

## Variability in oil tocopherol concentration and composition of traditional and high oleic sunflower hybrids (*Helianthus annuus* L.) in the Pampean region (Argentina)

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### RESUMEN

**Variabilidad en la concentración y composición de los tocoferoles en el aceite de híbridos de girasol tradicionales y "alto oleico" en Argentina.**

Los tocoferoles son antioxidantes naturales que aumentan la estabilidad de los aceites y cumplen una importante actividad biológica en humanos. No son conocidos estudios sobre la variabilidad del contenido y composición de tocoferoles en el aceite de híbridos de girasol tradicionales (HT) y "alto oleico" (HAO) sembrados en diferentes ambientes en Argentina. El objetivo de este trabajo fue detectar y examinar esa variabilidad. Las semillas fueron obtenidas de a) 7 HT sembrados en 4 localidades (Experimento I), y, b) 5 híbridos (3 HAO y 2 HT) sembrados en 3 localidades (Experimento II). La concentración de tocoferoles totales varió entre 634 y 1054  $\mu\text{g g aceite}^{-1}$ . El  $\alpha$ -tocoferol aportó con el 90.8 a 97.0% del contenido total de tocoferol. Las concentraciones de  $\alpha$ -tocoferol y tocoferol total estuvieron genéticamente determinadas (más del 80%). Para ambas variables, la interacción híbrido x localidad en el Experimento I fue significativa aunque de pequeño valor. La concentración media de tocoferoles totales entre ambientes y en cada ambiente fue mayor en los HAO que en los HT en el Experimento II, mientras que no hubo diferencias significativas entre HAO ni entre ambientes para esta variable, ni para  $\alpha$ -tocoferol. En ambos experimentos, la variabilidad en la concentración de tocoferoles para cada híbrido a través de los ambientes fue más alta que la ocurrida entre híbridos en un mismo ambiente. Para obtener aceite de girasol con alta concentración de tocoferoles deben seleccionarse tanto el genotipo como el ambiente.

**PALABRAS-CLAVE:** Aceite - Alto oleico - Girasol - *Helianthus annuus* L. - Tocoferol - Variabilidad.

### SUMMARY

**Variability in oil tocopherol concentration and composition of traditional and high oleic sunflower hybrids (*Helianthus annuus* L.) in the Pampean region (Argentina).**

Tocopherols are natural antioxidants that increase the stability of food fat and fulfill an important biological requirement in humans. There are no previous studies on the variability of tocopherol concentration and composition in the oil of sunflower traditional hybrids (TH) and high oleic sunflower hybrids (HOH) from different environments in Argentina. The objective of this work was to detect and

examine that variability. Seed samples were obtained from i) seven TH grown in four locations (Experiment I) and, ii) five hybrids (three HOH and two traditional ones) grown in three locations (Experiment II). Concentrations of total tocopherol in oil ranged from 634 to 1054  $\mu\text{g g oil}^{-1}$ .  $\alpha$ -tocopherol accounted for 90.8 to 97% of the total tocopherols. Total and  $\alpha$ -tocopherol concentrations were highly genetically determined (more than 80%). In Experiment I, a significant, although low in value, interaction between hybrid and location was found for total and  $\alpha$ -tocopherol concentrations. In Experiment II, mean values for total tocopherol concentration in HOH and in each environment were larger than in TH. Significant differences for total and  $\alpha$ -tocopherol concentration were not found among HOH and environments. The variation of tocopherol concentration for each hybrid across environments was higher than the variation among hybrids in the same environment. To obtain oil with high tocopherol concentration, both hybrid and environment must be selected.

**KEY-WORDS:** *Helianthus annuus* L - High oleic - Oil - Sunflower - Tocopherols - Variability.

### 1. INTRODUCTION

Tocopherols are natural compounds having vitamin E activity. Higher animals cannot synthesize vitamin E, so they need it in their food. Tocopherols also protect lipids in fats and oils along with protecting food from oxidation. A part of the tocopherols is lost during sunflower oil processing (Eitenmiller, 1997; Bramley *et al.*, 2000). Therefore, it is important to obtain oils with high tocopherol content.

Tocopherols occur in plants as a family of four derivatives named  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol. Tocopherols differ in their relative *in vitro* and *in vivo* antioxidant activities (Kamal-Eldin and Appelquist, 1996).

