HELIA, **25**, Nr. 36, p.p. 79-84, (2002) UDC 633.854.78:631.524.01

WILD PERENNIAL SUNFLOWER AS A POTENTIAL SOURCE OF REDUCED PALMITIC AND STEARIC FATTY ACIDS IN SUNFLOWER OIL

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Received: November 08, 2001
Accepted: June 03, 2002

SUMMARY

The trend in healthier human diets is to decrease the consumption of the saturated fatty acids. Sunflower oil, which is fourth in production among edible vegetable oils in the world, contains 65 g kg^{-1} palmitic and 45 g kg^{-1} stearic acids, both saturated fatty acids. These levels are high compared with rapeseed (*Brassica napus* L.) 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 ancestors of cultivated sunflower for potential sources of reduced palmitic and stearic fatty acids. Achene oil of one population of wild *H. giganteus* L. (GIG-102) from INRA, Montpellier, France, had 47 g kg^{-1} palmitic acid and 18 g kg^{-1} stearic acid. The combined 65 g kg^{-1} palmitic and stearic acids is 40% lower than the present level of these fats 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 averaged 48 g kg^{-1}, while stearic acid averaged 16 g kg^{-1}. This would indicate that palmitic and stearic acid concentrations are under genetic control with potential for incorporation into cultivated sunflower. Crossing this population with an inbred cultivated line produced F_1 plants with an achene oil that averaged 39 g kg^{-1} palmitic and 26 g kg^{-1} stearic acid. The inbred cultivated parent averaged 55 g kg^{-1} palmitic and 51 g kg^{-1} stearic acid. F_2 plants produced an achene oil that averaged 47 g kg^{-1} palmitic and 29 g kg^{-1} stearic acid, for a total of 76 g kg^{-1}. When F_1 plants were backcrossed to the cultivated inbred, BC_1F_1 plants produced an achene oil that averaged 47 g kg^{-1} palmitic and 28 g kg^{-1} stearic acid for a total of 75 g kg^{-1}. The inbred cultivated parent averaged 54 g kg^{-1} palmitic and 56 g kg^{-1} stearic acid, for a total of 110 g kg^{-1}. Preliminary information indicate that palmitic and stearic fatty acids in sunflower oil can be reduced by introducing genes from a wild perennial progenitor into cultivated sunflower. Further research will be needed to determine the inheritance of these fatty acids. Other agronomic traits will also

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have to be monitored during the introgression of genes for reduced saturated fatty acids into cultivated sunflower.

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

INTRODUCTION

In recent years consumers have become more concerned about the consumption of saturated fats in their diet. 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 50 species, 36 perennial and 14 annual (Schilling and Heiser, 1981). Wild species of the genus 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 around 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, 1998), but their stability and transfer into cultivated sunflower have not been documented.

This study evaluated a population of wild perennial *H. giganteus* (GIG-102) for low levels of palmitic and stearic acids, determined their stability, and explored the possibility of introgressing the lower saturated fatty acid genes into cultivated sunflower.

MATERIALS AND METHODS

The population was a perennial diploid, *H. giganteus* L. (GIG-102) from INRA, Montpellier, France, previously identified as having reduced levels of palmitic and

stearic acids (Seiler, 1998). Achenes were stored at 5°C and low humidity (<40%) until analyzed. Fatty acid composition of achene oil of the wild population was determined on a composite of 20 achenes. For F_1, F_2 and BC_1F_1 interspecific hybrids, 10-achene samples were analyzed. 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. Oil in the diethyl ether solution was converted to methyl esters using an organic base-catalyzed transesterification of the triacylglycerol by the addition of 200 µl of tetramethylammonium hydroxide (10% in methanol), followed by vortexing (Metcalfe and Wang, 1981). After 30 minutes, water was gently added to the reaction mixture, and the upper diethyl ether layer 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 Scientific1). The detection was a flame ionization detector (FID). The fatty acid standard (15a, NU-CHEK-PREP, INC1) contained the following acids: palmitic (C16:0), stearic (18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and arachidic (C20:0). Fatty acid peaks were identified by comparing the fatty acid methyl ester peaks and retention times of standards with sample peaks. Fatty acid concentrations were determined in two samples per population.

The stability of the lower saturated fatty acids for a selected population of wild *H. giganteus* was evaluated by growing progeny of the original population in a common environment (greenhouse) to determine if the low levels of saturated fatty acids observed in the achenes from its native habitat were expressed in progeny.

Interspecific F_1 hybrids were produced in the field using a nuclear male sterile, NMS HA89, as the female parent and the wild *H. giganteus* population (GIG-102) as the male parent. Pollen from the F_1 plants was used to backcross to NMS HA89, producing BC_1F_1 progeny. The male-fertile NMS HA89 was used as the check for normal levels of fatty acids for all generations.

