentionnées, les plantes BC_1F_1 ont donné l'huile d'achène, en moyenne, d'acides palmitiques de 45 g kg^{-1} et acides stéariques de 26 g kg^{-1}, c'est-à-dire la somme totale est de 71 g kg^{-1}, tandis que la ligne cultivée, utilisée comme parent contient, en moyenne, d'acides palmitiques de 65 g kg^{-1} et acides stéariques de 42 g kg^{-1}, c'est-à-dire la somme totale est de 107 g kg^{-1}. Les informations préliminaires indiquent que le contenu d'acides palmitiques et stéariques dans l'huile de tournesol pourrait être diminué par introduction du gène de sauvages populations annuelles aux lignes cultivées. Les recherches postérieures sont nécessaires afin de déterminer le procédé d'hérédité d'acides gras. Les autres traits agronomiques devraient être contrôlés pendant l'introgression de gènes d'acides gras