The total tocopherol concentration in oil of sunflower traditional hybrids, TH, (with a typical oleic acid content lower than 30%) typically ranges from 447 and 900  $\mu\text{g g oil}^{-1}$  (Gustone *et al.*, 1994) with extreme values varying from 389 to 1873  $\mu\text{g g oil}^{-1}$  (Nagao and Yamazaki, 1983; Dorrell and Vick,

1997; Miller and Fick, 1997; Cole *et al.*, 1998; Dolde *et al.*, 1999; Velasco *et al.*, 2002).

Few studies have considered tocopherol concentrations and their variability in the oil of TH and high oleic sunflower hybrids, HOH (with a typical oleic acid content higher than 70%). Genetic variability (Demurin *et al.*, 1996) and genetic and environmental variability in the relative proportion of different tocopherols in traditional sunflower oil were observed (Velasco *et al.*, 2002). De Villiers *et al.* (1989) found higher tocopherol concentration in two HOH (895-1002  $\mu\text{g g oil}^{-1}$ ) than in TH (710-790  $\mu\text{g g oil}^{-1}$ ) while Mascioli (2001) didn't find any differences among HOH, TH and middle oleic hybrids (typical oleic acid content between 50 and 70%).

Studies of variability in grain yield, oil content and oleic acid content (Aguero *et al.*, 1999) and of stability and adaptability in grain yield and quality traits in HOH (Lúquez *et al.*, 2002) have recently been assessed for several genotypes in the Argentine High Oleic Sunflower Trials. However, there are no studies on the variability of tocopherol concentration and composition in the oil of TH and HOH in the Pampean Region, which is the principal area where sunflowers are cultivated (ASAGIR, 2003). Argentina is the world biggest sunflower oil exporter.

The objective of this work was to detect and examine the variability of tocopherol concentration and composition in oil of traditional and high oleic sunflower hybrids cultivated in different locations in the Pampean Region, in Argentina.

## 2. EXPERIMENTAL

### 2.1. Plant materials

Two groups of sunflower, *Helianthus annuus* L. hybrids with different potential content of oleic acid: TH and HOH, were evaluated for grain yield ( $\text{kg ha}^{-1}$ ), oil content (%) and tocopherol concentrations in the oil ( $\mu\text{g g oil}^{-1}$ ) in two experiments.

*Experiment I:* It consisted of 7 TH belonging to the official comparative Yield Trials from Buenos Aires and La Pampa provinces: the stripped hull

hybrids ACA 884, Morgan 742 and Pyramid-1, and the black hull hybrids, Contiflor 11, Jaguel, Paraíso 20, and Rancul. These hybrids were provided by Asociación de Cooperativas Argentinas; Mycogen S.A., Cargill S.A.; Zéneca S.A.; Trébol Sur S.A.; Nidera S.A., and Novartis S.A., respectively.

*Experiment II:* It consisted of 3 commercial HOH belonging to the official High Oleic Yield Trials from Buenos Aires province, and 2 TH (testers). The HOH were Trisum 870, Aromo and Sideral, provided by Mycogen S.A., Nidera S.A. and Sursem S.A., respectively, and the TH were ACA 884 and Contiflor 9 (Mycogen S.A.).

### 2.2. Field trials

Field trials for Experiment I were conducted at four locations: Balcarce (37° 45' S, 58° 18' W), Bordenave (37° 51' S, 63° 01' W), Tandil (37° 14' S, 59° 15' W) and Realicó (34° 58' S, 64° 58' W), while field trials for Experiment II were conducted in three locations (Pieres, 38° 29' S, 58° 50' W, 9 de Julio, 35° 27' S, 60° 53' W and Tres Arroyos, 38° 20' S, 60° 15' W).

All experimental sites are located in the main sunflower-growing region of Argentina, the Pampean Region. Locations were selected because of contrasting mean meteorological conditions during the sunflower growing season (Table I) and because yield trials conducted in these locations in previous years presented large differences in mean yield (Pereyra, personal communication). The experimental design applied on each site was a completely random block design with three replicates in Experiment I and two replicates in Experiment II. Each plot consisted of six 6-m-long furrows, 0.70 m apart. Plant density was 71500 pl/ha. Harvesting was done when seeds had a humidity of 11%.

### 2.3. Measurements and data analysis

#### 2.3.1. Grain yield

This trait was determined in the central furrow of each plot and expressed in  $\text{kg ha}^{-1}$ .

