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EXPRESSIVITY OF TOCOPHEROL MUTATIONS IN SUNFLOWER

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SUMMARY

Influences of genetic background, ontogenesis and environment on tocopherol mutations in sunflower were estimated in this study. The content of α/β-tocopherols varied from 40/60 to 60/40% in seeds of different inbred lines containing the *tph1* gene. The lines containing the *tph2* gene ranged widely in α/γ-tocopherols, from 0/100 to 80/20%. A double mutation showed variability in different inbred lines in α/β/γ/δ-tocopherol contents from maximum expressivity of 0/0/60/40 to minimum 40/25/25/10%, due to incomplete expressivity of *tph2*. Seed maturation from 10 to 38 DAF influenced tocopherol composition in both normal and mutant genotypes by increasing the α-tocopherol content. The content varied from 81 to 97% in a normal genotype, from 33 to 50% in *tph1* mutation and from 0 to 6% in *tph2* mutation. Tocopherol mutations were shown to express their phenotype in different parts of a plant. All roots, hypocotyls, leaves, pollen and callus from the seeds, hypocotyls and leaves had normal, *tph1*, *tph2* and double mutation tocopherol profiles depending on the genotype. The only exception was the absence of *tph1* expressivity in the green tissue of the leaves. The experiment with day/night temperatures varying during seed development from 20/18 to 30/26°C showed an increased α-tocopherol content from 39 to 48% in *tph1* mutation. Both a normal genotype (about 97% of α-tocopherol) and *tph2* mutation (about 98% of γ-tocopherol) were constant in these two temperature regimes. Genetic background was the main factor that influenced the expressivity of *tph2* in sunflower.

Key words: expressivity, sunflower, tocopherol mutations

INTRODUCTION

Sunflower tocopherol complex is known to contain mostly the α-homologue, about 95%, which has the highest vitamin content and lowest antioxidant properties. Other oil crops mainly possess high percentages 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 achieved

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by breeding for enhanced tocopherol composition in sunflower seeds (Demurin *et al.*, 1996).

Two unlinked non-allelic genes, designated *Tph1* and *Tph2*, controlling tocopherol composition in sunflower seeds were identified for the first time in VNIIMK, Krasnodar, Russia (Demurin, 1993). Recessive alleles of the genes were found as spontaneous mutations using large-scale screening, selfing and the half-seed technique. Original inbred lines with modified tocopherol composition, LG15, LG17 and LG24, 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 exhibits epistasis over *tph1* through the expression of δ-tocopherol content amounting to about 8% in the recombinant double recessive homozygote LG24.

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 (Velasco *et al.*, 2003). The genetic identification by the allelic test showed the new medium β-tocopherol mutation to be allelic to *tph1* and the new high γ-tocopherol mutation to be allelic to *tph2* (Demurin *et al.*, 2004; Vera-Ruiz *et al.*, 2005). The δ-tocopherol content in the phenotype of a recombinant double mutation achieved in segregants up to 68% (Velasco *et al.*, 2004).

A linkage test in the F_2 and F_3 generations showed the *tph1* and *tph2* mutations to be independently inherited from the *Imr* gene for imidazolinone resistance (Demurin *et al.*, 2006).

The molecular genetic approach revealed a modifying cryptic recessive mutation, designated *d*, which has no effect in a normal genotype but increases the β-tocopherol content up to 70% in *tph1tph1 dd* homozygotes and to 40% in *tph1tph1 tph2tph2 dd* homozygotes. Three methyltransferase mutations, *m (tph1)*, *g (tph2)* and *d* were mapped to linkage groups 1, 8 and 4, respectively (Hass *et al.*, 2006; Tang *et al.*, 2006).

The present investigation was undertaken to estimate the effect of different factors on phenotypic variability of tocopherol mutations.

MATERIALS AND METHODS

Inbred lines from the genetic collection of VNIIMK were involved in this research. VK66 and VK373 were normal lines. The genotypes LG15 and VK571 contained the *tph1* gene. The genotypes LG17 and VK175 contained the *tph2* gene. LG24 and VK876 possessed a double *tph1, tph2* mutation.

