

IDENTIFICATION AND GENETIC CHARACTERIZATION OF NEW SOURCES OF BETA- AND GAMMA-TOCOPHEROL IN SUNFLOWER GERMPLASM

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SUMMARY

Sunflower seeds have more than 90% of the total tocopherols in the form of alpha-tocopherol, which exerts a weak antioxidant protection of the extracted oil. A partial substitution of alpha-tocopherol by another tocopherol derivative with greater antioxidant action would improve the oxidative stability of sunflower oil. The objective of the present research was to identify sources of modified tocopherol profile in sunflower germplasm. A total of 952 germplasm accessions were evaluated by analyzing the tocopherol profile of 12 half-seeds per accession by HPLC. The screening resulted in the identification of a source of increased beta-tocopherol content and a source of increased gamma-tocopherol content. In both cases the accessions corresponded to old populations of Peredovick. The line T589 presented a beta-tocopherol content from 30.4 to 48.5% of the total tocopherols, whereas the line T2100 had a gamma-tocopherol content from 87.9 to 93.9%. Genetic studies revealed that both traits were partially recessive in crosses with material with standard tocopherol profile. In both lines, the altered trait was determined by alleles at a single locus. Comparative studies with previously developed sunflower lines with similar levels of beta- and gamma-tocopherol will elucidate whether the new lines have the same or different genetic modifications within the tocopherol biosynthetic pathway.

Key words: inheritance, germplasm, modified tocopherol profile, tocopherols

INTRODUCTION

Tocopherols are the most important compounds with antioxidant activity in sunflower seeds. They act as antioxidants in biological systems (*in vivo* or vitamin E activity) as well as in oils, fats and foods containing them (*in vitro* antioxidant

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activity) (Kamal-Eldin and Appelqvist, 1996). The tocopherols occur as a family of four derivatives named alpha-, beta-, gamma-, and delta-tocopherol, differing in their relative *in vitro* and *in vivo* antioxidant activities. Alpha-tocopherol is the most efficient antioxidant *in vivo*, but it shows weak antioxidant potency *in vitro*. Conversely, gamma-tocopherol is the most powerful antioxidant *in vitro* but its *in vivo* activity is low. Beta and delta tocopherols exhibit intermediate properties (Pongracz *et al.*, 1995).

Standard sunflower seeds mainly contain alpha-tocopherol, which accounts for more than 90% of the total tocopherols. Beta- and gamma-tocopherols can be present in sunflower seeds, usually in amounts below 2% of the total tocopherols (Demurin, 1993; Dolde *et al.*, 1999). The modification of the tocopherol profile through a partial substitution of alpha-tocopherol, with weak *in vitro* antioxidant action, by other tocopherol derivatives is an important goal for developing sunflower oil with improved oxidative stability during storage and use (Škorić, 1992).

Sunflower germplasm with reduced concentration of alpha-tocopherol has been identified and characterized (Demurin, 1993; Demurin *et al.*, 1996). The authors isolated the line LG-15, with increased concentration of beta-tocopherol (50% of the total tocopherols), from the open-pollinated variety VNIIMK 8931, and the line LG-17, with increased concentration of gamma-tocopherol (95% of the total tocopherols), from a germplasm entry of the VIR world germplasm collection. Genetic characterization of both lines concluded that the increased levels of beta-tocopherol were produced by recessive alleles at the *Tph₁* locus, whereas the increased levels of gamma-tocopherol were the result of recessive alleles at the *Tph₂* locus (Demurin *et al.*, 1996).

The objective of the present research was to identify additional sources of variation for tocopherol profile in sunflower germplasm.

MATERIALS AND METHODS

A total of 952 accessions of sunflower were screened for tocopherol composition. The evaluation included part of the sunflower germplasm collection of the USDA, and the germplasm collection of the Institute for Sustainable Agriculture at Córdoba.

Tocopherol evaluation was nondestructively conducted in 1999 by analyzing 12 half seeds per accession. Tocopherols were extracted and separated by HPLC following the method described by Thies (1997) with the modifications of Goffman *et al.* (1999).

Half seeds exhibiting modified tocopherol profile (increased beta- or gamma-tocopherol contents) were identified. Selected half seeds were germinated and the corresponding plants were grown in the greenhouse in 1999. At flowering, each head was covered with a paper bag to avoid contamination with external pollen.

Half seeds from each of the plants were analyzed for tocopherol profile as described above to ensure that the plants bred true for the modified trait.

