

## A modifying gene affecting gamma-tocopherol content in sunflower

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### ABSTRACT

High levels of gamma-tocopherol confer greater oxidative stability to sunflower oil. Several lines with high gamma-tocopherol content have been developed and in all of them the trait has been found to be controlled by recessive alleles at the *Tph2* locus, underlying a gamma-tocopherol methyltransferase. Genetic studies involving crosses between standard lines and lines with high gamma-tocopherol content reported bimodal segregation patterns with clear-cut classes including low gamma-tocopherol (<10%) and high gamma-tocopherol (>85%) levels, respectively but not intermediate levels. Intermediate gamma-tocopherol content ranging from 10 to 85% has only been reported in the isolation process of the line IAST-1, derived from a mutagenic treatment. The objective of this research was to investigate the occurrence of intermediate gamma-tocopherol content in crosses involving the high gamma-tocopherol line IAST-1. Plants of the high gamma-tocopherol lines T2100 and IAST-1, and the standard line HA89 were crossed and the F<sub>2</sub> seed generation was analysed for seed tocopherol profile. F<sub>2</sub> seeds from all the F<sub>1:2</sub> families from the crosses between HA89 and T2100 followed bimodal distributions with clear-cut classes fitting a 3:1 (<5%:>90%) ratio, corresponding to the expected segregation of the recessive alleles *tph2*. In addition to the 3:1 ratio, a 13:3 (<80%:>90%) ratio was identified in F<sub>1:2</sub> families from the crosses between HA89 and IAST-1, which included F<sub>2</sub> seeds with intermediate levels of gamma-tocopherol (5 to 80%). Intermediate levels of gamma-tocopherol were also observed in some F<sub>1:2</sub> families derived from the crosses between T2100 and IAST-1. The results suggested the presence of a modifying gene that produced intermediate gamma-tocopherol levels in combination with the *tph2* alleles.

**Key words:** gamma-tocopherol – modifying gene – oil quality – tocopherols

### INTRODUCTION

Conventional sunflower seeds mainly contain alpha-tocopherol, which accounts for more than 90% of the total tocopherols. Several lines with modified tocopherol profiles have been developed. Demurin (1993) reported the lines LG-15 and LG-17, with increased levels of beta-tocopherol (50%) and gamma-tocopherol (95%), respectively. Both lines were developed from segregating accessions identified in a germplasm collection. Also in the course of germplasm evaluation, Velasco et al. (2004a) identified variations for beta- and gamma-tocopherol content, which allowed the development of the lines T589 and T2100, with increased levels of beta-tocopherol (>30%) and gamma-tocopherol (>85%), respectively. Additional variation for gamma-tocopherol content was created in sunflower by using chemical mutagenesis (Velasco et al., 2004b). The authors identified two M<sub>2</sub> seeds, derived from different M<sub>1</sub> plants, with increased gamma-tocopherol contents of 19.2% and 96.7%, respectively. M<sub>3</sub> progenies from the M<sub>2</sub> seed with 96.7% bred true for high gamma-tocopherol content, containing more than 90% gamma-tocopherol, which led to the development of the line IAST-540. M<sub>3</sub> progenies from the M<sub>2</sub> seed with 19.2% gamma-tocopherol segregated from zero to 84.6% gamma-tocopherol. Selection for high gamma-tocopherol content produced the line IAST-1, with stable high gamma-tocopherol content.

Genetic studies conducted by Demurin et al. (1996) 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). Similarly, Velasco and Fernández-Martínez (2003) reported the presence of recessive alleles at a single locus underlying the increased beta-tocopherol content in T589 and the high gamma-tocopherol content in T2100 seeds. Comparative genetic studies concluded that *tph1* alleles were present in both LG-15 and T589 lines (Demurin et al., 2004; Vera-Ruiz et al., 2005), and *tph2* alleles were present in the high gamma-tocopherol lines LG-17, T2100, IAST-540, and IAST-1 (Demurin et al., 2004; García-Moreno et al., 2006). The *Tph2* gene underlies a gamma-tocopherol methyltransferase (Hass et al., 2006). Genetic studies involving crosses between lines with high gamma-tocopherol content and lines with wild-type high alpha-tocopherol content have reported bimodal segregation patterns with clear-cut classes including low gamma-tocopherol (<10%) and high gamma-tocopherol (>85%) levels, respectively (Demurin et al., 1996; Velasco and Fernández-Martínez, 2003). Intermediate gamma-tocopherol content ranging from 10

to 85% in germplasm segregating for *tph2* alleles has only been reported so far in the isolation process of IAST-1. The objective of this research was to investigate the occurrence of intermediate gamma-tocopherol content in crosses involving the high gamma-tocopherol line IAST-1.

