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Comparison of fatty acid composition of oil from original and regenerated populations of wild *Helianthus* species

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Abstract

Monitoring and protecting germplasm in genebanks using *in situ* collections while preserving its original genetic integrity is a priority of germplasm curation. Many germplasm accessions need to be regenerated due to their demand and/or seed condition. The regeneration of wild *Helianthus* (sunflower) species poses several challenges due to the diversity of 53 wild species. Fatty acid composition of sunflower oil is an important quality factor for the crop. Since oil quality is environmentally influenced, and evaluation of this trait is usually performed on oil from achenes from the original accessions of wild sunflower species, we conducted a study on 72 accessions of eight annual and four perennial taxa of wild sunflower species to compare the oil quality of the original accessions and those regenerated for genebank maintenance. The results showed that the fatty acid composition profiles of achenes from the original and regenerated accessions are not the same. It seems that selection for specific fatty acids in several species will require the analysis of both populations to identify germplasm accessions for use in breeding programmes. It should be borne in mind that accessions of wild species are open-pollinated segregating populations, so one would expect some variability in each succeeding generation. While there may be differences between the original and regenerated accessions, the interrelationships of fatty acids are generally similar in wild and cultivated sunflower species, so there should be no detrimental effects on oil quality when using the wild species for other traits. As more regenerated accessions become available, a more precise relationship between the original and regenerated accessions should emerge.

Keywords: genebank maintenance; genetic resources; linoleic acid; oil quality; oleic acid; sunflower species

Introduction

Plant genetic resources management and conservation comprise several phases, including acquisition and maintenance of genetic integrity in germplasm collections (Chang, 1985). Preservation of cultivars, landraces and wild relatives of important plant species provides the basic foundation to promote and sustain agriculture and global food security (Campbell *et al*., 2010). The abundance of genetic diversity available in the global plant genetic resources genebanks is a valuable resource for potential new sources of resistance to biotic and abiotic stress for plant breeders (Soengas *et al*., 2009; Ulukan, 2011). Monitoring and protecting germplasm using *in situ* collections while preserving its original genetic integrity is a priority of germplasm curation (Bretting and Widrlechner, 1995).

The growth in size and species diversity of germplasm collections has led to several managerial problems (Engels and Ramanatha Rao, 1998; Brown *et al*., 1997).

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New introductions frequently arrive in the genebank with insufficient quantity or quality of seeds to be directly stored or distributed, and need to be regenerated. Regeneration may also be necessary from time to time due to the insufficiency of the quantity of seed for future distribution needs or the decline in the viability of the accession. Accessions of wild species are generally more costly to regenerate than those of cultivated species because of their life history, breeding system, genetic structure, ecology and lack of domestication (Brown *et al.*, 1997). The regeneration of wild *Helianthus* (sunflower) species poses several challenges because of the diversity of 53 wild species.

Wild *Helianthus* species have been utilized to enhance cultivated sunflower species, especially as sources of disease-resistance genes (reviewed by Seiler, 2012). The evaluation of the fatty acid composition of sunflower oil is important in hybrid sunflower production. Several studies have evaluated fatty acid composition from original accessions of wild sunflower species (Dorrell and Whelan, 1978; Thompson *et al.*, 1981; DeHaro and Fernandez-Martinez, 1991; Seiler, 1985, 1994; Seiler and Brothers, 1999). Knowledge of the fatty acid composition profile is important to see whether there are any detrimental effects on overall oil quality when wild species are used for disease resistance or other traits. Oil concentration and fatty acid composition, especially oleic and linoleic acids, of oil from wild and cultivated sunflower accessions vary greatly, mainly in response to the temperature during achene development (Canvin, 1965; Harris *et al.*, 1978; Seiler, 1986). There is a negative correlation between the latitude and the temperature. This results in lower oleic acid and higher linoleic acid concentrations in northern latitudes. This relationship is related to a high negative intercorrelation between linoleic and oleic acids ($r = > -0.900$) in both wild and cultivated sunflower species (DeHaro and Fernandez-Martinez, 1991; Seiler, 1986).

