

EVALUATION OF WILD SUNFLOWER SPECIES FOR TOCOPHEROL CONTENT AND COMPOSITION

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SUMMARY

Wild *Helianthus* species are an important reservoir of useful genes for sunflower breeding. The objective of the present research was to evaluate the existing variability for tocopherol content and composition in a germplasm collection of wild sunflower species. The germplasm evaluated consisted of 257 accessions from 36 *Helianthus* species, including both annual and perennial species. Eight seeds per accession were randomly picked and bulked for tocopherol analysis conducted by HPLC with fluorescence detection. Tocopherol content averaged 328 mg kg⁻¹ seed in the analyzed accessions, with an average profile of 99.0% alpha-tocopherol, 0.7% beta-tocopherol, and 0.3% gamma-tocopherol. In cultivated material, an average tocopherol content of 669.1 mg kg⁻¹ seed have been reported, made up of 92.4% alpha-tocopherol, 5.6% beta-tocopherol, and 2.0% gamma-tocopherol. The maximum total tocopherol content in wild sunflower germplasm corresponded to an accession of *H. maximiliani*, with 673 mg kg⁻¹ seed. Increased levels of beta-tocopherol were identified in one accession of *H. praecox* (11.2% of the total tocopherols) and one accession of *H. debilis* (11.8%). All other accessions contained less than 6.5% beta-tocopherol. Increased gamma-tocopherol levels were identified in one accession of *H. exilis* (7.4%) and two accessions of *H. nutalii* (11.0% and 14.6%, respectively). All other accessions contained less than 2% gamma-tocopherol. Although further research at an intrapopulation level is needed to confirm and isolate variants, the results of the present research indicated that wild *Helianthus* germplasm contains useful variability for tocopherol content and composition.

Key words: genetic diversity, germplasm evaluation, *Helianthus* spp., tocopherols, wild species, variability

INTRODUCTION

Tocopherols are fat-soluble compounds that exert an antioxidant action both *in vivo* (vitamin E activity) and *in vitro* (Kamal-Eldin and Appelqvist, 1996). They occur as a family of four derivatives named alpha-, beta-, gamma-, and delta-toco-

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pherol. The four tocopherols differ for their relative *in vitro* and *in vivo* antioxidant activities. Alpha-tocopherol has the highest vitamin E activity, but it is a weak *in vitro* antioxidant. Conversely, gamma-tocopherol is the most powerful antioxidant *in vitro* but its *in vivo* vitamin E activity is low. Beta and delta tocopherols has intermediate properties (Pongracz *et al.*, 1995).

The cultivated sunflower possesses a moderate content of seed tocopherols, predominantly made up of alpha-tocopherol. Velasco *et al.* (2002) reported an average tocopherol content of 669.1 mg kg⁻¹ seed, made up of 92.4% alpha-tocopherol, 5.6% beta-tocopherol, and 2.0% gamma-tocopherol, in a set of commercial hybrids cultivated in Spain. Both the increase of total tocopherol content in the seeds as well as the development of alternative tocopherol profiles are important breeding objectives in sunflower.

Genetic sources of modified tocopherol profiles have been identified in germplasm of cultivated sunflower (Demurin, 1993; Demurin *et al.*, 1996; Velasco *et al.*, 2004). These included germplasm with a high concentration of gamma-tocopherol, about 95% of the total tocopherols, as well as germplasm with increased levels of beta-tocopherol, about 50% of the total tocopherols. Both novel tocopherol profiles are of great value for developing sunflower cultivars with improved oxidative stability of the oil.

The objective of this research was to evaluate the variation for total tocopherol content and composition in germplasm of wild *Helianthus* species.