Table I  
Daily mean, maximum and minimum average temperatures calculated for the sunflower growing season (November-March, 1971-1990), and cumulated annual rainfall (1971-1990) for locations where field trial were conducted. Data <sup>1</sup> Experiment I, <sup>2</sup> Experiment II.

Location	Mean (°C)	Maximum (°C)	Minimum (°C)	Rainfall (mm)
Balcarce <sup>1</sup>	18.7	25.9	12.6	886.9
Bordenave <sup>1</sup>	20.1	27.6	13.1	713.9
Tandil <sup>1</sup>	18.8	25.5	12.4	940.8
Realicó <sup>1</sup>	22.6	31.3	14.3	797.1
Pieres <sup>2</sup>	19.4	26.6	11.6	975.0
9 de Julio <sup>2</sup>	21.6	28.2	14.9	1079.1
Tres Arroyos <sup>2</sup>	19.1	26.5	12.8	814.9

### 2.3.2. Oil content

This trait was determined by NMR (Nuclear Magnetic Resonance) (Robertson and Morrison, 1979) in grains harvested from free-pollinating plants from the central furrow of each plot with an Analyser Magnet Type 10 (Newport Oxford Instruments, Buckinghamshire, England; 37), according to AOCS Ai 2-75 method (1998). Oil concentration was expressed on a dry weight basis. Moisture content was determined according to the Association of Official Chemists methods (AOAC, 1990).

### 2.3.3. Tocopherol concentrations in the oil

Seeds of self fed plants were ground in a mill and extracted with n-hexane (Soxthel), first at room temperature for 4 hours, and at 69 °C for the next 4 hours. The solvent was evaporated in a rotary vacuum evaporator at 40 °C. Tocopherol contents of crude oils were determined by normal phase HPLC using the Hewlett Packard chromatography system (HP 1059 Series, Waldbronn, Germany) with a LiChrosorb Sil 60 (5µm, 250 mm x 4.00 mm, Merck, Darmstadt, Germany) column. Approximately 0.5g of each oil was dissolved in 5 ml of the mobile phase. Twenty micro liter samples were injected for each determination. The column was eluted with n-hexane: isopropanol (99.5:0.5, 99.3:0.7 v/v, HPLC solvent, J.T. Baker, Phillipsburg, USA) at a flow rate of 1.5 ml min<sup>-1</sup>. Tocopherols were detected by absorbance at 292 nm with an ultraviolet-visible detector (HP 1050 Series) and measured using a six-point external standard curve (A.O.C.S., 1998).

### 2.4. Statistical analyses

Means and standard deviations for grain yield, oil content and total and individual tocopherol concentrations were calculated.

An analysis of variance for a random complete block design (RCBD) combined across locations was performed for total and individual tocopherol concentrations. Hybrids, locations and the hybrid per location interactions were considered as random effects.

Genetic Determination Degree (GDD) was calculated for tocopherol concentration in the oil in order to know the proportion of phenotypic variation determined by genetic effects among sunflower hybrids. GDD was calculated as proposed by Mariotti (1986) and Fehr (1987):

$$GDD = \frac{\sigma^2 G}{\sigma^2 G + (\sigma^2 HLI)/l + (\sigma^2 E)/l * r}$$

where:  $\sigma^2 G$  is the genotypic variance among hybrids,  $\sigma^2 HLI$  the variance of hybrid \* location interactions,  $\sigma^2 E$  the error variance,  $l$  the number of locations, and  $r$  the number of replicates .

Regression analysis was used to determine the relationships between tocopherol concentration in oil and other variables in seeds (software Sigma Plot for Windows, version 4.10, SPSS, 1997).

Coefficients of variation (CV%) in the grain yield and the oil content were calculated, among hybrids and locations to analyze variations in tocopherol concentrations.

## 3. RESULTS AND DISCUSSION

### 3.1. Grain yield

#### Experiment I

Mean grain yield was 2705.4 kg ha<sup>-1</sup> (CV = 32.2%). In this experiment, grain yield ranged from 1865.5 in Tandil to 3552.0 kg ha<sup>-1</sup> in Balcarce. This maximum grain yield is higher than the best average on farm yield (Mercau *et al.*, 2001). Among hybrids, grain yield varied from 2398.7 kg ha<sup>-1</sup> for Jaguel to 2964.3 kg ha<sup>-1</sup> for Paraíso 20 (Table IIa).