Since oil quality is environmentally influenced and evaluation of this characteristic is usually performed on oil from achenes from the original accessions of wild sunflower populations, we conducted a study on 72 accessions of eight annual and four perennial taxa of wild sunflower species to compare the composition of four major fatty acids (palmitic, stearic, oleic and linoleic) of the original accessions with those regenerated for genebank maintenance at a common location.

Materials and methods

Plant materials

The collection of wild *Helianthus* germplasm accessions in the National Plant Germplasm System (NPGS) of the United States, held at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA, USA, is administered by the US Department of Agriculture, Agricultural Research Service, Plant Introduction Research Unit in cooperation with the Agricultural Experiment Station at Iowa State University, Ames, IA, USA (Marek *et al.*, 2012). The NCRPIS wild sunflower germplasm collection contains a total of 53 *Helianthus* species recognized in the recent Flora of North America (Schilling, 2006), including 39 perennial species represented by 842 accessions and 14 annual species represented by 1359 accessions.

All achenes used in this study were obtained from the NCRPIS, Ames, IA, USA. Prior to analysis, all achenes were stored at 4°C and 35% humidity. Original achenes were collected from various locations in the USA and Mexico under various local environmental conditions over a span of 10 years (Table S1, available online). Latitude (deg, min, sec), longitude (deg, min, sec) and elevation (m) data were obtained from passport information associated with each accession in the US Department of Agriculture, Agricultural Research Service (USDA-ARS), NPGS, Germplasm Resources Information Network database, Beltsville, MD.

Each accession of wild species represented an isolated, open-pollinated segregating population. All analyses were conducted on intact achenes from the original and regenerated accessions of 54 wild annual *H. annuus* L., one *H. debilis* Nutt. ssp. *cucumerifolius* (T&G) Heiser, one *H. debilis* Nutt. ssp. *tardiflorus* Heiser, two *H. debilis* Nutt. ssp. *vestitus* (Watson) Heiser, one *H. niveus* (Benth.) Brandegee ssp. *tephrodes* (Gray) Heiser, four *H. petiolaris* Nutt., four *H. praecox* Engelm. & A. Gray and one *H. praecox* Engelm. & A. Gray ssp. *hirtus* Heiser. In addition, four accessions of perennial species were analysed: one *H. decapetalus* L., one *H. divaricatus* L., one *H. giganteus* L. and one *H. nuttallii* T&G. Regenerated accessions were produced as needed and grown at the same location, but in different years. Furthermore, comparison between the original and regenerated achenes of a cultivated inbred line, HA 89, was analysed.

Regeneration conditions

All sunflower accessions were regenerated under the conditions of controlled pollination to maintain the genetic integrity of the original accessions. Wild *Helianthus* species were regenerated at the NCRPIS, Ames, IA, USA (42°02′05″ N latitude, 93°7′11″ W longitude; elevation 287 m). Dormancy is a persistent problem in many of the wild sunflower species. Achene samples were soaked in 3% H_2O_2 for 5 min, and then rinsed with cool running water and placed in small beakers containing

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25 mg ethephon/kg solution (Ethrel®; Bayer Crop Science, Leverkusen, Germany) overnight. The samples were rinsed again and transferred to germination boxes and vernalized for 2–8 weeks at 4°C before being transferred to a germinator at 20–30°C and 12 h light–12 h dark cycles. Seedlings were then transferred to flats and grown in a greenhouse before being transplanted to the field. It has been estimated that a minimum of 25–50 plants are needed to maintain a high level of genetic integrity in regenerated sunflower accessions (Cronn *et al.*, 1997). Accessions of the wild species were transplanted to the field and covered with screen cages before flowering, with honeybees being introduced into the cages for pollination to maintain isolation. After harvesting, drying and cleaning, achenes were stored at 4°C and 35% humidity.

