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# A NEW SUNFLOWER MUTANT WITH INCREASED LEVELS OF PALMITIC ACID IN SEED OIL

Velasco, L., Pérez-Vich, B. and Fernández-Martínez, J.M.*

*Instituto de Agricultura Sostenible (CSIC), Alameda del Obispo s/n, E-14004 Córdoba, Spain*

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SUMMARY

Sunflower oil with increased levels of saturated fatty acids has important applications in food industry. The objective of this research was to develop novel variations with increased saturated fatty acid levels by chemical mutagenesis. Seeds of four different accessions of Peredovik were treated with a solution of ethylmethane sulfonate. $M_2$ seeds from a single $M_1$ plant exhibited a large variation (5-29%) for palmitic acid content. The progenies of all selected $M_2$ seeds showed again continuous ranges of variation (10-30%) for palmitic acid content. Similar continuous segregation was observed in some $M_{3:4}$ and $M_{4:5}$ families derived from high palmitic half seeds (>25%), although other families had uniformly high palmitic acid content. Previous genetic studies of CAS-5, a high palmitic acid mutant which showed clear bimodal distributions in the $M_2$ generation, concluded that the trait was genetically controlled by alleles at three loci. The different segregation patterns observed in NP-40, the new high palmitic acid mutant, suggested that it was genetically different from CAS-5.

**Key words:** **fatty acids, *Helianthus annuus* L., mutagenesis, oil quality, palmitic acid**

## INTRODUCTION

Sunflower oils with increased levels of saturated fatty acids have extended applications in comparison with standard sunflower oil, on account of the higher oxidative stability that is of great value for high-temperature applications (Guinda *et al.*, 2003). Additionally, sunflower oils with increased saturated fatty acid content are semisolid at room temperature, and they can be directly used for margarine and shortening production, without need for previous hydrogenation (Fernández-Moya *et al.*, 2002). Partial hydrogenation of vegetable oils produces *trans* fatty acids that are suspected of raising the risk of coronary heart disease (Katan, 1998).

* Corresponding author: Phone: +34-957499204; Fax: +34-957499252; e-mail: cs9femaj@uco.es

Several sunflower lines with increased saturated fatty acid content (>25% of the total fatty acids compared with 12% in standard sunflower) have been developed by means of chemical or physical mutagenesis. Ivanov *et al.* (1988) reported for the first time a sunflower mutant (275HP) with high levels of saturated fatty acids, in this case the palmitic acid. Osorio *et al.* (1995) conducted a mutagenesis program that resulted in the development of the mutant CAS-3, with high stearic acid content, and the mutant CAS-5, with high palmitic acid content. Fernández-Martínez *et al.* (1997) reported the isolation of CAS-12, a mutant line with high palmitic acid content in high oleic acid background. Fernández-Moya *et al.* (2002) isolated the sunflower mutant CAS-14 with very high stearic acid content (up to 37%). More recently, while evaluating a world germplasm collection, Demurin (2003) identified a germplasm with high palmitic acid levels.

Genetic studies on high stearic acid sunflower mutants have identified two genes, *Es1* and *Es2*, in the mutant CAS-3 (Pérez-Vich *et al.*, 1999b) and a third gene, *Es3*, in the mutant CAS-14 (Pérez-Vich *et al.*, 2006). The inheritance of the high palmitic acid mutant 275HP was studied by Ivanov *et al.* (1988) and it was found to be partially recessive and gametophytic. The number of genes involved in the high palmitic acid content of this mutant has not been determined. The high palmitic acid content of the mutant CAS-5 was found to be controlled by alleles at three independent loci (*P1, P2, P3*), with partial dominance for low concentration and no maternal effects (Pérez-Vich *et al.*, 1999a). The high palmitic acid phenotype was produced when the allele *p1* and at least one of the other two alleles, *p2* or *p3*, were simultaneously present in the homozygous condition. The genetic control of the high palmitic acid trait in CAS-12 was found to be similar to that in CAS-5 (Pérez-Vich *et al.*, 2002).

The objective of this research was to develop further variation for increased saturated fatty acid levels by chemical mutagenesis.

## MATERIALS AND METHODS

About five hundred seeds of four different accessions of the open-pollinated Russian cultivar Peredovik were pre-soaked in water for 4 h and then soaked for 2 h in a mutagenic solution of 70 mM of ethylmethane sulfonate (EMS) prepared in a 0.1 M phosphate buffer at pH 7. After mutagenesis, seeds were thoroughly rinsed with running tap water for 16 h to rinse excess EMS and, after drying, they were immediately sown in the field at the experiment farm of Instituto de Agricultura Sostenible, Córdoba, in March 1999. $M_1$ plants were forced to self-fertilization by isolating their heads with paper bags. One thousand and eighty $M_1$ plants were harvested.

