NOVEL VARIATION FOR TOCOPHEROL PROFILE IN SUNFLOWER SEEDS

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Abstract

The tocopherol fraction of sunflower seeds is mainly made up of alpha-tocopherol, which accounts for more than 90% of the total tocopherols. The in vitro antioxidant efficiency of alpha-tocopherol is much lower than that exhibited by other tocopherol derivatives. Because a greater oxidative stability is demanded for most uses of sunflower oil, the present research aims to produce sunflower germplasm in which alpha-tocopherol is partly replaced by other tocopherols. The research comprised three stages: 1) germplasm evaluation, 2) mutagenesis, and 3) recombination. Germplasm evaluation for tocopherol profile led to the isolation of two germplasms with modified tocopherol profiles: T589, with increased concentration of beta-tocopherol (>30% of the total tocopherols), and T2100, with increased concentration of gamma-tocopherol (>85% of the total tocopherols). The use of chemical mutagenesis led to the isolation of two additional sources of increased gamma-tocopherol concentration: IAST-540 and IAST-1, both with more than 85% of the tocopherols in the gamma-tocopherol form. Different segregation patterns during the isolation process suggested genetic differences for increased gamma-tocopherol concentration between IAST-540 and IAST-1. Recombination between T589 and IAST-1 produced unexpected transgressive segregations for increased beta-tocopherol concentration (up to 77% of the total tocopherols) or increased delta-tocopherol concentration (up to 68% of the total tocopherols).

Introduction

Tocopherols are the most important compounds having 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 activity) (Kamal-Eldin and Appelqvist, 1996). The tocopherols occur as a family of four derivatives named alpha-, beta-, gamma-, and delta-tocopherol. Amongst the tocopherols, alpha-tocopherol is the most efficient antioxidant in vivo, but it shows the weakest antioxidant potency in vitro. Conversely, beta-, gamma-, and delta-tocopherol possess a lower vitamin E value, but they exert a considerably greater in vitro antioxidant protection than alpha-tocopherol (Pongracz et al., 1995).

Standard sunflower seeds mainly contain alpha-tocopherol, which accounts for more than 90% of the total tocopherols. Beta- and gamma-tocopherol 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 accession 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 develop additional sources of variation for tocopherol profile through a combined strategy based on germplasm evaluation, mutagenesis, and recombination. The most relevant results are summarized in the present manuscript. More detailed information can be found in Velasco et al. (2003, 2004a,b).

Materials and Methods

Germplasm Evaluation. 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 as described below by analyzing 12 half seeds per accession. 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 to ensure that the plants bred true for the modified trait.

Mutagenesis. The open-pollinated cultivar 'Peredovik,' formerly widely cultivated in Spain, was selected for mutagenesis. Four different accessions of 'Peredovik,' all of them with more than 95% of the total tocopherols in the form of alpha-tocopherol, were chosen. About five hundred seeds from each one were presoaked in water for 4 h and then soaked for 2 h with a mutagenic solution of 70 mM of ethyl methane 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 experimental farm of the Institute of Sustainable Agriculture, Córdoba in March 1999. M1 plants were self-pollinated by using paper bags to prevent uncontrolled pollination. One thousand-eighty M1 plants were harvested. Six M2 half-seeds per M1 plant were analyzed for tocopherol profile as described below. After identification of variants with increased levels of gamma-tocopherol in the analysis of M2 seeds, selection for high gamma-tocopherol content was conducted till the M5 seed generation.

Recombination. M4 plants with increased gamma-tocopherol concentration in the seeds, developed in the mutagenesis program, were crossed with plants of the line T589, with increased levels of beta-tocopherol, isolated in the germplasm evaluation program. F2 seeds from these crosses were analyzed for tocopherol profile, as described above. Recombinants with novel tocopherol profiles were identified in the F2 and confirmed in the F3 seed generation.

Tocopherol Analyses. Tocopherols were extracted and separated by HPLC following the method described by Thies (1997) with the modifications of Goffman et al. (1999).

Results and Discussion

Germplasm Evaluation. 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 these 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 a 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.

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 formerly cultivated in Spain of the open-pollinated variety Peredovik. Six out of seventy-eight half seeds analyzed of this accession had an increased gamma-tocopherol content ranging from 90.8 to 95.0% of the total tocopherols, compared to less than 1% in the other seeds of the accession. All the seeds with increased gamma-tocopherol content 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.

The inheritance of the increased beta-tocopherol trait in T589 and the increased gamma-tocopherol content in T2100 was studied in crosses with the standard line HA 89. The seeds of HA 89 had a very low concentration of beta-tocopherol (<2%). The cross HA 89 x 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 HA 89. 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 1a), indicating that the trait is controlled by alleles at a single locus. F1 seeds from the cross HA89 x T2100 had a gamma-tocopherol content from 1.3 to 4.7%, clearly above the range of variation found in HA89, which suggested partial dominance of standard over increased gamma-tocopherol content. F2 seeds segregated in a 3:1 (standard: increased gamma-tocopherol content) ratio (Fig. 1b), 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.