Fatty acid analyses

Fatty acid composition was determined using a 10–20 bulked achene sample from each accession. The sample was ground to a fine powder in a coffee bean mill, and a small portion (10–20 mg) was transferred to a disposable filter column (Fisher Scientific, Pittsburgh, PA, USA) and eluted with 3.5 ml of diethyl ether. The oil in the diethyl ether solution was converted to methyl esters through an organic-catalysed transesterification reaction of triacylglycerols by the addition of 200 µl of tetramethylammonium hydroxide (10% in methanol), followed by vortexing (Metcalfe and Wang, 1981). After 30 min, water was gently added to the reaction mixture, and the upper diethyl ether layer was transferred to a glass vial and capped. The sample was injected into a Hewlett-Packard 5890 II gas chromatograph (Palo Alto, CA, USA) containing a DB-23 capillary column (25 m × 0.25 mm; J&W Scientific, Folsom, CA, USA), at a flow rate of 1.9 ml/min helium gas at 200 kPa pressure. The injector temperature was 230°C and the detector temperature 250°C. The run temperature was held at 190°C for 5 min, then programmed to 220°C at 10°C/min, held at 220°C for 1 min, then programmed to 240°C at 20°C/min, and finally held at 240°C for 0.5 min, for a total time of 10.5 min. The detector was a flame ionization detector. A fatty acid standard, 21A (Nu-Chek-Prep, Inc., Waterville, MN, USA), was used as a reference that contained methyl esters of the following major fatty acids occurring in sunflower oil: palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2). Peaks of the fatty acids were identified by comparing those of fatty acid methyl esters, and retention times of the standard with sample peaks. The area under each fatty acid peak was calculated using the ChemStation software (Hewlett-Packard, Palo Alto, CA, USA). It was expressed as a percentage of the total area. Two bulked achene samples were analysed, with fatty acid analyses run in duplicate and the average values reported.

Data analysis

Data were analysed using the SAS statistical analysis software, version 9.3 (SAS, Institute, Inc., Cary, NC, USA). A general linear model (GLM) analysis of variance (ANOVA) was generated using the PROC GLM procedure, with species and regeneration as fixed effects and accessions as random effects. Means were separated using Duncan's new multiple range test. Pearson's correlation coefficients among the different fatty acids and geographic parameters for the original and regenerated accessions were generated using the PROC CORR procedure based on least-squares means.

Results

Due to the small number of perennial species available for evaluation, all species were combined for an ANOVA and correlation analysis. The ANOVA revealed that there were significant differences between the original and regenerated accessions of the wild annual and perennial species for the three of the four major fatty acids (palmitic, stearic, oleic but not linoleic acids; Table 1). Differences were observed in 75, 50, 92 and 67% of the accessions for the four fatty acids, respectively. The cultivated inbred line HA 89 differed only for oleic and linoleic acids.

Correlations among the fatty acids in the original and regenerated accessions were analysed, as well as between the original and regenerated accessions (Table 2). Saturated palmitic acid was significantly and positively correlated with saturated stearic acid in both the original ($r = 0.686$, $\alpha = 0.01$) and regenerated ($r = 0.678$, $\alpha = 0.01$) accessions, as well as between the original and regenerated accessions ($r = 0.703$, $\alpha = 0.01$). The correlation between palmitic and monounsaturated oleic acids in the original accessions was positive and significant ($r = 0.373$, $\alpha = 0.01$), but negatively correlated in the regenerated accessions ($r = -0.238$, $\alpha = 0.05$). No significant difference was observed when the correlation between the original and regenerated accessions was compared. Palmitic acid was negatively correlated ($r = -0.535$, $\alpha = 0.01$) with polyunsaturated linoleic acid in the original accessions and non-significantly in the regenerated accessions, whereas it was negatively and significantly correlated between the original and regenerated accessions ($r = -0.303$, $\alpha = 0.01$).