#### Experiment II

Mean grain yield was 2323.2 kg ha<sup>-1</sup> (CV = 22.4%). Grain yield ranged from 2175.7 kg ha<sup>-1</sup> in Pieres to 2488.2 kg ha<sup>-1</sup> in 9 de Julio. Among hybrids, grain yield varied from 1636.7 kg ha<sup>-1</sup> and 2892.3 kg ha<sup>-1</sup> for Sideral (HOH) and ACA 884 (TH) respectively. Grain yield variation among hybrids for each location was greater than variation among locations. On average, TH yielded more than HOH (2640.0 vs. 1866.2 kg ha<sup>-1</sup>). Grain yield variation across locations in HOH was greater than TH variation (CV = 18.5 and 9 %, respectively) (Table IIIa).

Grain yields registered in both experiments are frequently obtained in production fields in Argentina. The average grain yield obtained is higher than the average grain yield obtained in Argentina (1862 kg ha<sup>-1</sup> for the period 1985-1998, CIARA, 1998)

### 3.2. Oil content

#### Experiment I

Mean oil content was 48.1 (CV = 5.5%). This value is higher than the commercialization basis in Argentina (42%). Oil content ranged from 45.9 % in Tandil to 49.4 % in Bordenave. Among hybrids, oil content varied from 45.9 % for Morgan 734 to 50.7 % for Paraíso 20 (Table IIb). The oil content of black hull hybrids ranged from 47.8 % to 50.7 % while for stripped hull it ranged from 45.8 % to 46.7 %.

#### Experiment II

Mean oil content was 44.6% (CV = 4.8%). It ranged from 43.5 % in 9 de Julio to 45.4% in Tres Arroyos. Oil content varied among hybrids between

Table II  
 (a). Grain yield (kg ha<sup>-1</sup>) and (b) Oil content (% d.b.) for each hybrid and four locations in Experiment I.  
 B: Black hull hybrid, S: Stripped hull hybrid

## (a) Grain yield

Hybrids	Location				Average
	Balcarce	Bordenave	Realicó	Tandil	
Paraíso 20	4969.3	3026.0	1955.0	1906.7	2964.3
Pyramid-1	3224.0	2856.0	2207.7	2136.3	2606.0
Morgan 742	3241.3	3822.0	2705.7	1880.7	2912.4
Contiflor 11	3393.0	3427.3	1625.3	1911.0	2589.2
Rancul	3206.7	3434.0	2034.3	1778.7	2613.4
ACA 884	3830.7	3400.0	2324.3	1859.0	2853.5
Jaguel	2998.7	3523.7	1486.3	1586.0	2398.7
<b>Average</b>	<b>3552.0</b>	<b>3355.6</b>	<b>2048.4</b>	<b>1865.5</b>	

## (b) Oil content

Hybrids	Location				Average
	Balcarce	Bordenave	Realicó	Tandil	
Paraíso 20 <sup>B</sup>	52.2	53.7	48.6	48.3	50.7
Pyramid-1 <sup>S</sup>	48.3	47.9	46.5	44.2	46.7
Morgan 742 <sup>S</sup>	45.8	46.0	48.8	42.8	45.9
Contiflor 11 <sup>B</sup>	47.8	49.0	47.5	46.9	47.8
Rancul <sup>B</sup>	50.0	52.9	47.0	49.0	49.7
ACA 884 <sup>S</sup>	46.7	46.6	48.6	42.2	46.0
Jaguel <sup>B</sup>	50.7	50.0	49.6	48.1	49.6
<b>Average</b>	<b>48.8</b>	<b>49.4</b>	<b>48.1</b>	<b>45.9</b>	

Table III  
 Grain yield (kg ha<sup>-1</sup>) and (b) Oil content (% d.b.) for each hybrid and four locations in Experiment II. B: Black hull hybrid. S: Stripped hull hybrid. T: traditional hybrid, HOH, High oleic hybrid

## (a) Grain yield

Hybrids	Location			Average
	Pieres	9 de Julio	Tres Arroyos	
Sideral	1614	1994	1302	1637
Trisum	1914	2392	2184	2163
Aromo	2071	2683	2378	2377
Contiflor 9	2723	2694	2225	2547
ACA 884	2558	2679	3441	2892
<b>Average</b>	<b>2176</b>	<b>2488</b>	<b>2306</b>	