RESULTS AND DISCUSSION

A population of wild perennial *H. giganteus* (GIG-102) from INRA, France had a palmitic acid concentration of 47 g kg^{-1} and a stearic acid concentration of 18 g kg^{-1}, totaling 65 g kg^{-1} for both. This level is 40% lower than the concentration generally observed in oil of cultivated sunflower. This population was chosen to check the stability of the reduced saturated fatty acids trait, to determine if the genes controlling these acids were dominant, and to explore the possibilities of transferring these characters into cultivated sunflower.

Progenies of GIG-102 were grown in the greenhouse at 22-25°C and 16 hours of daylight. Plants were sib-pollinated. Saturated fatty acids in achene oil of the population were similar to the levels observed in the original population (Table 1).

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Table 1: Comparison of palmitic and stearic fatty acids from plants from the original habitat and those grown in common environment in the greenhouse

Environment	Palmitic acid g kg^{-1}	Stearic acid g kg^{-1}	Total fatty acids g kg^{-1}
Original habitat (10)[1]	47	18	65
Greenhouse (10)	48	16	64

[1]Number of plants evaluated

A cultivated line, fertile NMS HA 89, also grown in the greenhouse, had a palmitic acid concentration of 54 g kg^{-1} and a stearic acid concentration of 34 g kg^{-1} for a total of 88 g kg^{-1}. The low levels of saturated fatty acids observed in the original population appear to be stable indicating the lower levels of palmitic and stearic acids are under genetic control, and potentially can be introgressed into cultivated sunflower.

F_1 achenes produced in the field had an average palmitic acid concentration of 39 g kg^{-1} and a stearic acid concentration of 26 g kg^{-1} in the oil. These values were the average of 22 F_1 plants. The F_1 plants had a weak perennial growth habit. The cultivated inbred line, NMS HA 89, used to produce F_1 hybrids averaged 55 g kg^{-1} palmitic and 51 g kg^{-1} stearic fatty acids. The F_1 plants were self-pollinated to produce F_2 plants in the field. Achene oil of F_2 plants averaged 47 g kg^{-1} palmitic acid and 29 g kg^{-1} stearic acid totaling 76 g kg^{-1} of saturated fatty acids. These are averages of 45 plants.

F_1 plants were backcrossed in the field with cultivated NMS HA89 as the female to produce BC_1F_1 plants. Achene oil of the BC_1F_1 plants averaged 47 g kg^{-1} palmitic acid and 28 g kg^{-1} stearic acid, totaling 75 g kg^{-1}. These values were based on 36 observations from three backcross families. The cultivated NMS HA 89 line averaged 54 g kg^{-1} of palmitic acid and 56 g kg^{-1} of stearic acid.

CONCLUSIONS

Preliminary results indicate that palmitic and stearic acid concentrations in sunflower oil can be lowered by introgressing genes from a perennial diploid species into the cultivated crop. The genes appear to be stable after transfer. Further research will be needed to study the inheritance of the genes controlling palmitic and stearic fatty acids. Acceptable agronomic traits will also have to be bred into the lines and monitored during the introduction of the genes into cultivated sunflower.

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EL GIRASOL DE VARIOS AÑOS COMO FUENTE POTENCIAL DEL CONTENIDO REDUCIDO DE ACIDOS PALMITICO I ESTEARICO EN EL ACEITE DE GIRASOL

RESUMEN

La direccion basica a la alimentacion humana mas sana es el consumo reducido de acidos grasos saturados. El aceite de girasol, el cuarto entre los aceites comestibles de origen vegetal, contiene 65 g kg^{-1} de acido palmitico y 45 g kg^{-1} de acido estearico, que partenecen a los acidos grasos saturados. Esas cantidades son altas en comparacion con el aceite de colza (*Brassica napus* L.) que contiene 40 g kg^{-1} de acido palmitico y 20 g kg^{-1} de acido estearico. La reduccion de grasos saturados en el aceite del girasol clasico llevaria al aceite comestible mas sano. El objetivo de esta investigacion preliminar era el reconocimiento de la diversidad genetica rica de los antecesores silvestres para buscar las fuentes del contenido reducido de acidos palmitico y estearico. El aceite en las semillas de una poblacion de la especie silvestre *H. giganteus* L. (GIG-102) de INRA, Monpellier, Francia, contenia 47 g kg^{-1} de acido palmitico y 18 g kg^{-1} de acido estearico. Esa cantidad total de 65 g kg^{-1} de acidos palmitico y estearico es inferior por 40% al nivel de esas grasas en el eceite del girassol standard. El nivel de acidos grasos saturados quedo bajo mientras las plantas eran cultivadas en las condiciones de invernaculo estandardizadas. El valor medio del acido palmitico era de 48 g kg^{-1}, y del acido estearico l6 g kg^{-1}. Eso indica que el contenido de acidos palmitico y estearico es bajo el control, asi como que hay la posibilidad de transferir esta propiedad en el girasol cultivado. Por el cruce de esta poblacion con una linea consaguinea del girasol cultivado fueron obtenidas las plantas F_1 con el aceite en semillas que contenia el promedio de 39 g kg^{-1} de acido palmitico y 26 g kg^{-1} de acido estearico. La linea consaguinea paternal tenia el promedio de 55 g kg^{-1} de acido palmitico y 51 g kg^{-1} de acido estearico. Las plantas de la generacion F_2 tenian el aceite en semillas con el promedio de 47 g kg^{-1} de acido palmitico y 29 g kg^{-1} de acido estearico, o el total de 76 g kg^{-1}. Cuando las plantas F_1 eran cruzadas retrogradamente con la linea consaguinea del girasol cultivado, las plantas BC_1F_1 tenian el promedio de 47 g kg^{-1} de acido palmitico y 28 g kg^{-1} de acido estearico, o el total de 75 g kg^{-1}. La linea consaguinesa del girasol cultivado tenia el