Stearic acid was positively correlated with oleic acid ($r = 0.294$, $\alpha = 0.05$) in the original accessions, but not

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in the regenerated ones, whereas there was no significant correlation between the original and regenerated accessions. However, stearic acid was negatively correlated with linoleic acid in both the original ($r = -0.463$, $\alpha = 0.01$) and regenerated ($r = -0.187$, $\alpha = 0.05$) accessions. Furthermore, there was a significant negative correlation between the original and regenerated accessions ($r = -0.348$, $\alpha = 0.01$).

The highest correlation observed in the present study was the highly negative correlation between oleic and linoleic acids in both the original ($r = -0.892$, $\alpha = 0.01$) and regenerated ($r = -0.942$, $\alpha = 0.01$) accessions.

Table 1. Comparison of the fatty acid concentrations of the original and regenerated accessions of wild annual and perennial sunflower species

Species	Palmitic (g/kg)	Stearic (g/kg)	Oleic (g/kg)	Linoleic (g/kg)
Wild annual				
H. annuus (54)[a]				
Original	58.63a[b]	37.61a	178.22a	695.03a
Regenerated	52.26b	30.45b	210.13b	687.14a
H. debilis ssp. *cucumerifolius* (1)				
Original	65.01a	34.03a	212.56a	661.85a
Regenerated	52.25b	23.47b	126.38b	777.07b
H. debilis ssp. *tardiflorus* (1)				
Original	88.42a	39.88a	187.54a	649.91a
Regenerated	79.26b	40.04a	146.15b	698.19b
H. debilis ssp. *vestitus* (2)				
Original	84.86a	49.56a	269.13a	572.15a
Regenerated	77.44b	34.78b	159.26b	709.94b
H. petiolaris (4)				
Original	49.42a	26.44a	203.38a	686.28a
Regenerated	44.55a	23.29a	191.95b	708.83b
H. praecox (4)				
Original	77.72a	44.37a	270.41a	573.71a
Regenerated	70.93a	43.68a	166.92b	692.94b
H. praecox ssp. *hirtus* (1)				
Original	80.26a	36.38a	299.05a	557.58a
Regenerated	72.17b	37.54a	157.29b	712.39b
H. niveus ssp. *tephrodes* (1)				
Original	90.55a	53.62a	316.57a	521.35a
Regenerated	85.38b	42.05b	345.43b	502.85a
Wild perennial				
H. decapetalus (1)				
Original	86.35a	39.15a	193.22a	619.94a
Regenerated	79.43b	40.96b	147.74b	698.25a
H. divaricatus (1)				
Original	47.72a	30.02a	158.34a	741.56a
Regenerated	53.65b	27.24a	151.47a	747.12a
H. giganteus (1)				
Original	57.63a	19.33a	123.95a	771.25a
Regenerated	53.67b	15.77b	112.64b	793.83b
H. nuttallii (1)				
Original	46.82a	28.26a	161.28a	758.28a
Regenerated	48.86a	26.73a	232.09b	683.96b
Combined species				
Species (72)				
Original	60.61a	37.42a	181.60a	673.87a
Regenerated	54.60b	30.86b	203.52b	680.95a
Cultivated check				
HA 89 (1)				
Original	60.24a	40.82a	210.89a	659.78a
Regenerated	56.89a	38.65a	179.78b	697.23b

[a] Number of accessions evaluated. [b] Species means followed by the different letters within a column are significantly different at the $\alpha = 0.05$ probability level according to Duncan's new multiple range test.

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Table 2. Pearson's correlation coefficients (r) of the fatty acid composition of oil from the original and regenerated accessions of the wild annual and perennial sunflower species

		Palmitic	Stearic	Oleic	Linoleic
Palmitic					
Stearic	O	0.686**			
	R	0.678**			
	B	0.703**			
Oleic	O	0.373**	O 0.294*		
	R	−0.238*	R -0.056^{ns}		
	B	0.025^{ns}	B 0.052^{ns}		
Linoleic	O	−0.535**	O −0.463**	O −0.892**	
	R	-0.012^{ns}	R −0.187*	R −0.942**	
	B	−0.303**	B −0.348**	B −0.877**	

O, original accessions; R, regenerated; B, between the original and regenerated accessions.
*Significant at $\alpha = 0.05$ probability level; **significant at $\alpha = 0.01$ probability level, and ns = not significant.