Twelve $M_2$ half-seeds per $M_1$ plant were analyzed for fatty acid profile as described below. $M_2$ seeds from an individual $M_1$ plant showing different levels of increased palmitic acid content were germinated to evaluate the $M_3$ seed generation.

Evaluation and selection for high palmitic acid content was continued until the $M_5$ seed generation.

The fatty acid composition of oil was measured in half seeds by simultaneous extraction and methylation (Garcés and Mancha, 1993) followed by gas-liquid chromatography (GLC) using a Perkin-Elmer Autosystem gas-liquid chromatograph (Perkin-Elmer Corporation, Norwalk, CT, USA). A 2-m-long column packed with 3% SP-2310/2% SP-2300 on Chromosorb WAW (Supelco Inc., Bellefonte, PA, USA) was used. The oven, injector and flame ionization detector were held at 190, 275 and 250°C, respectively.

## RESULTS AND DISCUSSION

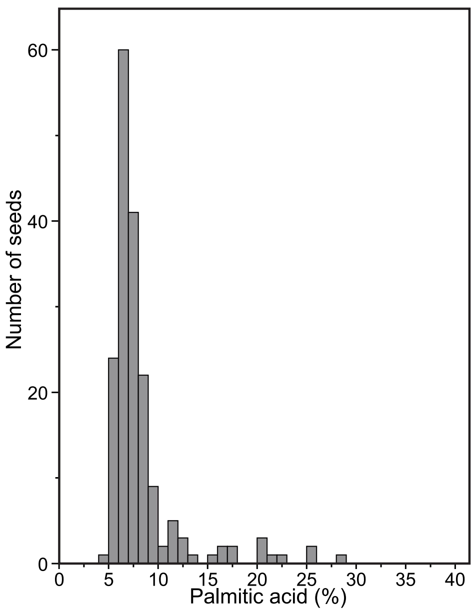


*Figure 1: Palmitic acid content (% of total fatty acids) in 181 $M_2$ half seeds from a mutagenic treatment of seeds of the cultivar Peredovik.*

The initial screening of 12 $M_2$ seeds per $M_1$ plant revealed that seeds of individual $M_1$ plants showed segregation for increased palmitic acid content. Therefore, all $M_2$ seeds from a particular plant were analyzed for fatty acid profile. They showed a wide range of variation for palmitic acid content, from 5.0 to 29.0% of the total fatty acids (Figure 1).

$M_2$ seeds with increased palmitic acid content (>10%) were germinated to evaluate their $M_3$ seed generation. Seed germination and plant survival were very poor, but four $M_2$ plants reached maturity and produced enough seeds for fatty acid analyses. The $M_3$ seeds from these plants showed again segregation for palmitic acid content, with continuous ranges of variation from around 10 to 30% (Figure 2).

Similar continuous segregation was observed in some $M_{3:4}$ families derived from high palmitic acid $M_3$ seeds (>25%), although other families had uniformly high palmitic acid content. Figure 3 shows the contrasting ranges of variation for palmitic acid content of two $M_{3:4}$ families derived from $M_3$ seeds with similarly high palmitic acid content, compared with the palmitic acid content of seeds of the high palmitic acid mutant CAS-5 (Osorio *et al.*, 1995) grown as a check in the same environment.

A similar pattern of palmitic acid variation was observed in the $M_{4:5}$ generation, *i.e.*, some selected families showed uniformly high palmitic acid content (>25%) but others again showed a variable proportion of seeds with low palmitic acid content, even below 10%. These results suggested a complex inheritance of the trait.

Studies on the previously developed high palmitic acid mutant CAS-5, for which the analysis of the $M_2$ generation and different genetic studies invariably revealed clear bimodal distributions (<15% and >25%, respectively) but never continuous variation, indicated a genetic control by alleles at three loci (Pérez-Vich *et al.*, 1999b). The occurrence of wide ranges of variation in both early and advanced generations of this new high palmitic acid sunflower mutant, named NP-40, suggests genetic differences with CAS-5. A comparative genetic study between the two lines is being initiated to elucidate genetic differences between the high palmitic acid mutants NP-40 and CAS-5.