### MATERIALS AND METHODS

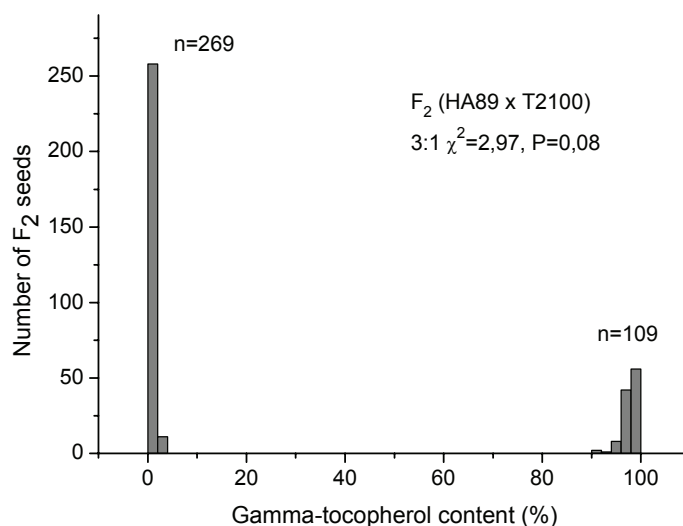
The study included the sunflower lines T2100 and IAST-1, with high gamma-tocopherol content (>85%), and the standard line HA89, with high alpha-tocopherol content (>95%). T2100 was developed from an accession of the open pollinated cultivar 'Peredovik' (Velasco et al., 2004a). IAST-1 was isolated after chemical mutagenesis on seeds of several 'Peredovik' accessions (Velasco et al., 2004b). HA89 is an oilseed maintainer line released by the Texas Agricultural Experiment Station and the USDA-ARS in 1971.

Twenty-four half seeds of HA89, T2100, and IAST-1 were nondestructively analyzed for tocopherol profile, germinated and planted in pots under open air conditions in spring 2005. Plants of the three lines were crossed following an incomplete diallel design. Half seeds of the parents as well as F<sub>1</sub> half seeds were analysed for tocopherol profile. F<sub>1</sub> and parent half seeds were sown in March 2006 and the corresponding plants were grown in pots under open air conditions. F<sub>1</sub> plants were self-pollinated to obtain the F<sub>2</sub> generation.

Twenty-four to 96 F<sub>2</sub> half seeds from 12 to 24 F<sub>1</sub> plants from each cross were analysed for tocopherol profile following the procedure reported by Velasco et al. (2004b).

### RESULTS AND DISCUSSION

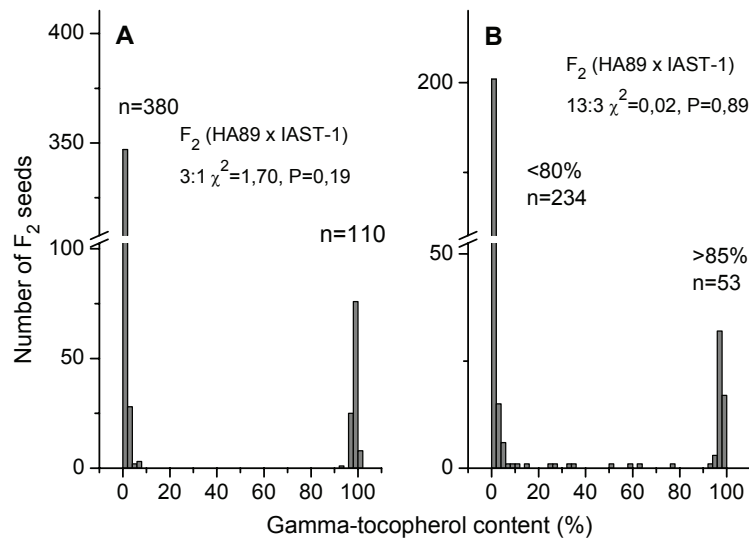
Seeds of the high gamma-tocopherol lines T2100 and IAST-1 showed uniformly high gamma-tocopherol content (>95% of the total tocopherols). Seeds of the standard line HA89 showed uniformly high alpha-tocopherol content (>95%). F<sub>2</sub> seeds from all the F<sub>1,2</sub> families from the crosses between HA89 and T2100 followed bimodal distributions with clear-cut classes characterized by low (<5%) and high (>90%) gamma-tocopherol content that fitted a 3:1 (low:high) segregation ratio (Fig. 1), corresponding to the expected segregation of the recessive alleles *tph2* (Demurin et al., 1996; Velasco and Fernández-Martínez, 2003).