Leaves, hypocotyls and roots were sampled from V6 plants. Five-week calli were obtained at 25°C in the dark. Explants were placed in Murashige-Skoog medium with BAP (0.5 mg/l), NAA (2 mg/l), hydrolyzed casein (500 mg/l), mesoinositol (100 mg/l), sucrose (30 g/l), agar (8 g/l), glycine (2 mg/l), vitamin B_1 (10 mg/l), vitamin B_6 (1 mg/l) and vitamin PP (1 mg/l) with pH from 5.6 to 5.8. After drying and crushing, the samples were analyzed for tocopherol composition.

Seed maturation was effected in two climatic chambers with different day/night temperature regimes, 20/18°C and 30/26°C per day/night. Day length was 16 h. Plants were grown in pots filled with the soil.

Tocopherol composition was determined with thin-layer chromatography (TLC) followed by Emmerie-Engel reaction without correction due to different rates of homologue staining (Popov *et al.*, 1991).

RESULTS AND DISCUSSION

Expressivity of mutations is known to be estimated on the basis of phenotypic variability exhibited under the influence of modifier genes of different genetic backgrounds, plant ontogenesis and environment factors.

Table 1: Expressivity of tocopherol mutations in different genetic backgrounds of inbred lines

Mutation	Expressivity	Tocopherol composition, %			
		α	β	γ	δ
tph1	max	40	60	0	0
	min	60	40	0	0
tph2	max	0	0	100	0
	min	80	0	20	0
tph1, tph2	max	0	0	60	40
	min	40	25	25	10

Genetic background of different lines was found to influence markedly the expressivity of *tph2* mutation and double recessive homozygote. The γ-tocopherol percentage in *tph2* varied from the maximum expressivity of 100% to a minimum level of 20% (Table 1). The double recessive homozygotes showed maximum expressivity in VK876 sub-line with 60% of γ- and 40% of δ-homologues. The minimum levels of expressivity corresponded to the tocopherol profiles with 40% of α-, 25% of β-, 25% of γ- and 10% of δ-form.

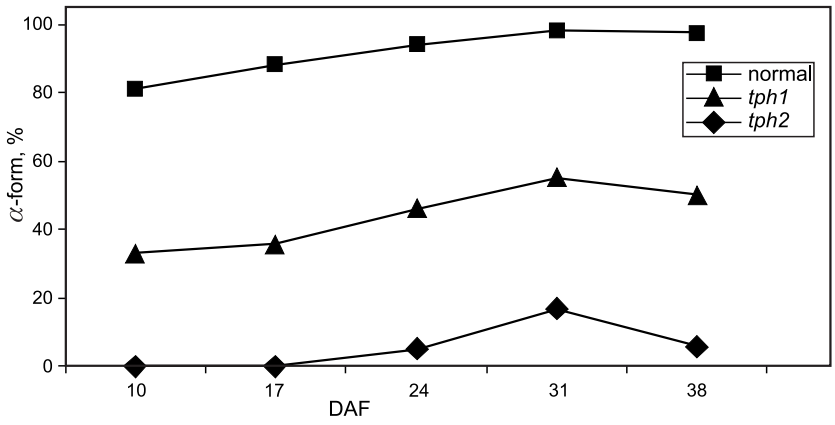

Figure 1: Influence of seed maturation (days after flowering) on α-tocopherol content of different genotypes

The range of variability of the phenotype possessing the *tph1* gene was even lower. The β-tocopherol percentage varied from 60 to 40%, with corresponding changes of α-tocopherol content from 40 to 60%.

Seed maturation from 10 to 38 days after flowering (DAF) influenced tocopherol composition in both normal and mutant genotypes by increasing the α-tocopherol content. In the normal genotype VK373, this increase varied from 81 to 97%, in LG15 with *tph1* from 33 to 50% and in LG17 with *tph2* from 0 to 6% (Figure 1).

Mutations in tocopherol composition can be detected in different parts of the plant (Table 2). All roots, hypocotyls, leaves, pollen grains and calli from the seeds, hypocotyls and leaves had normal, *tph1*, *tph2* and double mutation tocopherol profiles depending on the genotype. The only exception was the absence of *tph1* expressivity in the green tissue of the leaves in both single and double mutations.