Sunflower germplasm with similar tocopherol profiles to T589 and T2100 has been reported by Demurin (1993) and Demurin et al. (1996). The authors developed the inbred line LG 15, with similar increased levels of beta-tocopherol to T589, from the open-pollinated variety ‘VNIIMK 8931,’ as well as the inbred line LG 17, with similar increased levels of gamma-tocopherol to T2100, from the Russian germplasm accession VIR 44. A comparative evaluation of both sources of beta- and gamma-tocopherol is currently underway.

Mutagenesis. Two mutants with modified tocopherol profiles were identified after the analysis of M2 half seeds. One of the mutants was derived from one M2 seed with high gamma-tocopherol content (96.7%). The corresponding plant produced M3 seeds averaging 93.7% gamma-tocopherol, with a range of variation from 89.6% to 96.9%, similar to those exhibited by the germplasms T2100 and LG-17 (Demurin, 1993). The mutant was named IAST-540.

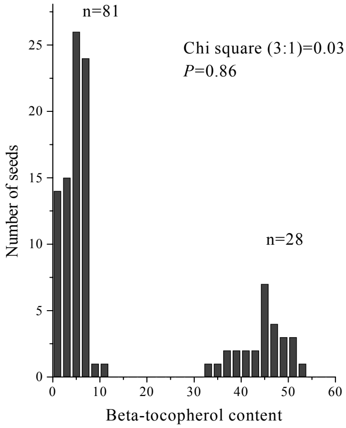

Figure 1a. Segregation for beta-tocopherol content in F2 seeds from the cross HA89 x T589

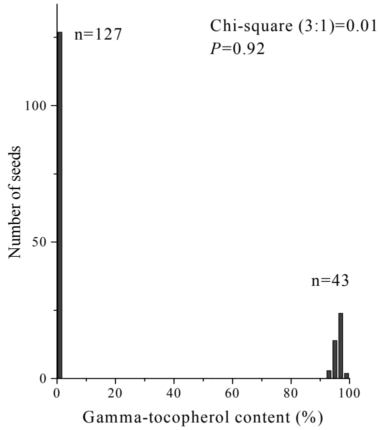

Figure 1b. Segregation for gamma-tocopherol content in F2 seeds from the cross HA89 x T2100

The second mutant was isolated from one M2 seed with 19.2% gamma-tocopherol. The M3 generation exhibited a wide variation for gamma-tocopherol content, from zero to 84.5%, with the remaining being alpha-tocopherol. Gamma-tocopherol in M3 seeds followed an apparent bimodal distribution, with about one fourth of the seeds (n=11, $\chi2$=1.42, p=0.23) having more than 65% gamma-tocopherol (Figure 2).

Selection for high gamma-tocopherol content (>80%) led to two M3 plants that produced M4 seeds segregating from 60.4% to 97.4% gamma-tocopherol. M4 seeds with more than 90% gamma-tocopherol produced plants that expressed the high gamma-tocopherol trait uniformly, with an average gamma-tocopherol content of 94.1% (392 M5 seeds from five M4 plants) and a range of variation between 87.6% and 97.2%. The mutant was named IAST-1. The wide segregation for intermediate levels of gamma-tocopherol found during the isolation of IAST-1 has not been observed in the other germplasms with high gamma-tocopherol content, neither in LG-17 developed by Demurin et al. (1996) nor in T2100 and IAST-540 developed in the present research. Such a different segregation pattern suggests genetic differences involved in the high gamma-tocopherol trait between IAST-1 and the other high gamma-tocopherol germplasms.

Recombination. Crosses between plants of IAST-1 and T589 produced F1 seeds with standard high alpha-tocopherol content and less than 2% of both beta- and gamma-tocopherol. On the contrary, F2 seeds exhibited a wide variation for tocopherol profile, including transgressive segregation for both beta- and delta-tocopherol, the latter being absent from T589 and present in small amounts (<2%) in seeds of IAST-1.

