

GENETIC IDENTIFICATION OF TOCOPHEROL MUTATIONS IN SUNFLOWER

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SUMMARY

Two new inbred lines, T589 (medium β -tocopherol content) and T2100 (high γ -tocopherol content), recently developed in CSIC, Cordoba, Spain, have been crossed to known *tph1* and *tph2* mutations which possessed the same phenotypes and which were obtained at VNIIMK, Krasnodar, Russia. Genetic identification of these recessive mutations with TLC profiles showed the new medium β -tocopherol mutation to be allelic to *tph1* and the new high γ -tocopherol mutation to be allelic to *tph2*.

Key words: allelic test, sunflower seeds, tocopherol mutations

INTRODUCTION

Tocopherols are the most powerful natural fat-soluble antioxidants (vitamin E). They exist in four homologous forms - α , β , γ and δ , which differ in number or position of methyl groups in their molecules. As a result there is an inversion in vitamin and antioxidant activity in the line.

Sunflower tocopherol complex is known to contain a prevalence content of α -homologue, about 95%, which has the highest vitamin and the lowest antioxidant properties while the main part of oil crops possesses the high percentage of other homologues, especially γ -tocopherol, making the complex more balanced.

It seems to be possible to increase the oil oxidative stability via high level of antioxidant protection by breeding for changed tocopherol composition in sunflower seeds (Kurnik, 1967; Rzechin, 1969; Demurin *et al.*, 1996). Obviously, the optimal content of different tocopherols is determined by the area of oil application.

Two non-allelic unlinked genes designated *Tph1* and *Tph2*, controlling tocopherol composition in sunflower seeds, were identified in VNIIMK, Krasnodar, Russia (Demurin, 1993). Recessive alleles of the genes were found by wide-scale screening and selfing in spontaneous mutations on the base of "half-seed technique". Original

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inbred lines with modified tocopherol composition have been developed. *Tph1* gene controls the ratio of α - and β - tocopherols, whereas *Tph2* gene affects that of α - and γ - homologues. The *tph2* mutation has an epistatic action over *tph1*.

Two new inbred lines T589, with medium β -tocopherol content, and T2100, with high γ -tocopherol content, have been recently developed in CSIC, Cordoba, Spain.

The main objective of this research includes allelic test of newly developed mutations to known *tph1* and *tph2* mutations.

MATERIALS AND METHODS

Our inbred lines VK571 (*tph1tph1* genotype), VK175 (*tph2tph2*) were used as male in allelic test crosses and VK876 CMS (*tph1tph1 tph2tph2*) as female.

Inbred lines T589, with medium β -tocopherol content, and T2100, with high γ -tocopherol content, were received from CSIC, Cordoba, Spain.

Crosses were made in a field plot of VNIIMK, Krasnodar, in the summer 2003 using hand emasculation (T589), male sterility (T2100) and CMS PET1 (VK876).

Tocopherol composition was determined with thin-layer chromatography (TLC) followed by Emmerie-Engel reaction without any correction to different rate of homologues staining (Popov *et al.*, 1991). Analyses were performed in each tenth seed on a sunflower head.

RESULTS AND DISCUSSION

Spontaneous recessive mutation *tph1* of the inbred line VK571 has about 50% of α - and 50% of β -homologues in seed tocopherol complex (Table 1). This mutation was found after self pollination of a plant of the variety VNIIMK 8931 in 1983 with the population allele frequency of about 0.01.

Table 1: Phenotypes of *tph1* and *tph2* mutations in sunflower (TLC)

Genotype, inbred line	Tocopherol, %			
	α	β	γ	δ
<i>Tph1Tph1</i> , wild type	95	2	3	0
<i>tph1tph1</i> , VK571	50	50	0	0
<i>tph2tph2</i> , VK 175	5	0	95	0
<i>tph1tph1 tph2tph2</i> , VK876	0	0	60	40

Spontaneous recessive mutation *tph2* of the inbred line VK175 has about 5% of α - and 95% of γ -homologues in seed tocopherol complex (Table 1). This mutation was found in the seeds of the specimen No. 44 of the VIR world germplasm collection (St.Petersburg) in 1986 with the population allele frequency of about 0.96.