The correlation between the original and regenerated accessions ($r = -0.878$, $\alpha = 0.01$) was also negative and significant.

The relationship between geographical features and the fatty acids was examined only in the original accessions. Since the regenerated accessions were grown at a common location, no valid correlation comparison can be made between the original and regenerated accessions since the geographical features of the regenerated accessions are not continuous variables.

Latitude was highly negatively correlated with palmitic ($r = -0.692$, $\alpha = 0.01$), stearic ($r = -0.693$, $\alpha = 0.01$) and oleic ($r = -0.315$, $\alpha = 0.01$) acids, but positively correlated with linoleic acid ($r = 0.438$, $\alpha = 0.01$).

Longitude was positively correlated with stearic ($r = 0.357$, $\alpha = 0.01$) and linoleic ($r = 0.196$, $\alpha = 0.01$) acids, and negatively correlated with oleic acid ($r = -0.323$, $\alpha = 0.01$), but not correlated with palmitic acid. There was no correlation between the four major fatty acids and elevation in the present study.

Discussion

Wild *H. annuus* is the most widely distributed annual species in the genebank collection; therefore, it represented the largest number of accessions of 54 wild annual *H. annuus* in the present study. Previous limited preliminary studies comparing achenes from original locations with those grown in a common environment have found significant differences in stearic, palmitic and oleic acids, but not linoleic acid (Seiler and Brothers, 2000). A somewhat similar study evaluating selected *H. annuus* accessions by comparing the original and regenerated accessions indicated a difference in linoleic acid, but not oleic acid (Seiler, 1983). These two studies were conducted in two very different areas, Iowa versus Texas. The regenerations took place in two completely different environments, and it is known from the literature that the synthesis of oleic and linoleic acids is highly influenced by environmental factors, especially temperature (Canvin, 1965; Harris *et al.*, 1978; Seiler, 1986; DeHaro and Fernandez-Martinez, 1991), and it has been reported here and in previous studies of wild and cultivated sunflower species (DeHaro and Fernandez-Martinez, 1991; Seiler, 1986) that there is a highly negative ($r = -0.942$) interrelationship between oleic and linoleic acids. The larger number of accessions evaluated in the present study may better represent the genetic diversity available and present a more accurate representation of what actually exists in these species. The differences between the studies could also be attributed to the small sample size, different populations examined and the breeding system of wild sunflower species being open-pollinated.

Since the original accessions were collected from various locations in the USA and Mexico under various local environmental conditions over a span of years, temperature-sensitive fatty acids, especially oleic and linoleic acids, were affected. For the regenerated accessions, the environmental temperature was more limited because these accessions were grown at a single location but in several different years, providing some environmental variations when comparing the original and regenerated accessions.

Studies comparing the fatty acid composition profile of the original and regenerated accessions are limited. One somewhat comparative study was carried out in safflower, in which greenhouse-grown wild species were compared with field studies of the same accessions; however, it did not indicate whether the seed used was original or regenerated. The author concluded that the two environments were different and the difference in

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the composition of fatty acids was due mainly to temperature (Knowles, 1972).

Velasco *et al.* (1997) indicated that it is difficult to evaluate the potential of different *Brassica* phenotypes for plant breeding by comparing the values of individual fatty acids, because they are intercorrelated and any breeding modification will affect the whole system. The intercorrelation among the major fatty acids, palmitic, stearic, oleic and linoleic, observed in the present study indicated that of the six possible combinations for the fatty acids of the original accessions, all had significant correlations, whereas of the six combinations for those of the regenerated ones and between the original and regenerated accessions, only four were significant. This implies that there was less intercorrelation among the fatty acids, but still a considerable amount of similarity existed between the two accessions based on the limited data of this study. It should be kept in mind that selecting for a specific fatty acid may have a detrimental effect on other non-selected fatty acids. The high number of significant correlations for the major fatty acids in the *Helianthus* species seems to confirm the intercorrelations observed in *Brassica* species by Velasco *et al.* (1997).