Table 2: Expressivity of tocopherol mutations in different plant parts

Mutation	Plant part				
	seed /callus	hypocotyl /callus	leaf /callus	root	pollen
wild type	- / -	- / -	- / -	-	-
tph1	+ / +	+ / +	- / +	+	+
tph2	+ / +	+ / +	+ / +	+	+
tph1, tph2	+ / +	+ / +	+* / +	+	+

* expressivity observed as *tph2* phenotype

It was shown for *tph1* and *tph2* mutations that heterozygotes could be clearly identified after pollen analysis from one head for mean tocopherol content (Table 3). That was possible due to monoheterozygotes production of the mixture of heterogeneous pollen grains of two types in 1 normal : 1 mutant ratio. Double heterozygotes produced a mixture of heterogeneous pollen grains of four types in 1 normal : 1 *tph1* : 1 *tph2* : 1 *tph1, tph2* ratio. This is suitable for backcrossing selection in developing of analogues of elite lines.

Table 3: Tocopherol profiles of pollen from a single head of different genotypes

Genotype	Tocopherol composition in pollen, %			
	α	β	γ	δ
wild type	85	0	15	0
Tph1tph1	70	20	10	0
tph1tph1	60	40	0	0
Tph2tph2	60	0	40	0
tph2tph2	15	0	85	0
Tph1tph1 Tph2tph2	40	10	40	10
tph1tph1 tph2tph2	0	0	50	50

The experiment with day/night temperature regimes during seed development at 20/18 and 30/26°C showed the *tph1* mutation to be increased in α-tocopherol content from 39 to 48%. Thus, the higher the temperature, the higher α-tocopherol and the lower β-tocopherol percentages. Both the normal genotype (about 97% of α-

tocopherol) and the *tph2* mutation (about 98% of γ-tocopherol) were constant in these two regimes (Figure 2).

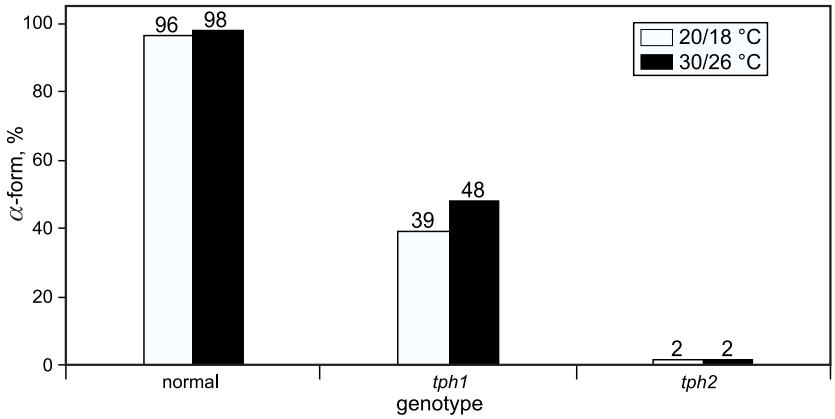

Figure 2: Influence of temperature during seed maturation on α-tocopherol content in different genotypes

There are two routes of α-tocopherol biosynthesis in plants. The route from γ- to α- may be the main route and that from δ- *via* β- to α-tocopherol may be a minor one (Furuya *et al.*, 1987). Obviously, different "5-carbon" and "7-carbon" methyltransferases are involved. The *tph1* mutation was recently shown to knock out the "5-carbon" type of enzyme: 2-demethylphytylplastoquinol methyltransferase (or MPBQ/MSBQ-MT) and the *tph2* mutation to knock out the "7-carbon" type of enzyme: γ-tocopherol methyltransferase (γ-TMT) (Hass *et al.*, 2006; Tang *et al.*, 2006).

Different phenotypes of the mutations, due to the different expressivity, can be explained with the general scheme of proposed genetic blocks in tocopherol biosynthesis where α-form is a terminal compound (Figure 3). For example, the higher the *tph2* expressivity in the double mutations, the higher the contents of γ- and δ-tocopherols. Conversely, the low level of *tph2* expressivity may lead to an additional accumulation of both α- and β-tocopherol. On the other hand, immature seeds were expected to contain increased percentages of precursors, *i.e.*, β-, γ- and δ-form, and a decreased content of α-tocopherol. Finally, the high temperature during seed development cannot change tocopherol profiles in the case of extremely high percentages of the terminal compounds: α-tocopherol in normal genotypes or γ-tocopherol in *tph2* mutations.

