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Genetic and environmental variation for tocopherol content and composition in sunflower commercial hybrids

L. VELASCO[1]*, J. M. FERNÁNDEZ-MARTÍNEZ[1], R. GARCÍA-RUÍZ[2] AND J. DOMÍNGUEZ[2]

[1] *Institute for Sustainable Agriculture (CSIC), Apartado 4084, E-14080 Córdoba, Spain*
[2] *Department of Plant Breeding and Agronomy, CIFA-Alameda del Obispo, Apartado 3092, E-14080 Córdoba, Spain*

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SUMMARY

Tocopherols are the most important compounds having antioxidant activity in sunflower seeds. The objective of the present research was to study the genetic and environmental variation for tocopherol content and composition in sunflower. Thirty-six sunflower hybrids were grown at 13 locations across all major areas of sunflower cultivation in southern Spain. Seed yield, 100 seed weight, oil content and tocopherol content and composition were determined. Tocopherol content ranged from 314·5 to 1024·5 mg/kg seed and from 562·8 to 1872·8 mg/kg oil. The tocopherol fraction was largely composed of alpha-tocopherol, which accounted for 88·4 % to 96·3 % of the total tocopherols. Both genotypic and environmental effects were significant for tocopherol content and composition. For alpha-, beta- and total tocopherol content, the effect of the genotype was larger than that of the environment, whereas the latter had a greater effect on gamma-tocopherol content. Genotype × location interaction was significant for alpha-, gamma- and total tocopherol content. Tocopherol content was not correlated with seed oil or seed yield, indicating the possibility of selecting for this trait without affecting the performance of the genotypes.

INTRODUCTION

Tocopherols are fat-soluble compounds with vitamin E activity that exert an effective inhibition of lipid oxidation in oils, fats and foods containing them (*in vitro* antioxidant activity) as well as in biological systems (*in vivo* antioxidant activity) (Kamal-Eldin & Appelqvist 1996). They occur as a family of four derivatives named alpha-, beta-, gamma- and delta-tocopherol. The four tocopherols differ in their relative *in vitro* and *in vivo* antioxidant activities. Alpha-tocopherol is the most efficient antioxidant *in vivo*, but it shows a weak antioxidant potency *in vitro*. Conversely, gamma-tocopherol is the most powerful antioxidant *in vitro* but its *in vivo* activity is low. Beta- and delta-tocopherols exhibit intermediate properties (Pongracz *et al*. 1995). Consequently, the antioxidant properties of an oil depend on both the total tocopherol content and its composition (Shintani & Dellapenna 1998).

Large variation for total tocopherol content has been reported in sunflower. Marquard (1990) reported a variation from 480 to 1128 mg/kg oil, whereas Dolde *et al*. (1999) found a tocopherol content ranging from 534 to 1858 mg/kg oil in a set of experimental and commercial breeding lines. The tocopherol fraction of sunflower seeds is predominantly made up of alpha-tocopherol, which accounts for more than 95 % of the total tocopherols (Demurin 1993; Dolde *et al*. 1999). Beta- and gamma-tocopherol can be present in sunflower seeds, usually in amounts below 2 % of the total tocopherols (Demurin 1993). The tocopherol content of sunflower seeds is affected by the genotype and the environment, although the scarce studies conducted to date have concluded different relative effects of both factors (Marquard 1990; Alpaslan & Gündüz 2000).

The objective of the present study was to investigate the genetic and environmental variation for tocopherol content and composition in a representative set of commercial sunflower hybrids cultivated in southern Spain.

MATERIALS AND METHODS

Plant material and experimental design

Thirty-three commercial hybrids of sunflower from 16 seed companies together with three experimental

* To whom all correspondence should be addressed. Email: ia2veval@uco.es

hybrids were used in this study. The commercial hybrids were: Turbo, Pilar (SES Ibérica); Coro, Sena (Verneuil); Emperador, Lanzador (Agrar Semillas); Albany (C. Asgrow); Deborah, Sunko, Arpón (Borges); Mikado (Rhône-Poulenc); Coriolis, Indiana (Battle); Xistral, Medallón, Jalón, Mencal, Aitana (Arlesa); Attila, PAU-9701, Sofía (Senasa); Alfar, Florasol (Monsanto); Megasun, Supersun (Advanta); Senador (Eurosemillas); Saxo, Kasol, Korona (Koipesol); Sanbro (Novartis); Espanil (Pioneer); Rodrigo, Trajano (Danisco).