MATERIALS AND METHODS

The plant material evaluated comprised 257 accessions representing 36 *Helianthus* species, 10 annuals and 26 perennials. The accessions were obtained from USDA-ARS Regional Plant Introduction Station (PI), Ames, IA, Institute of Field and Vegetable Crops (IFVC), Novi Sad, Serbia and Montenegro, and Station de Génétique et d'Amélioration des Plantes (SGAP), Montpellier, France. Perennial species were multiplied at the experiment farm of Instituto de Agricultura Sostenible at Córdoba, Spain. Annual species were grown in pots. Seeds for tocopherol analyses were obtained from sibbed heads in 2000.

Analysis of tocopherol content and composition, based on eight seeds per accession, was based on the method of Goffman *et al.* (1999). Half seeds were cut from each seed and the eight halves were weighed and placed into a 10 ml tube. After 2 ml of iso-octane were added, the half seeds were crushed with a stainless steel rod as fine as possible. The rod was then washed with 2 ml of iso-octane, which were collected in the tube. The samples were stirred and extracted overnight at room temperature in darkness (extraction time about 16 h). After extraction, the samples were stirred again, centrifuged, and filtered. 5 µl of the extract were analyzed by HPLC 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

Table 1: Number of accessions analyzed and average values for total tocopherol content (mg kg⁻¹ seed) and tocopherol profile (% of the total tocopherols) in 36 *Helianthus* species

Section	Series	Species	N	Total-T	α -T	β -T	γ -T
Helianthus		<i>H. annuus</i> L.	32	389.8	97.9	1.8	0.3
		<i>H. anomalous</i> S.F. Blake	2	317.5	97.4	2.6	0.0
		<i>H. argophyllus</i> Torr. & A. Gray	12	346.8	98.6	1.1	0.3
		<i>H. bolanderi</i> A. Gray	1	348.5	98.9	1.1	0.0
		<i>H. debilis</i> Nutt.	27	269.0	98.5	1.5	0.0
		<i>H. exilis</i> A. Gray	5	74.5	98.5	0.0	1.5
		<i>H. neglectus</i> Heiser	5	87.1	99.0	1.0	0.0
		<i>H. niveus</i> (Benth.) Brandegees	3	340.9	100.0	0.0	0.0
		<i>H. petiolaris</i> Nutt.	25	457.1	98.3	1.2	0.5
		<i>H. praecox</i> Engelm & A. Gray	17	290.0	99.0	1.0	0.0
Ciliares	<i>Ciliares</i>	<i>H. ciliaris</i> DC.	1	407.7	100.0	0.0	0.0
	<i>Pumili</i>	<i>H. cusickii</i> A. Gray	1	260.8	98.5	1.5	0.0
		<i>H. gracilentus</i> A. Gray	1	425.8	100.0	0.0	0.0
		<i>H. pumilus</i> Nutt.	1	275.6	100.0	0.0	0.0
Atrorubens	<i>Corona-solis</i>	<i>H. californicus</i> DC.	1	246.9	100.0	0.0	0.0
		<i>H. decapetalus</i> L.	15	349.2	99.4	0.2	0.4
		<i>H. divaricatus</i> L.	7	305.4	99.7	0.2	0.1
		<i>H. eggertii</i> Small	1	260.0	97.8	1.2	1.0
		<i>H. giganteus</i> L.	11	264.1	100.0	0.0	0.0
		<i>H. grosseserratus</i> Mart.	7	455.3	99.8	0.2	0.0
		<i>H. hirsutus</i> Raf.	1	222.7	100.0	0.0	0.0
		<i>H. maximilianii</i> Schrad.	13	473.2	99.4	0.6	0.0
		<i>H. mollis</i> Lam.	6	221.0	100.0	0.0	0.0
		<i>H. nuttallii</i> Torr. & A. Gray	20	286.4	98.6	0.1	1.3
		<i>H. salicifolius</i> A. Dietr.	2	227.6	98.7	1.3	0.0
		<i>H. schweinitzii</i> Torr. & A. Gray	1	333.0	100.0	0.0	0.0
		<i>H. strumosus</i> L.	6	306.4	99.8	0.0	0.2
	<i>H. tuberosus</i> L.	6	258.1	100.0	0.0	0.0	
	<i>Microcephali</i>	<i>H. glaucophyllus</i> D.M. Sm.	2	239.4	99.3	0.7	0.0
		<i>H. laevigatus</i> Torr. & A. Gray	3	344.6	99.6	0.3	0.1
		<i>H. microcephalus</i> Torr. & A. Gray	2	247.3	100.0	0.0	0.0
	<i>Atrorubentes</i>	<i>H. atrorubens</i> L.	1	307.7	100.0	0.0	0.0
		<i>H. occidentalis</i> Riddell	6	422.7	99.9	0.1	0.0
		<i>H. pauciflorus</i> Nutt.	9	363.6	99.7	0.3	0.0
<i>Angustifolii</i>	<i>H. simulans</i> E. Watson	3	238.5	99.5	0.5	0.0	
	<i>H. x laetiflorus</i> Pers.	1	254.4	100.0	0.0	0.0	