Half seeds from plants breeding true for increased beta-tocopherol content or increased gamma-tocopherol content as well as half seeds of the standard line HA89 were sown in 2000 and the plants grown in the greenhouse. Plants with increased beta-tocopherol content and plants with increased gamma-tocopherol content were reciprocally crossed with plants of HA-89. Crossing was done by emasculating florets of the female parent followed by pollination of their stigmas with pollen from the male parent. Half seeds of the parents as well as F₁ seeds were analyzed for tocopherol profile and germinated. The plants were grown at the experimental farm of the Institute for Sustainable Agriculture at Córdoba (Southern Spain) in the spring of 2001. F₁ plants were self-pollinated to obtain the F₂ generation. F₂ half seeds as well as seeds of the parents were analyzed for tocopherol profile. The chi-square test was used to evaluate proposed segregation ratios.

RESULTS AND DISCUSSION

Increased beta-tocopherol content

Two half seeds with increased beta-tocopherol content (>30% of the total tocopherols) were identified within the accession PI 307937 during the screening of 12 half seeds per accession. After such results, the rest of the available seeds were nondestructively analyzed, resulting in a total of 15 seeds with increased beta-tocopherol content out of 100 seeds analyzed. Beta-tocopherol content ranged from 32.1 to 46.7% in the seeds with increased levels of this tocopherol derivative, while the other seeds of the accession showed standard tocopherol profile, with less than 5% of beta-tocopherol. All the seeds with increased beta-tocopherol content produced plants that expressed the character uniformly, with beta-tocopherol content in the seeds ranging from 30.4 to 48.5% of the total tocopherols. T589 was formed by bulking equal numbers of seeds from these plants. Similar levels of beta-tocopherol were observed in seeds of T589 in subsequent generations.

The inheritance of the increased beta-tocopherol trait in T589 was studied through crosses with the standard line HA89. The seeds of HA89 had a very low concentration of beta-tocopherol (<2%), while the seeds of T589 presented increased levels of beta tocopherol (>30%). The cross HA89 × T589 produced F₁ seeds exhibiting a beta-tocopherol content from 3.7 to 8.6%, which represents a slight increase in comparison with the levels observed in HA89 (Figure 1). These results suggest partial dominance of standard over increased beta-tocopherol content. F₂ seeds segregated in a 3:1 (standard:increased beta-tocopherol content) ratio (Figure 2), indicating that the trait is controlled by alleles at a single locus.

The line T589 was developed from the accession PI 307937 of the USDA Sunflower Germplasm Collection, which corresponds to an entry of the Russian open

pollinated variety Peredovick. Demurin (1993) and Demurin *et al.* (1996) have previously reported the development of the sunflower line LG-15, with increased beta-tocopherol content, which was developed from the open pollinated variety VNIIMK 8931. According to the authors, the line has a beta-tocopherol content of about 50%, which is the phenotypic expression of the recessive homozygote of the gene *Tph₁*. It has to be elucidated whether the modified tocopherol profile in the seeds of T589 was caused by the same alleles at the same locus as in LG-15. Apparently, both lines exhibit some differences that might indicate different genetic modifications. Thus, beta-tocopherol content in LG-15 has been reported to be recessive (Demurin *et al.*, 1996), whereas it is partially recessive in T589. Similarly, the range of variation of the increased levels of beta-tocopherol in T589 is relatively wide, from 30 to 50%, whereas the trait has been reported to be more stable in LG-15.