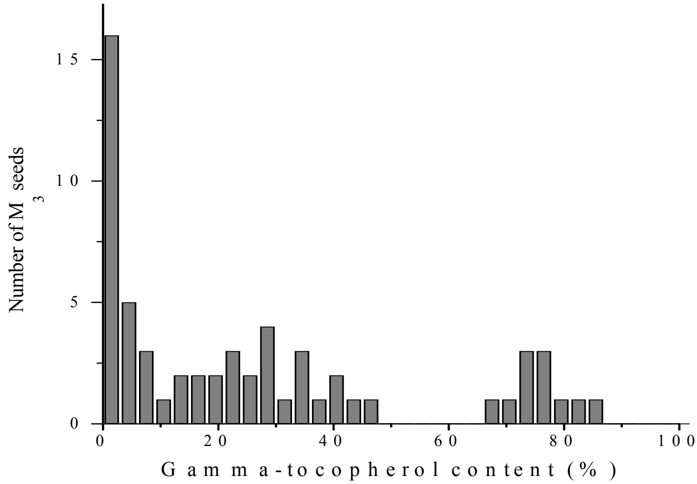

Figure 2. Histogram of gamma-tocopherol content (% of the total tocopherols) in M3 seeds of an M2 plant derived from an M2 half seed with 19.2% gamma-tocopherol.

The analysis of 647 F2 seeds from five F1 plants revealed ranges of variation from zero to 77.0% for beta-tocopherol, and from zero to 67.9% for delta-tocopherol (Figure 3). Seeds of T589 from plants grown in the same environment had a beta-tocopherol content from 30.1% to 55.1%. Selection for high beta-tocopherol content in the F2 generation led to F3 progenies that expressed the transgressive high beta-tocopherol content uniformly, with an average tocopherol profile made up of 30.2 ± 3.2% alpha-tocopherol and 69.8 ± 3.2% beta-tocopherol. The line was named IAST-5. Beta-tocopherol content in IAST-5 was considerably higher that that found in the other two lines with increased beta-tocopherol content developed so far, LG15 (Demurin et al., 1996) and T589, suggesting that increased beta-tocopherol content in IAST-5 may respond to a different genetic control.

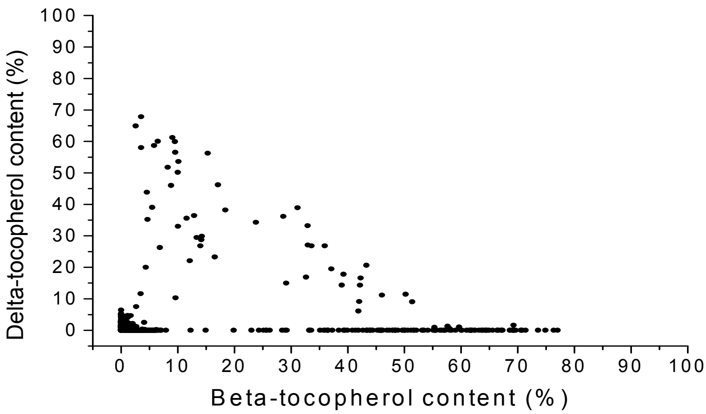

Figure 3. Scatter plot of beta- vs. delta-tocopherol content (% of the total tocopherols) in F2 seeds from the cross of plants of the sunflower mutant IAST-1 (>90% gamma-tocopherol) with plants of the germplasm line T589 (30.1% to 55.1% beta-tocopherol).

F2 seeds with transgressive high delta-tocopherol content produced plants that expressed the trait uniformly, with an average tocopherol profile in F3 seeds of 4.3 ± 1.9% alpha-tocopherol, 3.2 ± 3.1% beta-tocopherol, 34.3% ± 7.9% gamma-tocopherol, and 58.2 ± 7.1% delta tocopherol. The range of variation for delta-tocopherol content was found within 47.0% and 73.0%. The line was named IAST-4. Demurin (1993) reported that the combination of the recessive allele for increased beta-tocopherol at the Tph1 locus and the recessive allele for high gamma-tocopherol at the Tph2 locus resulted in the transgressive production of 8% delta-tocopherol. A higher delta-tocopherol content of 22% was reported by Demurin et al. (1996) when both recessive alleles were transferred to the inbred line VK66. However, no levels similar to those of the IAST-4 germplasm have been reported. The high levels of up to 73% delta-tocopherol in IAST-4 suggest the presence in this line of other alleles different to those reported by Demurin et al. (1996).

Conclusions

A large variation for tocopherol profile in sunflower has been developed through the combined use of germplasm evaluation, mutagenesis, and recombination. Among the six new lines developed, T2100, IAST-540, and IAST-1 are characterized by a high gamma-tocopherol content (>85%), T589 and IAST-5 have increased beta-tocopherol content (>30% and >65%, respectively), and IAST-4 shows increased levels of delta-tocopherol (>45%). Such a large variation will be of great value for tailoring novel sunflower oils for specific applications demanding a greater resistance to oxidative processes than that exhibited by current sunflower oil types. Additionally, these lines represent a useful collection of genetic modifications at different steps of the tocopherol biosynthetic pathway, of great utility for genetic and biochemical studies on plant tocopherols. The lines T589 and T2100 have already been released and they are available for the industry and the scientific community (Velasco et al., 2004b).

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