## (b) Oil content

Hybrids	Location			Average
	Pieres	9 de Julio	Tres Arroyos	
Sideral <sup>HOH,B</sup>	48.1	44.0	48.2	46.8
Trisum <sup>HOH,B</sup>	42.7	42.0	44.4	43.0
Aromo <sup>HOH,B</sup>	47.6	44.6	46.7	46.3
Contiflor 9 <sup>T,B</sup>	44.2	45.1	44.2	44.5
ACA 884 <sup>T,S</sup>	42.0	41.8	43.7	42.5
<b>Average</b>	<b>44.9</b>	<b>43.5</b>	<b>45.4</b>	

42.5 for ACA 884 and 46.8 for Sideral. Mean HOH oil content was greater than TH oil content (45.3 vs. 43.5%) (Table IIIb).

As expected, in both experiments, mean oil content in black hull hybrids (high oil content potential, 47.3%) was higher than in the stripped hull variety (low oil content potential 45.3 %).

### 3.3. Tocopherol concentrations in oil

#### *Experiment I*

The total tocopherol concentration mean was  $826 \mu\text{g g oil}^{-1}$  (CV = 12.8%) and ranged from 708 to  $936 \mu\text{g g oil}^{-1}$  in Realicó and Tandil respectively. In sunflower, Purdy (1986) and Dorrell and Vick (1997) reported values that ranged from 570 to  $770 \mu\text{g g oil}^{-1}$ . Total tocopherol concentration varied from 765 and  $938 \mu\text{g g oil}^{-1}$  for Jaguel and Paraíso 20 respectively (Table IVa). CV (12.8%) of tocopherol content was higher than CV of oil content (5.5%) but lower than CV of grain yield (32.2%), this last trait was considered highly variable. The CV of tocopherol content in this experiment was lower than the CV of 23% calculated from data obtained by Nagao and Yamazaki (1983), who worked with different genotypes and environments in Japan. Also, it was close to the CV of 19% obtained by Jáky *et al.* (1980), who worked with sunflower varieties from Hungary and Russia, and higher than the CV = 7.4% obtained by Kandil *et al.* (1990), who worked with different sunflower hybrids in contrasting environments (Germany and Egypt, summer and winter).

$\alpha$ -tocopherol and  $\beta$ -tocopherol were detected in all samples while  $\gamma$ -tocopherol was only detected in 31% of the samples. The  $\alpha$ -tocopherol concentration mean was  $791 \mu\text{g g oil}^{-1}$  and represented the major proportion of total tocopherols (90.8%) (Table IVb).  $\beta$ -tocopherol and  $\gamma$ -tocopherol concentrations were 32.0 and  $4.5 \mu\text{g g oil}^{-1}$  and represented 3.9 and less of 1% of the total tocopherols respectively (Tables IVc and d).

#### *Experiment II*

Mean total tocopherol concentration was  $840 \mu\text{g g oil}^{-1}$  (CV = 8.4%) and ranged from 795 to  $870 \mu\text{g g oil}^{-1}$  in 9 de Julio and Tres Arroyos respectively. Total tocopherol concentrations varied from 776 and  $926 \mu\text{g g oil}^{-1}$  for ACA 884 and Sideral respectively. In HOH, this trait was higher than the TH mean ( $875$  vs.  $786 \mu\text{g g oil}^{-1}$ ) in each location (Table Va).

The CV value (8.4%) was higher than the CV of oil content (4.8%) but lower than the grain yield (CV = 22.4%). It was also lower than in Experiment I. Mean values for this trait in HOH and TH were similar to those found by De Villiers *et al.* (1989), who reported  $949 \mu\text{g g oil}^{-1}$  in two HOH in relation to  $710$ - $790 \mu\text{g g oil}^{-1}$  in TH. However, Holló *et al.* (1998) reported no differences in the composition

and concentration of tocopherols in the oil of TH and HOH. Mascioli (2001) reported no differences in tocopherol concentrations in the oil among one TH, one HOH and one hybrid with a medium oleic acid content.

$\alpha$ -tocopherol and  $\beta$ -tocopherol were detected in all samples.  $\alpha$ -tocopherol mean concentration was  $815 \mu\text{g g oil}^{-1}$  and represented the highest proportion of total tocopherols (97%).  $\beta$ -tocopherol concentration was  $25.4 \mu\text{g g oil}^{-1}$  and represented 3.0% of the total tocopherols (Table Va and b). It was not possible to measure the  $\gamma$ -tocopherol concentration. Only small traces were detected in 23% of the samples.

Relative  $\alpha$ - and  $\beta$ -tocopherol concentrations with respect to total tocopherol concentration were similar in TH and HOH.

In both experiments, some of samples (25 % in Experiment I and 13 % in Experiment II) showed tocopherol content values higher than those reported by Gustone *et al.* (1994) as typical values of *Helianthus annuus*. Extreme values for this trait were close to results reported by Marquard, (1990) ( $480$  to  $1128 \mu\text{g g oil}^{-1}$ ) while maximum values were lower than the values found by Velasco *et al.*, (2002) ( $562.8$  to  $1872.8 \mu\text{g g oil}^{-1}$ ).

In both experiments, the  $\alpha$ -tocopherol concentration represented the highest proportion of total tocopherol concentrations. These results are similar to those reported by Demurin *et al.* (1996); Dorrell and Vick (1997); Miller and Fick (1997) and Cole *et al.* (1998).

### 3.4. Variance analysis and Genetic Determination Degree (GDD) determination of tocopherol concentrations in the oil

#### 3.4.1. General

Error Mean square (EMS) at Bordenave was 7.5 higher than the minor EMS in other experimental locations. Therefore, in order to avoid distortions in the results (Box, 1954; Cochran and Cox, 1992), this location was not considered for the combined ANOVA.

#### 3.4.2. Total tocopherol

##### *Experiment I*

Significant ( $P \leq 0.01$ ) differences among hybrids and among locations ( $P \leq 0.0001$ ) were detected. Hybrid\*Location interaction (HLI) was significant ( $P \leq 0.0034$ ), meaning that some hybrids showed different ranking in different locations. This interaction, however, had a low value.

Calculated GDD was 77.6%, which showed that 77.6% of the phenotypic variation observed among hybrids was a genotypic variation.

CV (%) values across locations for each hybrid ranged from 8.4 to 18.3%, while CV (%) values

Table IV  
 (a) total tocopherol, (b)  $\alpha$ -tocopherol, (c)  $\beta$ -tocopherol and (d)  $\gamma$ -tocopherol in oil ( $\mu\text{g g oil}^{-1}$ ) for each hybrid and four locations in Experiment I

## (a) total tocopherol

Hybrids	Location				Average
	Balcarce	Bordenave	Realicó	Tandil	
Paraíso 20	935	987	777	1054	938
Pyramid-1	841	868	807	987	876
Morgan 742	820	827	634	1002	821
Contiflor 11	865	778	688	885	804
Rancul	818	799	695	847	790
ACA 884	734	810	698	911	788
Jaguel	753	781	657	867	765
<b>Average</b>	824	836	708	936	

(b)  $\alpha$ -tocopherol

Hybrids	Location				Average
	Balcarce	Bordenave	Realicó	Tandil	
Paraíso 20	887	957	756	1022	906
Pyramid-1	794	821	780	936	833
Morgan 742	783	805	621	940	787
Contiflor 11	820	752	671	849	773
Rancul	775	771	680	819	761
ACA 884	694	767	677	875	753
Jaguel	705	739	635	822	725
<b>Average</b>	780	802	689	895	

(c)  $\beta$ -tocopherol

Hybrids	Location				Average
	Balcarce	Bordenave	Realicó	Tandil	
Paraíso 20	43	30	21	32	31.5
Pyramid-1	46	44	27	47	41.0
Morgan 742	35	22	13	53	30.8
Contiflor 11	31	25	12	35	25.8
Rancul	32	23	15	27	24.3
ACA 884	37	40	22	36	33.8
Jaguel	42	42	22	41	36.8
<b>Average</b>	38.0	32.3	18.9	38.7	

(d)  $\gamma$ -tocopherol

Hybrids	Location				Average
	Balcarce	Bordenave	Realicó	Tandil	
Paraíso 20	5.3	–	–	–	5.3
Pyramid-1	0	3.7	–	0.8	1.5
Morgan 742	2.3	–	–	9.0	5.7
Contiflor 11	14.0	1.0	–	0.8	5.3
Rancul	10.7	5.0	–	2	5.9
ACA 884	3.0	3.7	–	–	3.4
Jaguel	6.0	–	–	3.7	4.9
<b>Average</b>	5.9	3.4	–	3.3	

Table V  
 (a) total tocopherol, (b)  $\alpha$ -tocopherol and, (c)  $\beta$ -tocopherol in oil ( $\mu\text{g g oil}^{-1}$ )  
 for each hybrid and three locations in Experiment II (TH and HOH)

## (a) total tocopherol

Hybrids	Location			Average
	Pierres	9 de Julio	Tres Arroyos	
Sideral	925	910	942	926
Trisum	923	774	895	864
Aromo	855	775	879	836
Contiflor 9	759	798	833	797
ACA 884	812	716	799	776
<b>Average</b>	855	795	870	

(b)  $\alpha$ -tocopherol

Hybrids	Location			Average
	Pierres	9 de Julio	Tres Arroyos	
Sideral	888	869	926	894
Trisum	904	750	874	843
Aromo	843	736	856	812
Contiflor 9	736	776	813	775
ACA 884	774	690	782	749
<b>Average</b>	829	764	850	

(c)  $\beta$ -tocopherol

Hybrids	Location			Average
	Pierres	9 de Julio	Tres Arroyos	
Sideral	38	41	16	31.7
Trisum	19	24	21	21.3
Aromo	12	40	23	25.0
Contiflor 9	24	22	20	22.0
ACA 884	38	26	18	27.3
<b>Average</b>	26.2	30.6	19.6	

among hybrids for each location ranged from 8.2 to 8.8%.

Experiment II

Significant ( $P \leq 0.01$ ) differences among hybrids and among locations ( $P \leq 0.05$ ) were detected. Hybrid\*Location interaction was not significant, showing that hybrids had similar values in different locations. No significant differences among hybrids, locations and HLI ( $P \geq 0.1$ ) were detected in the group HOH. Calculated GDD was 85%.

CV (%) values across locations for each hybrid ranged from 1.7 to 9.2 % while CV values across hybrids for each location varied from 6.9 to 9.9%. Maximum CV (%) value across hybrids was lower than in Experiment I and similar to CV values across locations in this experiment.

3.4.3.  $\alpha$ -tocopherol concentrationExperiment I

Significant ( $P \leq 0.01$ ) differences among hybrids and among locations ( $P \leq 0.0001$ ) were detected. Hybrid\*Location interaction was significant ( $P \leq 0.017$ ).

Calculated GDD was 81%, a similar value to the GDD for total tocopherol concentration.

CV (%) values trough locations for each hybrid ranged from 7.7 to 16.6%, and CV values trough hybrids for each location ranged from 8.3 to 9.3%.

Experiment II

Significant ( $P \leq 0.025$ ) differences among hybrids and among locations ( $P \leq 0.05$ ) were detected. Hybrid\*Location interaction was not significant. Calculated GDD was 85%.

The high values of GDD for total and  $\alpha$ -tocopherol concentrations in both experiments suggest that it could be possible to recommend the sowing of hybrids in a broad range of locations because although Hybrid\*Location interaction was significant, it had a low value.

CV (%) values across locations for each hybrid ranged from 3.2 to 9.7%, and CV values across hybrids for each location ranged from 6.5 to 8.7%.

#### 3.4.4. $\beta$ -tocopherol concentration

##### Experiment I

Significant ( $P \leq 0.0001$ ) differences among locations were detected while the differences among hybrids and Hybrid\*Location interaction were not significant.

##### Experiment II

No significant differences among locations, hybrids and Hybrid\*Location interaction were detected.

### 3.5. Correlations between total and individual tocopherol concentrations and grain yield and oil content

##### Experiment I

Total tocopherol concentration and grain yield were not significantly correlated ( $P = 0.9$ ).

$\alpha$ -tocopherol concentration was positively correlated with total tocopherol concentration ( $r = 0.992$ ;  $P < 0.0001$ ;  $n = 84$ ; slope = 0.9; intercept = 21).  $\beta$ -tocopherol concentration was significant ( $r = 0.544$ ;  $P < 0.0001$ ; slope = 5; intercept = 666) and positively correlated with total tocopherol concentrations but the high value of the slope means that increases in the total tocopherol concentration correspond with low increases of  $\beta$ -tocopherol concentration.  $\gamma$ -tocopherol and total tocopherol concentration were not correlated.

Total tocopherol concentration and oil content were not significantly correlated, meaning that both traits could be independently selected. The lack of correlation could probably be explained by the broad variation in oil content of the different hybrids

when data were pooled (Table IIb). However, in three hybrids, ACA 884, Pyramid-1 and Morgan 742, the total tocopherol concentration variations inversely accounted for oil content variations (Table VI), with the different proportion of variation explained for each one of them (43.8%; , 45.3% and 76.7% for ACA 884, Pyramid-1 and Morgan 742, respectively). Such a negative correlation has been found in sunflower (Marquard, 1990; Nolasco *et al.*, 2004) and several mustards (Marquard, 1985). In the other hybrids, no significant correlation between both traits was observed ( $P \geq 0.16$ ) (Table VI). These results were similar to those reported by Velasco *et al.* (2002).

##### Experiment II

Total and  $\alpha$ -tocopherol concentrations diminished when grain yield increased. Lineal relationships accounted for the variation of total tocopherol to a low extent (38.6%;  $P < 0.0001$ ;  $n = 30$  and 46.5%,  $P < 0.0001$ ;  $n = 30$  for total and  $\alpha$ -tocopherol, respectively). This variation was mainly explained by the negative relationship for each location across hybrids (Table VIIa). Such results partly agree with Nolasco *et al.* (2004) who found a weak negative relationship between total and  $\alpha$ -tocopherol concentration and yield per plant. Total and  $\alpha$ -tocopherol concentrations increased at the Tres Arroyos location when oil content increased (Table VIIb). This result disagrees with previous research (i.e. Marquard, 1985, y Marquard, 1990) where negative relationships between tocopherol concentration and oil content are found). This inverse trend may be explained by a dilution of tocopherols in a higher oil quantity (Nolasco *et al.*, 2004).

$\alpha$ -tocopherol concentration was positively and significantly correlated with total tocopherol concentration ( $r = 0.99$ ;  $P < 0.0001$ ;  $n = 40$ ).  $\beta$ -tocopherol and total tocopherol concentration were not correlated.

In both experiments, variations in  $\alpha$ -tocopherol concentration accounted for variations in total tocopherol concentrations. The correlations between  $\beta$ - and  $\gamma$ -tocopherol, which have a higher antioxidant activity and a lower vitamin activity than  $\alpha$ -tocopherol (Miller and Fick, 1997), were weak.

Results suggest that it would be possible to modify total and  $\alpha$ -tocopherol concentrations in TH with low variations across regions. . Environmental

Table VI  
Correlation coefficient and significance between total,  $\alpha$  and  $\beta$ -tocopherols and oil content (% d.b.) for each of seven hybrids grown in four locations in Experiment I. \*:  $P < 0.05$ , \*\* $P < 0.01$

Parameter	Hybrid						
	Paraíso 20	Pyramid-1	Morgan 742	Contiflor 11	Rancul	ACA 884	Jaguel
Total tocopherol	NS	-0.67*	-0.88**	NS	NS	-0.67*	NS
$\alpha$ -tocopherol	NS	-0.71*	-0.87**	NS	NS	-0.69*	NS
$\beta$ -tocopherol	NS	NS	-0.71*	NS	NS	NS	NS

Table VII  
**Correlation coefficient and significance between (a) total,  $\alpha$ - and  $\beta$ - tocopherols and grain yield (kg ha<sup>-1</sup>) and, (b) total,  $\alpha$ - and  $\beta$ - tocopherols and oil content (% d.b.) for each of three locations where five hybrids were grown in Experiment II.**  
 \*P < 0.05, \*\*P < 0,01

(a) Grain yield			
Parameter	Lociation		
	Pierres	9 de Julio	Tres Arroyos
Total tocopherol	-0.78**	-0.65*	-0.66*
$\alpha$ -tocopherol	-0.75*	-0.63*	-0.67*
$\beta$ -tocopherol	NS	NS	NS

  

(b) Oil content			
Parameter	Lociation		
	Pierres	9 de Julio	Tres Arroyos
Total tocopherol	NS	NS	0.69*
$\alpha$ -tocopherol	NS	NS	0.69*
$\beta$ -tocopherol	NS	NS	NS

variability existing in the Pampean Region, in Argentina, is sufficient to produce differences due to environmental effects. It would be necessary to make trials in other environments in different regions of Argentina to detect the variability in tocopherol concentrations among HOH.

So, to produce sunflower oil content with high tocopherol concentrations the genotype is as important as the environment. In order to complete the information supplied in this paper, it is necessary to determine how environmental factors affect tocopherol concentrations and to know the relationship among them, among other quality traits and grain yield. The information given in this paper would allow plant breeders to interpret the variability in tocopherol concentrations obtained in different environments.

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