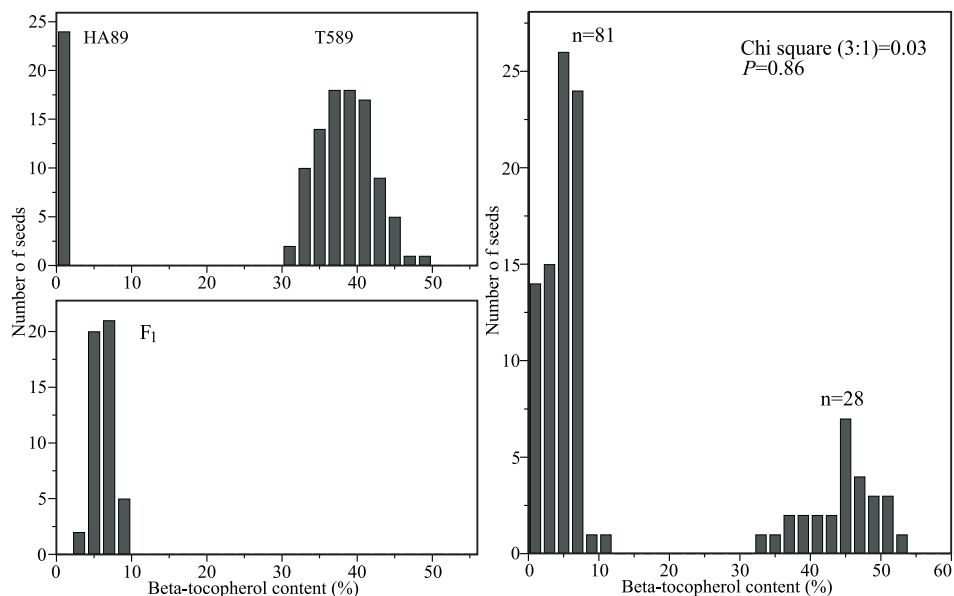


Figure 1: Histograms of beta-tocopherol content in seeds of HA89, T589, and their F₁ Figure 2: Segregation for beta-tocopherol content in F₂ seeds from the cross HA89 × T589

Increased gamma-tocopherol content

Increased levels of gamma-tocopherol were identified in the accession CO-77-256 of the sunflower germplasm collection of the Institute for Sustainable Agriculture at Córdoba. This entry corresponded to a local population of the open pollinated variety Peredovick, formerly cultivated in Spain. Six out of seventy-eight half analyzed seeds of this accession had an increased gamma-tocopherol content ranging from 90.8 to 95.0% of the total tocopherols, compared with less than 1% in the other seeds of the accession. All the seeds with increased gamma-tocopherol con-

tent produced plants that expressed the character uniformly, exhibiting a range of variation from 87.9 to 93.9% of the total tocopherols. T2100 was formed by bulking equal numbers of seeds from the plants having increased gamma-tocopherol content in the seeds. Similar levels of gamma-tocopherol were observed in seeds of T2100 in subsequent generations.

Plants of T2100 were crossed with plants of HA89 to study the inheritance of the increased levels of gamma tocopherol. Seeds of HA89 contained less than 1% of gamma tocopherol, while those of T2100 contained uniformly more than 88% of this tocopherol derivative. F₁ seeds had a gamma-tocopherol content from 1.3 to 4.7%, clearly above the range of variation found in HA89 (Figure 3). These results suggested partial dominance of standard over increased gamma-tocopherol content. F₂ seeds segregated in a 3:1 (standard:increased gamma-tocopherol content) ratio (Figure 4), which demonstrated that the trait is controlled by alleles at a single locus. No seeds with intermediate gamma-tocopherol levels were recovered in the F₂ generation.

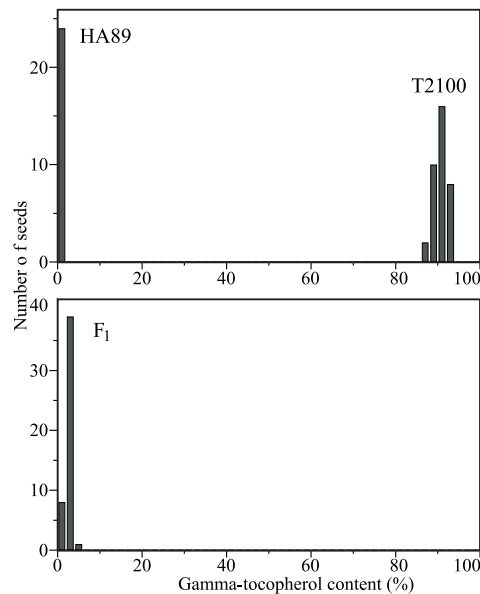


Figure 3: Histograms of gamma-tocopherol content in seeds of HA89, T2100, and their F₁

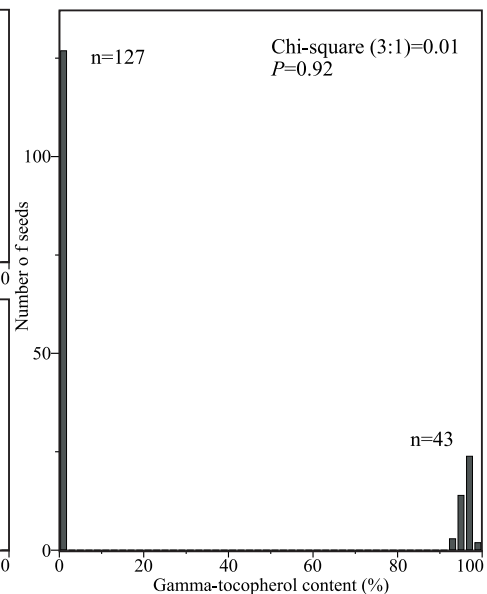


Figure 4: Segregation for gamma-tocopherol content in F₂ seeds from the cross HA89 × T2100

The sunflower line LG-17, with increased concentration of gamma tocopherol, has been previously reported (Demurin, 1993; Demurin *et al.*, 1996). The line was developed from a germplasm entry and it exhibited a gamma-tocopherol content of 95%, close to the levels of the line T2100. Genetic studies revealed that the increased gamma-tocopherol content in LG-17 was controlled by recessive alleles at one locus, *Tph₂*. Comparative studies between the lines LG-17 and T2100 have not been conducted yet.