The hybrids were planted in 2000 in 13 locations covering all the major sunflower-growing areas of southern Spain. The experimental design at all locations was a 6×6 lattice with three replications. Plots consisted of four rows 10 m long and a spacing of 0·7 m between rows, with a plant density of about 57 000 to 60 000 plants/ha. The plants from the two central rows of each plot were harvested by hand and seed yield was calculated. Further, hundred-achene weight and the proportion of kernel in the achene, expressed as percentage of the total achene weight, were measured in samples from all plots.

Chemical analyses

Achene oil content was determined by nuclear magnetic resonance (NMR) of dried achenes (Granlund & Zimmerman 1975). Seed (kernel) oil content was estimated with the previously determined kernel content of the achene.

For tocopherol extraction, eight randomly selected achenes were husked and crushed as fine as possible with a stainless steel rod. Tocopherol content and composition were determined by high-performance liquid chromatography (HPLC) as described by Thies (1997), using a fluorescence detector at 295 nm excitation and 330 nm emission and iso-octane/tert-butylmethylether (94:6) as eluent at an isocratic flow rate of 0·7 ml/min. Chromatographic separation of the tocopherols was performed on a LiChrospher 100 diol column (250 mm × 3 mm I.D.) with 5-μm spherical particles, connected to a silica guard column (LiChrospher Si 60, 5 mm × 4 mm I.D.). Quantitative determination of tocopherols was done by using external calibration curves. Total tocopherol content was calculated as the sum of alpha-, beta- and gamma-tocopherol contents. Total tocopherol content was expressed both as mg/kg seed and mg/kg oil.

Statistical analyses

The data were analysed as a random effects model using the analysis of variance. The lattice designs were first analysed separately for each environment and, when needed, adjusted treatment means were computed. For each variable, Barlett's test (Gomez & Gomez 1984) was used to verify homogeneity of error variances from the different environments. A combined analysis over environments for each variable was then made using the adjusted treatment means of homogeneous groups of environments. The pooled experimental error was calculated following Cochran & Cox (1957).

Correlation coefficients were calculated from the means over the 13 environments using for each environment the adjusted treatment means calculated after analysis of variance of the lattice design. Chi-square test was used to verify the goodness of fit of observed data to the normal distribution (Gomez & Gomez 1984).

RESULTS

Variability for tocopherol content and composition

The samples analysed exhibited a wide range of variation for tocopherol content, from 314·5 to 1024·5 mg/kg seed (mean of 669·1 mg/kg seed) and from 562·8 to 1872·8 mg/kg oil (mean of 1115·2 mg/kg oil). Mean values for total tocopherol content in the 36 hybrids averaged over 13 environments, referred to in terms of both seed weight and seed oil weight, followed normal distributions ($P = 0{\cdot}05$; Fig. 1). The average tocopherol content ranged from 511·5 to 823·3 mg/kg seed and from 821·7 to 1416·4 mg/kg oil. The hybrids with the highest tocopherol content, referred to in terms of both seed and oil weight, were Xistral (823·3 and 1416·4 mg/kg, respectively), Lanzador (807·8 and 1349·4 mg/kg, respectively), Emperador (774·9 and 1321·2 mg/kg, respectively) and Attila (771·8 and 1320·9 mg/kg, respectively). There was also variation between locations, with mean tocopherol contents ranging from 570·9 to 776·4 mg/kg seed and from 971·6 to 1263·3 mg/kg oil.