Genetic background was found to influence the expressivities of both *tph2* mutation and its double recessive homozygote. Tocopherols of the double recessive homozygote with VK876 background included only γ - and δ - homologues (Table 1).

The inbred line T589 possessed medium β -tocopherol content of about 50% and T2100 had high γ -tocopherol content of 100% (Table 2).

Table 2: Phenotype of medium β - and high γ -tocopherol inbred lines from Cordoba, Spain (TLC), grown in Krasnodar

Inbred line	Tocopherol, %			
	α	β	γ	δ
T 589	50	50	0	0
T 2100	0	0	100	0

The lines from Cordoba T589 and T2100 were used as female in cross No. 1 or as male in cross No. 2.

All F_1 seeds in each combination showed mutant phenotype. In case of T589 it was medium β -tocopherol content of about 50% (Table 3). Hybrid seeds in the cross with T2100 exhibited high content of γ -tocopherol of 100% (Table 4).

Table 3: Allelic test on *tph1* mutation

	Female	Male	Hybrid
Cross No. 1	T589 \times VK571		F_1
Phenotype	mutant, medium β	mutant, medium β	mutant, medium β
Genotype	<i>tph1tph1</i>	<i>tph1tph1</i>	<i>tph1tph1</i>
Cross No. 2	VK876 \times T589		F_1
Phenotype	mutant, medium δ	mutant, medium β	mutant, medium β
Genotype	<i>tph1tph1</i> <i>tph2tph2</i>	<i>tph1tph1</i> <i>Tph2Tph2</i>	<i>tph1tph1</i> <i>Tph2tph2</i>

Table 4: Allelic test on *tph2* mutation

	Female	Male	Hybrid
Cross No. 1	T2100 \times VK175		F_1
Phenotype	mutant, high γ	mutant, high γ	mutant, high γ
Genotype	<i>tph2tph2</i>	<i>tph2tph2</i>	<i>tph2tph2</i>
Cross No. 2	VK876 \times T2100		F_1
Phenotype	mutant, medium δ	mutant, high γ	mutant, high γ
Genotype	<i>tph1tph1</i> <i>tph2tph2</i>	<i>Tph1Tph1</i> <i>tph2tph2</i>	<i>Tph1tph1</i> <i>tph2tph2</i>

CONCLUSIONS

The genetic identification in F_1 allelic test for recessive characters with recently developed inbred lines T589 and T2100 showed the new medium β -tocopherol mutation to be allelic to *tph1* and new high γ -tocopherol mutation to be allelic to *tph2*.

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**IDENTIFICACIÓN GENÉTICA DE LAS MUTACIONES
TOCOFERÓLICAS EN GIRASOL**

RESUMEN

Dos nuevas líneas consanguíneas, T589 (mediano contenido de β -tocopherol) y T2100 (alto contenido de γ -tocopherol), que se formaron recientemente en CSIC en Córdoba (España), se cruzaron con las mutaciones conocidas de *tph1* y *tph2*. Estos cruzamientos son semejantes desde el punto de vista de fenotipo con los cruzamientos realizados en VNIIMK, Krasnodar, Rusia. Los datos sobre la identificación genética de estas mutaciones recesivas mediante los perfiles TLC, han demostrado que la nueva mutación para el mediano contenido de β -tocopherol es alelomorfa en relación con *tph1* y que la nueva mutación para el alto contenido de γ -tocopherol es alelomorfa en relación con *tph2*.

**IDENTIFICATION GÉNÉTIQUE DE LA MUTATION DE
TOCOPHÉROL DANS LA CULTURE DE TOURNESOL**

RÉSUMÉ

Deux nouvelles lignes cultivées, T589 (niveau moyen de β -tocophérol) et T2100 (niveau élevé de γ -tocophérol) sont récemment créées à Instituto de Agricultura Sostenible (CSIC), à Córdoba en Espagne par le croisement des mutations connues *tph1* et *tph2*, qui possèdent les phénotypes identiques obtenus à (VNIIMK) à Krasnodar, en Russie. Les données sur l'identification génétique de ces mutations récessives ont démontré que la nouvelle mutation de niveau moyen de β -tocophérol est allélomorphe par rapport de *tph1* et la nouvelle mutation de niveau élevé de γ -tocophérol est allélomorphe par rapport de *tph2*.