**Fig. 1.** Gamma-tocopherol content (% of the total tocopherols) in F<sub>2</sub> seeds from the cross between the high gamma-tocopherol line T2100 and the standard line HA89.

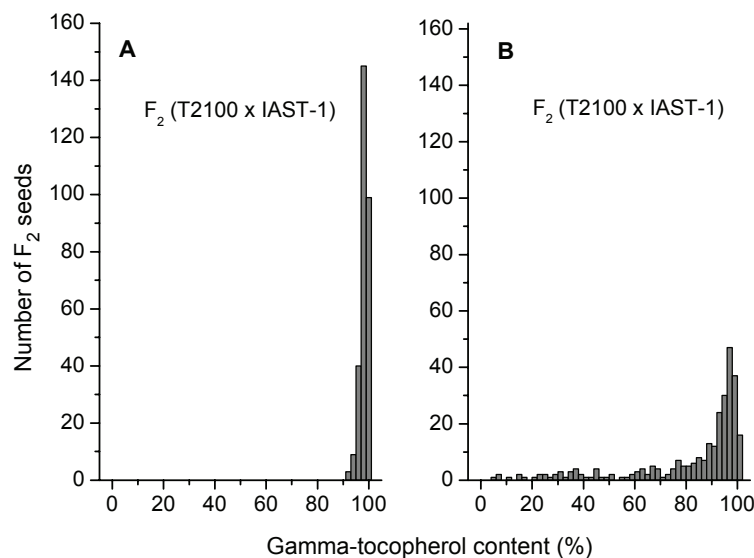
Two different segregation patterns were identified in F<sub>1,2</sub> families from the crosses between the standard line HA89 and the high gamma-tocopherol line IAST-1. The first segregation pattern was similar to that observed for the cross between HA89 and T2100, with F<sub>2</sub> seeds distributed into low and high gamma-tocopherol classes that fitted a 3:1 segregation ratio (Fig. 2A). The second segregation pattern showed the particularity of the presence of F<sub>2</sub> seeds with intermediate levels of gamma-tocopherol content (5 to 80%). The high gamma-tocopherol (>90%) class included 3 out of every 16 F<sub>2</sub> seeds (Fig.

2B), suggesting the presence of a second recessive gene that produced intermediate gamma-tocopherol levels in combination with the *tph2* alleles in a homozygous condition.



**Fig. 2.** Gamma-tocopherol content (% of the total tocopherols) in F<sub>2</sub> seeds from the cross between the high gamma-tocopherol line IAST-1 and the standard line HA89.

Two different patterns of gamma-tocopherol distribution were also identified in F<sub>2</sub> seeds from F<sub>1:2</sub> families derived from the crosses between the high gamma-tocopherol lines T2100 and IAST-1. F<sub>2</sub> seeds had uniformly high gamma-tocopherol content in some F<sub>1:2</sub> families (Fig. 3A), whereas other families showed segregation for a wide range of intermediate gamma-tocopherol levels (Fig. 3B).



**Fig. 3.** Gamma-tocopherol content (% of the total tocopherols) in F<sub>2</sub> seeds from the cross between the high gamma-tocopherol lines T2100 and IAST-1.

A previous genetic study concluded that the high gamma-tocopherol lines T2100 and IAST-1 shared the alleles *tph2*, as both the F<sub>1</sub> and F<sub>2</sub> seed generations from crosses between them showed uniformly a high gamma-tocopherol content (García-Moreno et al., 2006). The present research work suggested the presence of a modifying gene affecting gamma-tocopherol content in IAST-1. The modifying gene produced a reduction in gamma-tocopherol content from high (>90%) to intermediate (5 to 80%) levels in

seeds with expected allelic configuration *tph2tph2*. This effect was observed in some F<sub>1</sub> plants from the crosses of IAST-1 with HA89 and T2100, but not in others. Additionally, the genetic effect of the modifying gene was not expressed in seeds of the IAST-1 parent grown in the same environment. Modifying genes affecting high oleic acid content have been reported in sunflower, leading to suppression of the trait (Lacombe et al., 2001) or a strong distortion of segregation patterns (Fernández-Martínez et al., 1989). Further characterization of the modifying gene affecting high gamma-tocopherol content in sunflower is currently under way.

#### ACKNOWLEDGEMENTS

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