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GENETIC VARIABILITY OF TOCOPHEROL COMPOSITON IN SUNFLOWER SEEDS

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SUMMARY

Two non-allelic unlinked genes designated tph-1 and tph-2, controlling tocopherol composition in sunflower seeds, were identified for the first time. Recessive alleles of the genes were found by wide-scale screening and selfing as spontaneous mutations on the base of "half-seed technique". Inbred lines with modified tocopherol composition have been developed. The tph-1 gene controls the ratio of alfa- and beta-tocopherols whereas tph-2 gene controls that of alfa- and gamma-tocopherols.

Key words: Sunflower, genes for tocopherol composition

INTRODUCTION

The improvement of oil stability during storage and use, regarding the oxidation of unsaturated fatty acids which results in the accumulation of toxins and loss of commercial quality, can be effectively reached by the increase of natural antioxidants content in oil. Scientists have given more attention to this problem since the widely-used synthetic antioxidant BHA has been identified as cancerogenic.

Tocopherols are the most powerful natural fat-soluble antioxidants (vitamin E). They exist in four forms - alpha, beta, gamma and delta, which differ in activity.

Sunflower tocopherol complex is known to contain a high percentage of alfa-tocopherol which has the lowest antioxidant properties (Table 1).

It would be very profitable to improve oil quality, e.g., to increase seed storage term and oil "shelf life", by genetic changes of tocopherol composition in sunflower seeds.

These possible genetic changes are expected to be connected with mutations in methylation steps because alfa-tocopherol is synthesized via methylation of other tocopherols.

Table 1. Tocopherol composition in some oils (from reference 2)

Crop	Tocopherol, %			
	Alfa	Beta	Gamma	Delta
Sunflower	96	2	2	0
Olive	93	0	7	0
Groundnut	50	2	44	4
Rape	26	6	65	3
Maize	22	3	68	7
Soyabean	10	3	63	24
Sesame	4	2	83	11

MATERIAL AND METHODS

Twelve varieties of oilseed sunflower, 40 specimens of VIR world germplasm collection, and about 200 inbred lines were studeid.

The micromethod of tocopherol determination in the part of cotyledon without germ damage, the so-called "half-seed technique", was applied. After separation of individual forms of tocopherols on TLC-plates these substanses were determined by Emmerie-Engel reagent on the base of the use of ferric chloride for oxidation of tocopherols followed by the measurement of red-colour complex of ferrous ion and dipyridyl.

Single seeds were used in the analysis of F_1, F_2, BCP_1 and BCP_2 populations. Segregation ratios were tested by the chi-square goodness of fit.

RESULTS AND DISCUSSION

Tocopherol composition in 1000 single seeds of the cross-pollinated genotypes and in seed bulk of inbred lines was uniform. The content of alfa-tocopherol ranged from 90 to 99% in the sum with beta and gamma isomers. Delta tocopherol was not observed.

The seeds with a reduced content of alfa-tocopherol of about 90% were grown and self-pollinated. Only one of them, derived from the variety VNIIMK 8931, gave the progeny segregating for the tocopherol compositon into two phenotypic classes in a ratio 3:1. The inbred line LG-15 was develped on the base of the seeds from the second class.

The phenotype of the line LG-15 had 50% alfa- and 50% beta-tocopherol. In the crosses with common lines it was shown that the trait of increased content of beta-tocopherol in seeds is the phenotype of the recessive gomozygote of the gene tph-1 (abbreviation for "tocopherol").

Another inbred line with modified tocopherol composition, LG-17, was obtained by selfing the specimen no.44 of VIR world sunflower germplasm collection. The phenotype of the line LG-17 had 5% alfa- and 95% gamma-tocopherol.

A hybridological analysis showed the presence of recessive gene tph-2 controlling this trait.

The allelic interactions for tph-1 and tph-2 genes corresponed to partial dominance. The differences between homo- and heterozygotes were about 3% of alfa-tocopherol. The dominance degree was estimated at 87%.

Table 2. Inheritance of tocopherol composition in two mutant sunflower lines

Genotype, generation	Number of seeds per phenotype				
	Wild type	LG-15	LG-17	LG24	Ratio ($p>0.05$)
P_1 (LG-15)	0	50	0	0	
P_2 (LG-17)	0	0	50	0	
F_1	50	0	0	0	
BCP_1	29	31	0	0	1:1:0:0
BCP_2	32	0	25	0	1:0:1:0
F_2	175	41	49	16	9:3:3:1

Gene identification in the crosses of the line LG-17 showed the genes tph-1 and tph-2 to be non-allelic and unlinked (Table 2). The most interesting observation in F_2 was the occurrence of a new phenotypic class with 8% delta-tocopherol which corresponds in its frequency 1/16 to the double recessive gomozygote. From the seeds of this class the inbred line LG-24 was derived.

Table 3. Mutant lines for tocopherol composition in sunflower seeds

Line	Genotype	Tocopherol, %			
		Alfa	Beta	Gamma	Delta
Common sunflower	$\frac{Tph-1}{Tph-1}\ \frac{Tph-2}{Tph-2}$	95	3	2	0
LG-15	$\frac{tph-1}{tph-1}\ \frac{Tph-2}{Tph-2}$	50	50	0	0
LG-17	$\frac{Tph-1}{Tph-1}\ \frac{tph-2}{tph-2}$	5	0	95	0
LG-24	$\frac{tph-1}{tph-1}\ \frac{tph-2}{tph-2}$	8	0	84	8

Thus, a system of mutant lines for tocopherol compositon has been composed (Table 3). Four phenotypic classes can be distinguished quite well. Gene interaction is considered as complementary. In order to understand the epistatic action of the tph-2 mutation on the tph-1 mutation, an alternative scheme of tocopherol biosynthesis should be used where alfa-form can be syntesized either via beta- and delta- or via gamma-tocopherols. Presently, there are three main directions or research:

- introduction of recessive alleles of these genes into commercial lines to produce isogenic lines and hybrids with modified tocopherol composition in seeds;
- investigation of oil oxistability with genotypes differing in tocopherol and fatty acid composition to clarify the aims of breeding for new types of sunflower oil;
- study on mechanisms of genetic blocks in pathways of tocopherol biosynthesis and expression of the mutations obtained.

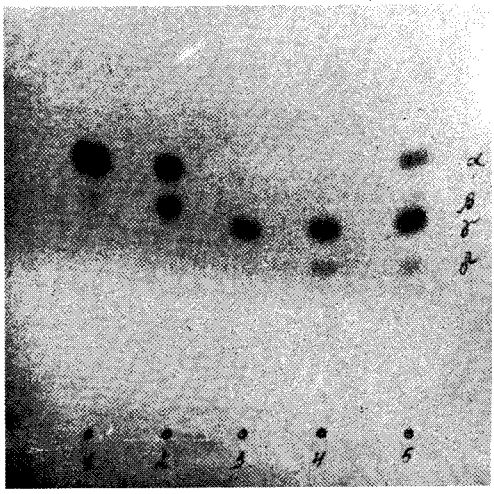

Figure 1.
Chromatography of tocopherols from mature seeds
1. common sunflower
2. tph-1 mutant
3. tph-2 mutant
4. tph-1 and tph-2 combined
5. soybean

REFERENCE

Haumann B.F., 1990. Antioxidants: Firms seeking products they can label as "natural". International News on Fats, Oils and Related Materials (from the American Oil Chemists' Society), I2: IOO2-IoI3.

Muller-Mulot V.W. et al., 1976. Schnellmethode zur quantitativen Bestimmung individueller Tocopherole in Olen und Fetten. Fette, Seifen, Anstrichmittel, 7: 257-262.

VARIABILIDAD GENÉTICA DE LA COMOSICIÓN DE TOCOFEROL EN LAS SEMILLAS DE GIRASOL

RESUMEN

Se han identificado por primeva vez genes ligados y no alélicos designados como tph-1 y tph-2 que controlan la composición de tocoferol en las semillas de girasol. Alelos recesivos de los genes fueron encontrados en gran escala como mutaciones espontáneas en un screening después de autofecundar y utilizar la técnica de la media semilla. Lineas presas con comosición modificada de composición de tocoferol han sido desarrolladas. El gen tph-1 controla la relación de los tocoferoles alfa y beta mientras que el gen tph-2 control los niveles de tocoferoles alfa y gamma.

VARIABILITÉ GÉNÉTIQUE DE LA COMPOSIION DU TOCOPHÉROL DANS LES GRAINES DE TOURNESOL

RÉSUMÉ

Pour la première fois, nous avons identifié deux gènes non allèliques et indópendants contrôlant la composition du tocophérol dans les graines de tournesol. Nous les avons désignés par tph-1 et tph-2. Des allèles récessifs de ces gènes ont été trouvés grâce à un screening à large échelle et par des mutations spontanées après autofécondation en utilisant la technique dite de demie graine. Nous avons développé des lignées comportant des variatiors de composition en tocophérol. Le gène tph-1 contrôle le rapport alpha / beta-tocophérol alors que le gène tph-2 contrôle le rapport alpha / gamma-tocophérol.