rate of 0.8 ml min^{-1} . Chromatographic separation of the tocopherols was performed on a LiChrospher 100 diol column ($250 \text{ mm} \times 3 \text{ mm I.D.}$) with $5\text{-}\mu\text{m}$ spherical particles, connected to a silica guard column (LiChrospher Si 60, $5 \text{ mm} \times 4 \text{ mm I.D.}$). External calibration curves were used for quantitative determination of tocopherols.

RESULTS AND DISCUSSION

Tocopherol content averaged 328 mg kg^{-1} seed in the analyzed accessions of wild *Helianthus* spp. (Table 1), compared with a tocopherol content of 669.1 mg kg^{-1} seed reported for cultivated sunflower (Velasco et al., 2002). Wild accessions of *H. annuus* averaged 389.8 mg kg^{-1} seed, which was below the average levels of other species, particularly *H. grosseserratus*, *H. petiolaris* and *H. maximiliani*. The latter species included the accession with the largest tocopherol content, 673 mg kg^{-1} seed (accession PI 586899).

The average tocopherol profile of the evaluated germplasm consisted of 99.0% alpha-tocopherol, 0.7% beta-tocopherol, and 0.3% gamma-tocopherol, which is similar to the typical tocopherol profile of the cultivated sunflower (Demurin, 1993). However, wild *Helianthus* species included an interesting variability for beta- and gamma-tocopherol contents. Thus, increased levels of beta-tocopherol were identified in one accession of *H. praecox* (11.2% of the total tocopherols, accession 1342 from IFVC) and one accession of *H. debilis* (11.8%, accession 1566 from IFVC). All other accessions contained less than 6.5% beta-tocopherol. Increased gamma-tocopherol levels were identified in one accession of *H. exilis* (7.5% of the total tocopherols, accession 671 from SGAP) and two accessions of *H. nutalii* (11.0%, accession PI 531054 and 14.6%, accession PI 531043). All other accessions contained less than 2% gamma-tocopherol. Although further research at an intrapopulation level is needed to confirm and isolate variants, these results indicated that wild *Helianthus* germplasm contains useful variability for tocopherol profile.

Previous studies have reported that wild *Helianthus* species contain useful variation for the fatty acid composition of the oil, including variants for increased oleic acid (Seiler, 1985), high linoleic acid (De Haro and Fernández-Martínez, 1991), or low levels of total saturated fatty acids (Seiler, 1994, 1996). The results of the present research suggest that useful variation for tocopherol content and composition can also be found within germplasm of wild *Helianthus* spp.