CONCLUSIONS

The modifier genes of genetic backgrounds, seed maturation and tissue-specific expression in different parts of a plant, such as root, hypocotyls, leaves, pollen and callus, and temperature during seed maturing were shown to influence the expressivity of *tph1* and *tph2* tocopherol mutations in sunflower. The main factor was genetic background.

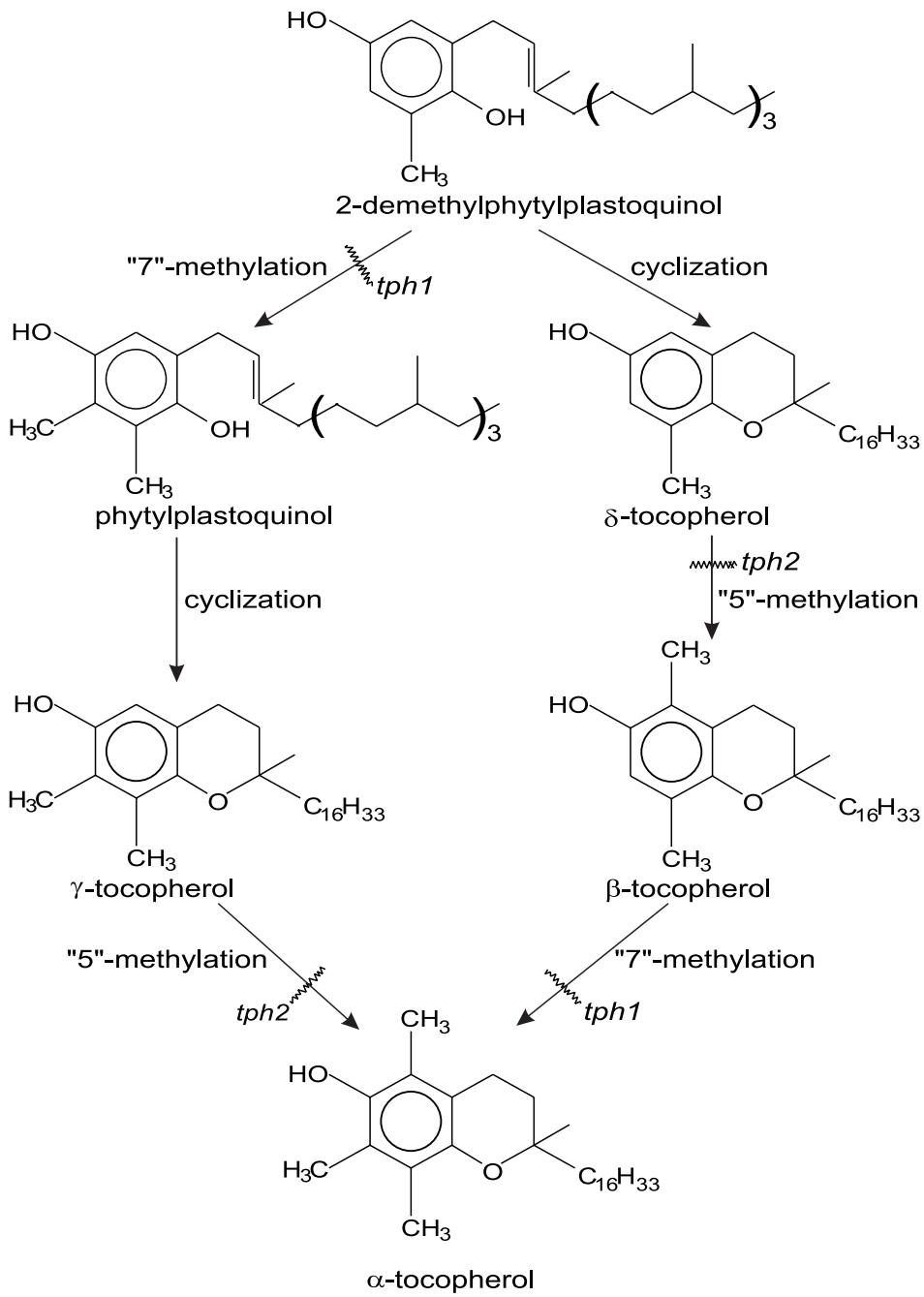

Figure 3: Proposed genetic blocks in tocopherol biosynthesis with tph1 and tph2 mutations in sunflower