promedio de 54 g kg^{-1} de acido palmitico y 56 g kg^{-1} de acido estearico, o el total de 110 g kg^{-1}. Los datos preliminares indican que el contenido de acidos palmitico y estearico en el aceite de girasol puede ser reducido por la introduccion de genes de un antecesor silvestre en el girasol cultivado. Por la investigacion ulterior sera determinado el modo de herencia de esos acidos grasos. Durante la introduccion de genes para el contenido reducido de acidos grasos saturados en el girasol cultivado sera necesario de seguir tambien otras propiedades agronomicas importantes.

TOURNESOL SAUVAGE VIVACE EN TANT QUE SOURCE POTENTIELLE DE CONTENU RÉDUIT D'ACIDES GRAS PALMITIQUE ET STÉARIQUE DANS L'HUILE DE TOURNESOL

RÉSUMÉ

Dans le but d'obtenir une alimentation humaine plus saine, la tendance générale est de réduire la consommation d'acides gras saturés. L'huile de tournesol, quatrième au monde parmi les huiles végétales comestibles contient 65 g kg^{-1} d'acide palmitique et 45 g kg^{-1} d'acide stéarique qui sont tous les deux des acides gras saturés. Ces quantités sont élevées si on les compare à celles contenues dans l'huile de colza (*Brassica napus* L.): 40 g kg^{-1} d'acide palmitique et 20 g kg^{-1} d'acide stéarique. La diminution des acides gras dans l'huile de tournesol traditionnelle contribuerait à la production d'une huile comestible plus saine. Le but de cette étude préliminaire était d'examiner la riche diversité génétique des ancêtres sauvages du tournesol de culture pour y trouver des sources potentielles de réduction du contenu d'acides palmitique et stéarique. L'huile de la graine d'une population de l'espèce sauvage *H. giganteus* L. (GIG-102) de l'INRA, Montpellier, France contenait 47 g kg^{-1} d'acide palmitique et 18 g kg^{-1} d'acide stéarique. Cette quantité totale de 65 g kg^{-1} d'acides palmitique et stéarique est inférieure de 40% au niveau de ces acides gras dans l'huile de tournesol standard. Le niveau d'acides gras saturés constaté est resté bas quand les plantes ont été cultivées dans des serres et dans des conditions uniformes. La valeur moyenne d'acide palmitique était de 48 g kg^{-1} et celle de l'acide stéarique de 16 g kg^{-1}. Ceci démontre que le contenu d'acides palmitique et stéarique est sous contrôle génétique et qu'il existe une possibilité de transmission de ces caractéristiques dans le tournesol cultivé. Le croisement de ces populations avec une ligne inbred cultivée a produit les plantes F_1 dont le contenu d'huile dans l'akène contenait en moyenne 39 g kg^{-1} d'acide palmitique et 26 g kg^{-1} d'acide stéarique. La ligne inbred du parent contenait en moyenne 55 g kg^{-1} d'acide palmitique et 51 g kg^{-1} d'acide stéarique. Le contenu d'huile dans l'akène des plantes de la génération F_2 était d'en moyenne 47 g kg^{-1} pour l'acide palmitique et de 29 g kg^{-1} pour l'acide stéarique, ou un total de 76 g kg^{-1}. Quand les plantes F_1 ont été de nouveau croisées avec la ligne de tournesol de culture, les plantes BC_1F_1 contenaient en moyenne 47 g kg^{-1} d'acide palmitique et 28 g kg^{-1} d'acide stéarique ou un total de 75 g kg^{-1}. La ligne inbred de tournesol cultivé contenait en moyenne 54 g kg^{-1} d'acide palmitique et 56 g kg^{-1} d'acide stéarique ou un total de 110 g kg^{-1}. Les données préliminaires montrent que le contenu d'acides palmitique et stéarique dans l'huile de tournesol peut être diminué si on introduit le gène d'un ancêtre sauvage dans le tournesol cultivé. Des recherches ultérieures seront nécessaires pour que soit déterminé le processus de transmission de ces acides gras. Il faudra observer d'autres caractéristiques agronomiques importantes lors de l'introduction de gènes à contenu réduit d'acides gras saturés dans le tournesol cultivé.