The location of the original accessions has an influence on the fatty acid composition profile. In a previous study of the relationship between latitude and fatty acid composition profiles of 63 original accessions of six *Helianthus* species from Canada, latitude was shown to be negatively correlated with palmitic, stearic and oleic acids, but positively correlated with linoleic acid (Seiler and Brothers, 1999). In a wider-ranging study in which 215 original accessions of 13 wild species from North America were analysed for fatty acid composition, latitude was also shown to be negatively correlated with palmitic, stearic and oleic acids, but positively correlated with linoleic acid (Seiler, 1994). In the present study, latitude was shown to be negatively correlated with palmitic, stearic and oleic acids, but positively correlated with linoleic acid, similar to the relationships observed in the other studies. The latitude ranged from 18° to 47° N in the present study, whereas it ranged from 49° to 52° N in the Canadian study, and from 38° to 49° N in the other North American study. It appears from these studies that the correlation between latitude and fatty acid composition profiles is reasonably consistent over a range of latitudes.

Generally, the cooler northern latitudes produce higher concentrations of linoleic acid in the oil than the warmer southern latitudes (DeHaro and Fernandez-Martinez, 1991). This was confirmed by the positive relationship observed between higher, more northern latitudes having higher linoleic acid concentrations and conversely lower latitudes having higher oleic acid concentrations. This relationship is consistent for both wild and cultivated sunflower species (DeHaro and Fernandez-Martinez, 1991; Seiler, 1986).

Longitude was positively correlated with stearic and linoleic acids, negatively correlated with oleic acid, but not with palmitic acid in the present study. In the previous study of Canadian accessions, longitude was shown to be positively correlated with only palmitic acid and no other fatty acids. In the study of other North American accessions, longitude was shown to be negatively correlated only with palmitic and stearic acids, but positively correlated with linoleic acid. Longitude ranged from 73° to 121° W in the present study, whereas it ranged from 95° to 113° W in the Canadian study, and from 85° to 110° W in the North American study. Within the range of the longitudes evaluated so far, there does not appear to be a consistent correlation between fatty acid composition profiles and longitude.

A study of the relationship between elevation and fatty acid composition profiles of oil from Canadian accessions indicated that palmitic and stearic acids were positively correlated, whereas linoleic acid was negatively correlated, and oleic acid was not correlated. Elevation ranged from 0 to 2400 m in the present study, whereas it ranged from 225 to 1061 m, being a more limited range, in the Canadian study. On the basis of these limited observations and the range of elevations analysed, there seems to be no consistent correlation between elevation and fatty acid composition profiles of oil from the original accessions.

The present study indicates that fatty acid composition profiles of achenes from the original and regenerated accessions are not the same. It appears that selection for a specific fatty acid will require examining both accessions before selecting the one to breed for the desired trait. The more the regenerated populations become available, the more the confident statistic for selection purposes will become available. Another thing to keep in mind is that accessions of wild species are open-pollinated segregating populations, so one would expect a certain amount of variability in each of the succeeding generations.

Fatty acid composition was influenced by temperature, especially for oleic and linoleic acids during achene filling in both the original and regenerated accessions. Since the original accessions came from a wider range of latitudes, which is related to temperature, they potentially have more variability since the regenerated accessions were grown in a common environment with a fixed latitude and a narrower temperature range.

On the basis of the interrelationships of the fatty acids of the original and regenerated accessions as measured by correlation coefficients, there appears to be a generally similar pattern in both the original and regenerated accessions and between them. This interrelationship of the

fatty acids is similar in the cultivated sunflower species, so there should be no detrimental effects on oil quality when using the wild species for other traits. As more regenerated accessions become available, a more precise relationship between the original and regenerated accessions and fatty acid composition should emerge.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262114000677

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