CONCLUSIONS

Two novel sources of modified tocopherol profile have been developed after the evaluation of large germplasm collections. The line T589 had increased beta-tocopherol content, while the line T2100 presented increased gamma-tocopherol content. Both lines presented tocopherol profiles similar to the lines LG-15 and LG-17, respectively, developed by Demurin (1993), although some apparent differences were observed. Such differences will have to be characterized through a comparative genetic evaluation of both sources of modified beta- and gamma-tocopherol content.

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IDENTIFICACIÓN Y CARACTERIZACIÓN GENÉTICA DE NUEVAS FUENTES DE BETA- Y GAMMA-TOCOFEROL EN GERMOPLASMA DE GIRASOL

RESUMEN

Las semillas de girasol contienen más del 95% de tocoferoles en forma de alfa-tocoferol, cuya acción antioxidante en el aceite extraído es débil. La sustitución parcial de alfa-tocoferol por otros tipos de tocoferoles con mayor poder antioxidante mejoraría la estabilidad oxidativa del aceite de girasol. El objetivo de este trabajo fue identificar germoplasma de girasol con modificación en el perfil de tocoferoles de las semillas. Un total de 952 entradas de germoplasma se evaluaron mediante análisis por HPLC del perfil de tocoferoles de 12 medias semillas por entrada. La evaluación dio como resultado la identificación de una entrada con niveles elevados de beta-tocoferol y otra entrada con niveles elevados de gamma-tocoferol. En ambos casos, las entradas de germoplasma correspondieron a antiguas poblaciones de Peredovick. Tras una generación de autofecundación, la línea T589 presentó un contenido de beta-tocoferol entre 30.4 y 48.5%, mientras que la línea T2100 mostró un contenido de gamma-tocoferol entre 87.9 y 93.9%. Estudios genéticos demostraron que ambos caracteres se comportan como parcialmente recesivos en cruzamientos con material con perfil estándar de tocoferoles. En ambas líneas, los nuevos caracteres estuvieron controlados por alelos en un único locus. Estudios comparativos con líneas similares previamente desarrolladas determinarán si las nuevas líneas poseen las mismas o diferentes alteraciones genéticas en la ruta biosintética de los tocoferoles.

IDENTIFICATION ET CARACTÉRISATION GÉNÉTIQUE DE NOUVELLES SOURCES BETA ET GAMMA DE TOCOPHÉROL DANS LE GERMOPLASME DU TOURNESOL

RÉSUMÉ

Dans la graine de tournesol, plus de 90% du contenu entier de tocophérol se trouve sous la forme de tocophérol alpha qui, par extraction d'huile, offre une faible protection antioxydante. Une substitution partielle du tocophérol alpha par d'autres dérivés de tocophérol ayant une action antioxydante plus puissante améliorerait la stabilité oxydante de l'huile de tournesol. Le but de ce travail était d'identifier les sources du profil de tocophérol modifié dans le germoplasme du tournesol. Neuf cent cinquante-deux échantillons de germoplasmes ont été évalués par l'analyse sur HPLC du profil de tocophérol de 12 demi-graines par échantillon. L'analyse a identifié une source de contenu accru de tocophérol bêta et une source de contenu accru de tocophérol gamma. Dans les deux cas, les échantillons correspondaient aux anciennes populations de la sorte Peredovick. Dans la ligne T589, le contenu de tocophérol bêta comptait de 30,4 à 48,5% du contenu entier de tocophérol alors que dans la ligne T2100 le contenu de tocophérol gamma était de 87,9 à 93,9%. Les examens génétiques ont montré que ces deux propriétés étaient partiellement récessives dans les croisements avec le matériel qui avait un profil de tocophérol standard. Dans les deux lignes la propriété modifiée était déterminée par les allèles sur locus unique. Une étude comparative avec des lignes de tournesol créées antérieurement et ayant un contenu de tocophérol bêta et gamma semblable montrera si ces nouvelles lignes ont les mêmes modifications génétiques ou si celles-ci sont différentes dans le cadre de la trajectoire biosynthétique de tocophérol.