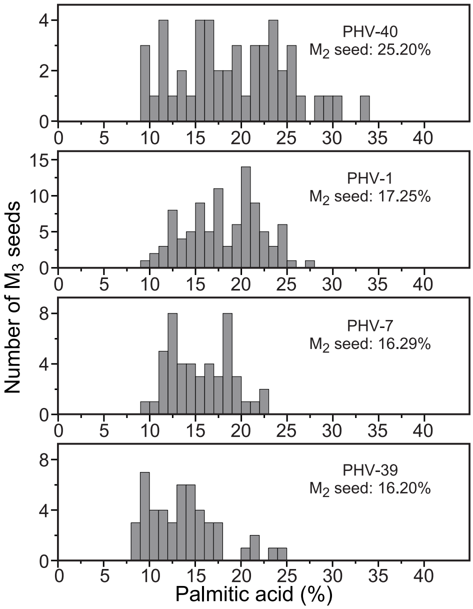


*Figure 2: Palmitic acid content (% of total fatty acids) in $M_3$ half seeds from four $M_2$ plants. The palmitic acid content of the $M_2$ half seed from which each $M_2$ plant was derived is given for each $M_{2:3}$ family.*

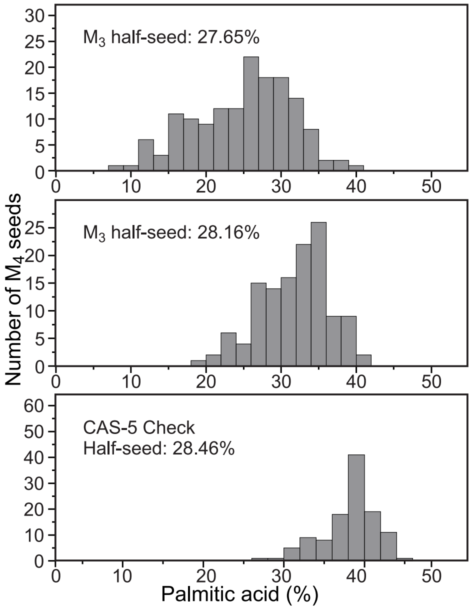


*Figure 3: Palmitic acid content of two $M_{3:4}$ families derived from $M_3$ seeds with high palmitic acid content, and the high palmitic acid mutant CAS-5 grown as a check in the same environment.*

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## UN NUEVO MUTANTE DE GIRASOL CON ALTO CONTENIDO EN ÁCIDO PALMÍTICO EN EL ACEITE

RESUMEN

El aceite de girasol con niveles elevados de ácidos grasos saturados tiene importantes aplicaciones en la industria alimentaria. El objetivo de este trabajo fue el desarrollo de nueva variabilidad para niveles elevados de ácidos grasos saturados en semillas de girasol mediante mutagénesis química. Se trataron semillas de cuatro entradas diferentes de la variedad Peredovik con una solución de metilsulfonato de etilo. Las semillas $M_2$ de una planta $M_1$ individual mostraron una amplia variación (5-29%) para contenido en ácido palmítico. La descendencia de las semillas $M_2$ seleccionadas mostraron de nuevo rangos continuos de variación (10-30%) para contenido en ácido palmítico. Algunas familias $M_{3:4}$ y $M_{4:5}$ procedentes de medias semillas con alto contenido en ácido palmítico (>25%) mostraron asimismo segregación continua para el contenido en este ácido graso, si bien hubo familias que mostraron un alto contenido uniforme en ácido palmítico. Estudios genéticos previos sobre el mutante con alto contenido en ácido palmítico CAS-5, que mostró una clara distribución bimodal en la generación $M_2$, concluyeron un control genético basado en alelos en tres loci. Los diferentes patrones de segregación para ácido palmítico observados en el nuevo mutante, denominado NP-40, sugieren diferencias genéticas entre este nuevo mutante y CAS-5.

## UN NOUVEAU MUTANT DE TOURNESOL AVEC UN TAUX D'ACIDE PALMITIQUE AUGMENTE DANS L'HUILE DE LA GRAINE

RÉSUMÉ

L'huile de tournesol avec des niveaux élevés d'acides gras saturés a d'importantes applications dans l'agro-industrie. L'objectif de cette recherche était de développer de nouvelles variations pour les niveaux d'acides gras saturés par mutagénèse. Les semences de 4 accessions différentes de "Peredovik" ont été traitée avec une solution d'"ethylmethane sulfonate". Les semences $M_2$ provenant d'un plant simple $M_1$ ont révélées une grande variation (5-29%) pour la teneur en acide palmitique. Les descendances de toutes les semences sélectionnées $M_2$ montre à nouveau des variations continues (10-30%) en acide palmitique. Des ségrégations similaire a été observées dans certaines familles de $M_{3:4}$ et $M_{4:5}$ derivées de demi-graines à hautes teneurs en acide palmitique (>25%), bien que d'autres familles aient une teneur d'acide palmitique stable. De précédentes études génétiques sur la haute teneur en acide palmitique issue du mutant CAS-5 qui a démontré de claires distributions bimodales dans la génération $M_2$, concluaient sur un contrôle génétique allélique à trois loci. Les différents modèles de ségrégations observées sur ce nouveau mutant NP-40 suggèrent des différences génétiques avec CAS-5.