The tocopherol fraction was predominantly made up of alpha-tocopherol, which accounted for 276·7 to 961·8 mg/kg seed, i.e. from 88·4% to 96·3% of the total tocopherols. The rest was beta-tocopherol, from 14·5 to 60·5 mg/kg seed (2·7% to 8·8% of the total tocopherols), and gamma-tocopherol, from 6·2 to 32·5 mg/kg seed (0·7% to 3·9% of the total tocopherols). No delta-tocopherol was detected in the samples.

Analysis of variance

The combined analysis of variance for alpha-, beta- and gamma-tocopherol content as well as total tocopherol content, referred to in terms of both seed weight and seed oil weight, is presented in Table 1. Genotypic and environmental effects were highly significant ($P = 0{\cdot}01$) for all tocopherol traits. Estimates of variance components for alpha-, beta- and total tocopherol content revealed that genotypic variance was greater than environmental variance for these traits. Conversely, a predominance of environmental variance

Table 1. *Mean squares of combined analysis of variance and estimates of variance components*

Source No. environments (e)	D.F.[1]	Alpha-T 7	Beta-T 9	Gamma-T 11	Total T (seed) 9	Total T (oil) 10
Genotype	35	14 377**	181·1**	2·1**	22 770**	75 932**
Environment	e-1	42 405**	27·7**	82·3**	46 939**	81 777**
G × E	35 (e-1)	2511*	7·1	1·2**	2630**	7241**
Pooled error	55 (e)	2000	12·2	0·3	2007	5554
σ^2_G		565	6·44	0·03	746	2290
σ^2_E		369	0·19	0·75	410	690
$\sigma^2_{G\times E}$		170	0·00	0·30	208	562

[1] Degrees of freedom vary according to number of environments.
*,** Significant at the 0·05 and 0·01 level, respectively.

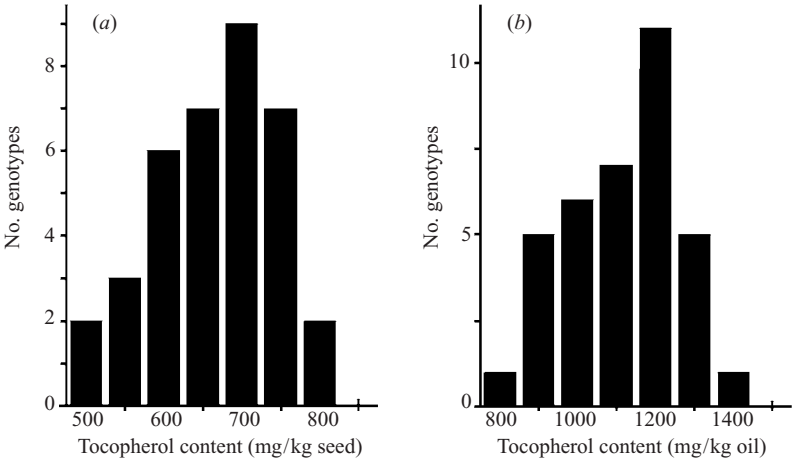

Fig. 1. Histograms of total tocopherol content expressed as (A) mg/kg seed and (B) mg/kg oil in 36 commercial sunflower hybrids (data averaged over 13 environments).

was observed for gamma-tocopherol content, suggesting that the trait is largely influenced by the environment.

Genotype × location interactions were statistically significant for total tocopherol ($P=0·01$), gamma-tocopherol ($P=0·01$) and alpha-tocopherol content ($P=0·05$), but not for beta-tocopherol content, confirming the low weight of environmental effects on this trait.

Correlations between tocopherols and other traits

Total tocopherol content in the seed was positively correlated with alpha- and beta-tocopherol contents ($P=0·01$), but its correlation with gamma-tocopherol content was not significant at $P=0·05$ (Table 2). Alpha-tocopherol content was positively correlated with beta-tocopherol content, whereas the latter was in turn positively correlated with gamma-tocopherol content. Correlation coefficient between alpha- and gamma-tocopherol content was positive but not significant at $P=0·05$. Seed oil content was not significantly correlated with seed tocopherol content.