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EXPRESIÓN DE GENES DE MUTACIÓN DE TOCOFEROL EN GIRASOL

RESUMEN

En este trabajo ha sido estudiada la influencia del fondo genético, ontogénesis y el entorno en mutaciones de tocoferol en girasol. El contenido de α/β-tocoferol, varía entre 40/60 y 60/40% en la semilla de diferentes líneas consanguíneas (inbred) que contenían el gen *tph1*. Las líneas con el gen *tph2*, varían ampliamente en contenido de α/γ-tocoferol, de 0/100 a 80/20%. Una mutación doble mostró la variabilidad en el contenido de α/β/γ/δ-tocoferol en diferentes líneas consanguíneas (inbred) que varía entre la expresión máxima de 0/0/60/40 a la expresión mínima de 40/25/25/10%, lo que es consecuencia de expresividad incompleta del gen *tph2*. La maduració de semilla de 10 a 38 días después de floración, ha influido en el contenido de tocoferol, en caso de los normales, tanto como en los genotipos mutantes, a través del incremento de α-tocoferol. El contenido variaba de 81 a 97% en genotipo normal, de 33 a 50% en la mutación *tph1*, y de 0 a 6% en mutación *tph2*. Las mutaciones de tocoferol expresaban su fenotipo en diferentes partes de la planta. Todas las raíces, hipocotiles, hojas, polen y callos de la semilla, hipocotiles y hojas, tenían muta-

ciones de tocoferol normales, *tph1*, *tph2* y dobles, dependiente del genotipo. La única excepción fue falta de expresión *tph1* en el tejido verde de las hojas. En el experimento en el cual la variación entre las temperaturas diurna y nocturna durante el desarrollo de la semilla fue de 20/18 a 30/26°C, se produjo el incremento del contenido de α-tocoferol 39 hasta 48% en la mutación *tph1*. El genotipo normal (alrededor de 97% α-tocoferol) tanto como la mutación *tph2* (alrededor de 98% de γ-tocoferol) fueron constantes en esos dos regímenes temperaturales. El fondo genético fue el factor principal que influyó en la expresión de *tph2* en girasol.

EXPRESSION DU GÈNE DE MUTATION DU TOCOPHÉROL CHEZ LE TOURNESOL

RÉSUMÉ

Cette étude avait pour objet les effets de l'origine génétique, de l'ontogenèse et de l'environnement sur les mutations du tocophérol dans le tournesol. Le contenu de α/β- tocophérol variait de 40/60 à 60/40% dans les akènes de différentes sources pures contenant le gène *tph1*. Les lignées contenant le gène *tph2* variaient largement dans le contenu de α/γ- tocophérol, de 0/100 à 80/20%. Une mutation double a révélé une variabilité dans le contenu de α/β/γ/δ- tocophérol dans différentes sources pures pour une expression maximale de 0/0/60/40 à une expression minimale de 40/25/25/10%, ce qui est dû à l'expression incomplète du gène *tph2*. La maturation de l'akène de 10 à 38 jours après la floraison a eu un effet sur le contenu de tocophérol dans les génotypes normaux comme dans les génotypes mutants par l'augmentation du contenu de α-tocophérol. Le contenu variait de 81 à 97% dans un génotype normal, de 33 à 50% dans la mutation *tph1* et de 0 à 6% dans la mutation *tph2*. Les mutations de tocophérol exprimaient leur phénotype dans différentes parties de la plante. Toutes les racines, hypocotyles, feuilles, pollen et calus des akènes, les hypocotyles et les feuilles avaient un *tph1*, *tph2* et des mutations doubles du tocophérol normaux selon le génotype. La seule exception était l'absence d'expression de *tph1* dans le tissu vert des feuilles. Les variations de 20/18 à 30/26°C des températures nocturne et diurne au cours du développement des akènes pendant l'expérience ont révélé une augmentation de 39 à 48% du contenu de α-tocophérol dans la mutation *tph1*. Le génotype normal (environ 97% de α-tocophérol) ainsi que la mutation *tph2* (environ 98% de γ-tocophérol) ont été constants sous les deux régimes de température. L'origine génétique est le principal facteur ayant eu un effet sur l'expression *tph2* dans le tournesol.

Presented at: