Estimation of breeding potential for tocopherols and phytosterols in sunflower

Alicia Ayerdi Gotor[1], Monique Berger[1], Françoise Labalette[2], Sylvie Centis[3], Jean Daydé[1], Anne Calmon[1]

[1]E.I. Purpan, Laboratoire d'Agro-Physiologie, 75 voie du TOEC, BP 57611, 31076 Toulouse, France, alaygo@hotmail.com; monique.berger@purpan.fr; jean.dayde@purpan.fr; anne.calmon@purpan.fr

[2] ONIDOL, 12 Av. Georges V Paris 75008, France, f.labalette@prolea.com

[3] ASEDIS-SO, 39 chemin Virebent, Toulouse 31200, France, sylvie.centis.aubay@gnis.fr

ABSTRACT

Sunflower (*Helianthus annuus* L.) oil is a good source of tocopherols and phytosterols, interesting bioactive molecules with beneficial health effects. The objective of this work was to determine the possibility of breeding sunflowers with improved oil quality for these traits. Seven B lines (females) and seven R lines (males) were crossed to obtain 49 F_1 hybrids progenies. The F_1 hybrids were then grown for two consecutive years (2005 and 2006) on six locations / year. General combining abilities (GCA) for total tocopherol content were higher than for the total phytosterol content. In both B and R parental lines, there was a positive correlation between the parental lines values and their GCA for total tocopherol content, suggesting additive effects and the possibility of genetic gain for this parameter. This was not the case for total phytosterol content, which displayed important year effects on GCA. The specific combining abilities (SCA) were very important, particularly the negative ones, for the total tocopherol content indicating that some dominance effects could change the predicted hybrid performance. The results of this study indicate the existence of genetic variance for total tocopherol content, but phytosterol content variability is lower that that of tocopherols and subjected to year interactions.

Key words: breeding – GCA– phytosterols – tocopherols – sunflower.

INTRODUCTION

Several studies have shown that tocopherols and sterols can have many positive health effects: they prevent cancer (Bramley et al., 2000), reduce blood cholesterol level (von Bergmann et al., 2005; Patel and Thompson, 2006) and they are effective antioxidants (Niki and Noguchi, 2004). An increasing interest in such active molecules has promoted research in the natural sources of these substances. Tocopherols are lipid antioxidants, vitamin E-active substances, with four isomers (α-, β-, γ-, and δ-tocopherol) with species-dependent proportions. Naturally, sunflower oil has more than 95% of α-tocopherol (Ayerdi Gotor et al., 2006a), the most efficient Vitamin E bioactive tocopherol homologue. In literature, references can be found on sunflower tocopherol mutants with a high content in β-, δ-, or γ-tocopherol (Velasco et al., 2004a,b; Demurin et al., 2007). The total content can vary between 300 to 1873 $mg \cdot kg^{-1}$ of oil (Demurin et al., 1996; Velasco et al., 2002; Nolasco et al., 2006). Phytosterol content in sunflower oil varies from 200 to 700 $mg \cdot 100g^{-1}$ of oil (Vlahakis and Hazebroek, 2000; Ayerdi Gotor et al., 2007) and β-sitosterol is the major form (40-60%).

Genotype as well as environment can influence the total tocopherol content in sunflower oil. Temperature is one of the most influential environmental factors (Velasco et al., 2002; Ayerdi Gotor et al., 2006b; Nolasco et al., 2006). Phytosterols content is less influenced by genotype or by environmental factors (Ayerdi Gotor et al., 2006b; Roche et al., 2006).

In spite of the growing importance of tocopherols and phytosterols as micronutrients and as natural oil stabilizers, few studies have focused on breeding programs for these minor components in oilcrops, especially on sunflower. It has been shown in rapeseed that genetic progress is possible for the oil tocopherol content (Goffman and Becker, 2001a,b). In these studies, tocopherol content and composition inheritances were highly associated with additive gene action.

Breeding for tocopherol and phytosterol contents can increase the market value of sunflower oil by means of health-promoting effects associated with these nutriments. The objective of the present study was to determine the feasibility of breeding for these molecules in sunflower oil.

MATERIALS AND METHODS

Plant material

Seven restorers (males) and seven females (cytoplasmic male sterile) parental lines of sunflower, *Helianthus annuus* L., were selected for their high and low tocopherol and phytosterol content. The F1 hybrids seeds were produced in a 7 X 7 factorial design (NCII). Crosses were made in Chile during winter 2005. Three of the 49 hybrids formed were not viable and produced no seed. The fourteen parental lines were provided by six sunflower breeders: Caussade semences, Maïsadour semences, Monsanto Dekalb SAS, RAGT-R2n, Soltis and Syngenta seeds.

Field trials

The progenies (from grains F_1) were cultivated in the summers of 2005 and 2006 in six different places throughout France (Table 1), with two blocks in each place. Hybrids were randomized in the blocks to limit the effect of interactions between plants. Just before flowering, the buds were covered with microperforated bags to ensure self-pollinated achenes; these bags were taken away at the end of flowering. F_2 achenes from F_1 plants were collected at maturity, the lab samples (for analysis) were made with 5 plants from the same plot.

Table 1. F_2 hybrid growing places in 2005 and 2006.

Breeder company	Place (French department)	Geographical location
Caussade semences	Cayrac (81)	44º6'N 1º28'E
Maïsadour semences	Conan (41)	47º48'N 1º15'E
Monsanto Dekalb SAS	Savenès (82)	43º49' N 1º11'E
RAGT-R2n	Villampuy (28)	48º2'N 1º30'E
Soltis	Mondonville (31)	43º40'N 1º17'E
Syngenta seeds	Saint sauveur (31)	43º45'N 1º24'E

Chemical analysis

Oil extraction

Grains were ground in a sample mill (KnifeTec 1095; Foss Tecator AB, Sweden) for 2 periods of 10 s. Around 15 g of ground seeds were placed in a 33ml cartridge with Fointainebleu sand for extraction in an accelerated solvent extractor apparatus (ASE-200, Dionex, France) with the following extraction conditions: 120ºC, 10 min of static extraction, 95% Hexane (n-hexane Prolabo-Subra, France) and 5% Propanol-2 (HPLC grade, SDS, France) under a pressure of 100 bar. Oil was recovered after solvent evaporation under low pressure with a rotavapor (HS 40 Huber, Bioblock Scientific, Heildolph, Germany). Lipid extracts were weighed and conserved at –18°C to minimize oxidative reactions before analysis.

Tocopherol determination

Complete separation of all native tocopherols was achieved using high-performance liquid chromatography (HPLC) (SpectraPhysics; TSP, USA) (ISO 9936, 1997). A normal-phase LiChrosorb Si60 column was used. The mobile phase was hexane/isopropanol (99.7:0.3 v/v) and the solvent flow was 1 mL/min. One gram of oil sample was diluted in 25 mL of hexane and 20 µL were injected. Tocopherols were identified by comparison of retention times with their respective standards (Tocopherol Kit; ChromaDex, USA). Total tocopherol content was calculated as the sum of α- , β-, γ- and δ-tocopherol contents and expressed in mg kg^{-1}oil.

Phytosterol determination

The analyses of sterol required a saponification with KOH 0.5M and a purification on an aluminium oxide basic (Panreac, Spain) column. The total and the individual sterol contents were analyzed by GC, after silylation with trimethylsylil (TMS) ether derivatives. 1µl of the TMS solutions were injected on a silica capillary column (ZB-5) in a gas chromatograph (Clarus 600, Perkin Elmer, USA) fitted with a flame ionization detector. Sterols were identified by their retention time relative to betulin internal standard. They were quantified using the ratio obtained between betulin (Internal standard, Sigma-Aldrich, France) and sterol standards. Sterols were expressed in mg 100 g^{-1} oil (NF EN ISO 12228, 1999).

RESULTS AND DISCUSSION

Total tocopherol content in the parental lines varied between 548.0 to 1096.4 mg·kg^{-1} oil. Total phytosterol content varied between 260.7 to 455.7 mg 100g^{-1} oil. Mean values of F_2 seeds of the 6 growing places and the two years are in Table 2.

Table 2. Mean values, standard deviation (SD) and range of tocopherol and phytosterol total contents for the 6 locations in 2005 and 2006 of the 46 F1 hybrids

Minor component	2005			2006		
	Mean	SD	Range	Mean	SD	Range
α-tocopherol (mg·kg^{-1} oil)	452.3	52.6	346.9- 570.2	516.5	65.0	390.4-679.6
Total tocopherol (mg·kg^{-1} oil)	469.8	55.8	354.8- 590.0	469.8	76.8	427.7- 733.9
β-sitosterol (mg·100g^{-1} oil)	215.7	14.3	176.4- 241.7	227.5	23.4	183.6- 317.1
Total phytosterol (mg·100g^{-1} oil)	315.7	20.6	254.7-366.4	319.6	27.6	262.2- 398.8

The general combining ability (GCA) for each parent was calculated as the difference between the mean of its half sib offsprings and the mean of its overall hybrids, which was calculated separately for each growing place. A mean GCA was then calculated over the six places. The correlations between parental line values and the corresponding mean GCA of their offsprings are shown Fig. 1. GCA for the total tocopherol content was greater than for total phytosterol content, in accordance with the fact that phytosterol variability is less important than that of tocopherols (Ayerdi Gotor et al., 2006b).

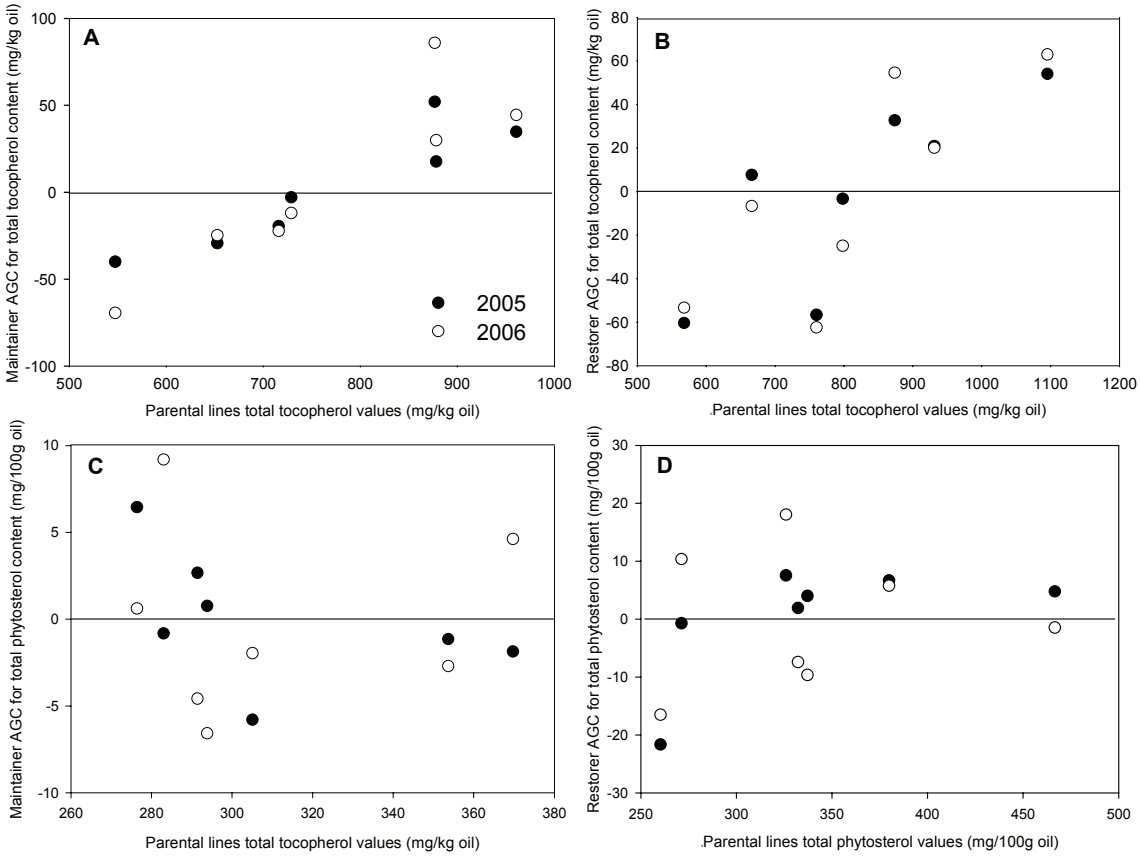

Fig. 1. Correlations between parental values and general combining ability (GCA) in 2005 and 2006. A - B: total tocopherol contents (mg/kg oil), female lines and restorer lines, respectively; C - D: total phytosterol contents (mg/100g oil), female lines and male lines, respectively.

For the total tocopherol content, the GCA and parental values were positively correlated for both female and restorer lines. The tendency was the same in the two years. Such correlations were not observed for the total phytosterol content, which also showed an important year effect.

For each location by year, the specific combining ability (SCA) was calculated as differences between a given hybrid mean and its two parental half sib means deviations from the general mean. Then the means were given by year over the six locations.

Table 3. Range of specific combining ability (SCA) of the 49 hybrids for 2005 and 2006

Year	SCA total tocopherol content (mg/kg oil)		SCA total phytosterol content (mg/100g oil)	
	Maximum	Minimum	Maximum	Minimum
2005	40.25	-52.84	58.94	-29.25
2006	57.18	-117.92	56.49	-48.32

SCA range was larger in 2006 than in 2005 for total tocopherol content. On the contrary, the SCA range showed a lesser year effect for total phytosterol content. Negative SCA for phytosterol and tocopherol content were of a greater amplitude in 2006.

New statistical treatment is currently under development to improve the accuracy of the genetic parameters obtained with these data. This work will soon be completed with heritability information from data of the F3-F4 hybrid seeds from three F2 families selected for their F1 highest GCA (negative or positive, female or restorer) grown during summer, 2007.

These first results suggested that total tocopherol content is influenced by an additive effect showing the possibility of a genetic gain for this parameter. For the total phytosterol content there was a larger year effect on GCA, so genetic gain could be less important. The SCA values indicated that total tocopherol and phytosterol contents could be affected by dominance effects, which could change the predicted hybrid performance. Both families of minor components were subjected to year interactions. These results open up possibilities for breeders to improve sunflower composition by increasing the content of these interesting compounds for human health.

ACKNOWLEDGEMENTS

The authors thank Caussade Semences, Maïsadour Semences, Monsanto Dekalb SAS, RAGT-R2n, Soltis, Syngenta Seeds for field experiments and supply of sunflower plant material. Financial support was provided by ASEDIS-SO, ONIDOL (French oilseeds organization) and Midi-Pyrenees Region. We also thank Cognis-France for their technical support.

REFERENCES

Association Française de Normalisation. 1999. European norm, NF EN ISO 12228 May 1999; French norm T 60-258: Animal and vegetable fats and oils - Determination of individual and total sterols contents - Gas chromatographic method. AFNOR. Paris. 18 p.

Ayerdi Gotor, A., M. Berger, F. Labalette, S. Centis, J. Daydé, and A. Calmon. 2006a. Etude de la variation des teneurs et compositions des composés mineurs dans l'huile de tournesol au cours du développement du capitule: Partie I - Tocophérols. OCL 13: 206-212.

Ayerdi Gotor, A., M. Berger, F. Labalette, S. Centis, J. Daydé, and A. Calmon. 2006b. Variability of minor components in sunflower (*Helianthus annuus* L.) oil from a multi-local study. p. 248-252. In: Proc. 17th Int. Symp. Plant Lipids, East Lansing, MI, USA.

Ayerdi Gotor, A., E. Farkas, M. Berger, F. Labalette, S. Centis, J. Daydé, and A. Calmon. 2007. Determination of tocopherols and phytosterols in sunflower seeds by NIR spectrometry. Eur. J. Lipid Sci. Technol. 109:525-530.

Bramley, P.M., I. Elmafda, A. Kafatos, F.J. Kelly, Y. Manios, H.E. Roxborough, W. Schuch, P.J.A. Sheehy, and K.H. Wagner. 2000. Vitamin E. J. Sci. Food Agr. 80: 913-938.

Demurin, Y., D. Škorić, and D. Karlovic. 1996. Genetic variability of tocopherol composition in sunflower seeds as a basis of breeding for improved oil quality. Plant Breed. 115:33-36.

Demurin, Y.N., S.G. Efimenko, and T.M. Peretyagina. 2007. Expressivity of tocopherol mutations in sunflower. Helia 29:55-62.

Goffman, F.D., and H.C. Becker. 2001a. Diallel analysis for tocopherol contents in seeds of rapeseed. Crop Sci. 41:1072-1079.

Goffman, F.D., and H.C. Becker. 2001b. Genetic analysis of tocopherol content and composition in winter rapeseed. Plant Breed. 120:182-184.

International Standard Organization, 1997. ISO 9936: Animal and vegetable fats and oils - Determination of tocopherols and tocotrienols contents - Method using high-performance liquid chromatography. ISO. Genève. 11 p.

Niki, E., and N. Noguchi. 2004. Dynamics of antioxidant action of vitamin E. Accounts Chem. Res. 37: 45-51.

Nolasco, S.M., L.A.N. Aguirrezábal, J. Lúquez, and C. Mateo. 2006. Variability in oil tocopherol concentration and composition of traditional and high oleic sunflower hybrids (*Helianthus annuus* L.) in the Pampean region (Argentina). Grasas y Aceites 57: 260-269.

Patel, M.D., and P.D. Thompson. 2006. Phytosterols and vascular disease. Atherosclerosis 186:12-19.

Roche, J., A. Bouniols, Z. Mouloungui, T. Barranco, and M. Cerny. 2006. Management of environmental crop conditions to produce useful sunflower oil components. Eur. J. Lipid Sci. Technol. 108:287-297.

Velasco, L., J.M. Fernández-Martínez, R. García-Ruiz, and J. Domínguez. 2002. Genetic and environmental variation for tocopherol content and composition in sunflower commercial hybrids. J. Agr. Sci. 139:425-429.

Velasco, L., B. Pérez-Vich, and J.M. Fernández-Martínez. 2004a. Novel variation for the tocopherol profile in a sunflower created by mutagenesis and recombination. Plant Breed. 123:490-492.

Velasco, L., B. Pérez-Vich, and J.M. Fernández-Martínez. 2004b. Development of sunflower germplasm with high delta-tocopherol content. Helia 27:99-106.

Vlahakis, C., and J. Hazebroek. 2000. Phytosterol accumulation in canola, sunflower, and soybean oils: Effects of genetics, planting location, and temperature. J. Am. Oil. Chem. Soc. 77: 49-53.

von Bergmann, K., T. Sudhop, and D. Lütjohann. 2005. Cholesterol and plant sterol absorption: recent insights. J. Am. Cardiol. 96:10D-14D.