There was no significant correlation between total tocopherol content and seed weight or seed yield. Conversely, gamma-tocopherol content exhibited a strong negative correlation with seed weight (Table 2), indicating that smaller seeds contain higher levels of this tocopherol derivative. Gamma-tocopherol content was not significantly negatively correlated ($P>0·05$) with seed yield, which might simply be an indirect effect of the strong positive correlation between seed weight and seed yield.

DISCUSSION

Variation for total tocopherol content in the material evaluated in the present study is very similar to that previously reported by Dolde *et al.* (1999), from 534 to 1858 mg/kg oil, in the analysis of a wide collection of

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Table 2. *Correlation coefficients between individual and total tocopherol contents, oil content, seed weight and seed yield (means over 13 environments)*

	Beta-T	Gamma-T	Total-T	Oil	Seed weight	Seed yield
Alpha-T	0·70**	0·25	0·99**	0·06	0·05	−0·04
Beta-T		0·59**	0·75**	0·12	−0·09	−0·09
Gamma-T			0·30	0·16	−0·74**	−0·30
Total-T				0·07	0·04	−0·04
Oil					−0·31	−0·35*
Seed weight						0·53**

experimental and commercial breeding lines. Lower values for tocopherol content in sunflower oil, however, are found in the literature. After reviewing different sources, Kamal-Eldin & Andersson (1997) reported an average tocopherol content of 698 mg/kg oil, which is much lower than that found in the present study. The reason for such a difference might be the utilization in that study of data based on refined, commercial oils. The refining process, especially the step of deodorization, involves the removal of a considerable amount of tocopherols (Gogolewski *et al.* 2000).

The predominance of alpha-tocopherol in standard sunflower seeds is in agreement with previous data in the literature (Demurin 1993; Kamal-Eldin & Andersson 1997; Dolde *et al.* 1999). All the samples analysed in the present study also contained minor levels of beta- and gamma-tocopherol. Dolde *et al.* (1999), however, detected no gamma-tocopherol in the sunflower materials analysed.

Both genotypic and environmental effects were significant for tocopherol content and composition. For alpha-, beta- and total tocopherol content, the effect of the genotype was larger than that of the environment, whereas the latter had a greater effect on gamma-tocopherol content. The influence of genotypic and environmental effects on tocopherol content and composition has been scarcely studied in sunflower. In a study conducted with six sunflower varieties, Marquard (1990) concluded that total tocopherol content was influenced significantly by both location and genotype, the effect of location being more important than the effect of genotypes. Alpaslan & Gündüz (2000) evaluated 10 sunflower varieties during 2 years, concluding that both the genotype and the environment influenced significantly the tocopherol content. In that study, the effect of varieties was larger than that of years. In soya beans, Dolde *et al.* (1999) reported that both genotype and planting location as well as their interaction affected total tocopherol content, with genotype having the largest effect. In rapeseed, Goffman & Becker (2002) found significant effects of genotype, environment and their interaction on individual and total tocopherol contents, with greater effect of the environment. Similar results were obtained by Goffman & Becker (2001*a*, *b*) in the same crop.

In a previous study, Marquard (1990) identified a strong negative correlation between oil and tocopherol contents in sunflower seeds. Such a negative correlation has not been observed in the present research, which suggests the possibility of selecting for increased tocopherol levels without affecting the oil content of the seeds. In rapeseed, Goffman & Becker (2002) identified no correlation between oil and tocopherol contents, but a positive correlation between oil and gamma-tocopherol contents in a collection of rapeseed genotypes. In the same crop, Goffman & Becker (2001*a*) found positive correlation coefficients between oil and tocopherol contents in six out of 10 F_2 populations evaluated.

In summary, the present study revealed an important variability for tocopherol content and composition in cultivated sunflower. Although total tocopherol content was significantly influenced by the environment, the greater magnitude of the genotypic effect in comparison with the genotype × environment interaction suggests the feasibility of selecting for increased tocopherol content in sunflower. Such a selection would not affect the general performance of the lines, as no correlation of tocopherol with seed yield or oil content was detected.

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