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EVALUACIÓN DEL CONTENIDO Y COMPOSICIÓN DE TOCOFEROL EN LAS ESPECIES SILVESTRES DE GIRASOL

RESUMEN

Las especies silvestres del género *Helianthus* constituyen una importante reserva de genes útiles para la mejora del girasol. El objetivo de este trabajo fue la evaluación de la variabilidad existente para contenido total y composición en tocoferoles en una colección de germoplasma de especies silvestres de girasol. Esta colección estuvo compuesta por 257 entradas de 36 especies de *Helianthus*, incluyendo tanto especies anuales como perennes. Se escogieron al azar ocho semillas de cada entrada y se analizaron conjuntamente para contenido y composición en tocoferoles, mediante empleo de HPLC con detector de fluorescencia. El contenido medio en tocoferoles fue de 328 mg kg⁻¹ semilla, con un perfil de tocoferoles consistente en 99.0% alfa-tocoferol, 0.7% beta-tocoferol, y 0.3% gamma-tocoferol. El contenido medio descrito en material cultivado es de 669.1 mg kg⁻¹ semilla, compuesto por 92.4% alfa-tocoferol, 5.6% beta-tocoferol, y 2.0% gamma-tocoferol. El contenido máximo en tocoferoles en germoplasma de girasol silvestre se encontró en una entrada de *H. maximilianii*, con 673 mg kg⁻¹ semilla. Se encontraron niveles elevados de beta-tocoferol en una entrada de *H. praecox* (11.2%) y en una entrada de *H.*

debilis (11.8%). El resto de entradas presentó menos de 6.5% de beta-tocoferol. Se encontraron niveles elevados de gamma-tocoferol en una entrada de *H. exilis* (7.4%) y dos entradas de *H. nutalii* (11.0% y 14.6%, respectivamente). El resto de entradas presentó menos del 2% de gamma-tocoferol. Aunque es necesaria una investigación adicional a nivel intrapoblacional para confirmar y aislar genotipos con perfiles modificados de tocoferoles, los resultados de este trabajo indican que el germoplasma de especies silvestres de *Helianthus* contiene valiosa variabilidad para contenido y composición en tocoferoles.

EVALUATION DE CONTENU ET COMPOSITION DE TOCOPHÉROL CHEZ LES ESPECES SAUVAGES DE TOURNESOL

RESUME

Les espèces sauvages du genre *Helianthus* sont un réservoir utile de gènes dans le développement de tournesol cultivé. Le but de cette recherche était d'évaluer la variabilité existante de contenu et composition de tocophérol dans une collection de germe plasmique d'espèces sauvages de tournesol. La collection examinée contenait 257 génotypes de 36 espèces de plantes annuelles et vivaces du genre *Helianthus*. Huit graines de chaque génotype étaient recueillies par échantillon aléatoire afin d'effectuer l'analyse de tocophérol faite par HPLC de détection fluorescente. Le contenu moyen de tocophérol de génotypes analysés était de 328 mg kg⁻¹ de graine, en moyenne de 99,0% alpha-tocophérol, 0,7% bêta-tocophérol et 0,3% gamma-tocophérol. Les valeurs référentielles du matériel cultivé étaient de 669,1 mg kg⁻¹ de graine, de 92,4% alpha-tocophérol, 5,6% bêta-tocophérol et 2,0% gamma-tocophérol. Le total maximum (de 673 mg kg⁻¹ de graine) était constaté chez un génotype d'espèce *H. maximilianii*. Le contenu élevé de bêta-tocophérol était confirmé chez un génotype d'espèce *H. praecox* (11,2% du total de tocophérol) et d'espèce *H. debilis* (11,8%). Tous les autres génotypes avaient moins de 6,5% de bêta-tocophérol. Le contenu élevé de gamma-tocophérol était confirmé chez un génotype d'espèce *H. exilis* (7,4%) et deux génotypes d'espèce *H. nutalii* (11,0% et 14,6%). Tous les autres génotypes avaient moins de 2% de gamma-tocophérol. Malgré les besoins de recherches supplémentaires d'intra population pour confirmer et isoler les variants, les résultats de cette recherche ont montré que le germe plasmique d'espèces sauvages de tournesol contenait une variabilité utile de contenu et composition de tocophérol.