Collection and Evaluation of Wild Perennial *Helianthus pumilus* Achenes for Oil Concentration and Fatty Acid Composition

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ABSTRACT

- The genus *Helianthus* consists of 52 species and 19 subspecies with 14 annual and 38 perennial species. The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from the wild species, which have provided a continued source of desirable agronomic traits. There has been an increased interest in using wild species in breeding programs, but there have been concerns about the introgression of low oil content and quality from the wild species. *Helianthus pumilus* (Dwarf sunflower) is a perennial species with the potential to improve oil and fatty acid composition based on its xerophytic habitat.
- The objective of the study was to undertake an exploration to Colorado and Wyoming, USA to collect achenes from the entire distributional range of *H. pumilus* and assess the potential of the populations for improving oil content and quality in cultivated sunflower.
- The sunflower exploration took place from August 7 to August 20, 2005 and covered 5150 kilometers in Colorado and Wyoming. Achenes of 45 populations were collected and deposited in the wild sunflower germplasm collection at the USDA-ARS, NPGS, North Central Regional Plant Introduction Station, Ames, Iowa, where they are maintained and distributed. Voucher specimens are maintained at the USDA-ARS wild sunflower species herbarium at Fargo, North Dakota. Achenes were collected from 10 to 100 plants within each population and were bulked into a single sample. For each population, a composite sample of 10 randomly sampled achenes was analyzed for fatty acid composition using organic base-catalyzed transesterification of fatty acid methyl esters and capillary gas chromatography. Oil content was determined on a 2-ml achene sample using nuclear magnetic resonance.
- The exploration was successful in collecting representative populations from the entire distributional range. The *H. pumilus* populations had an average oil content of 25.4%, considerably lower than cultivated sunflower, which averages 45% oil. The highest oil content of an *H. pumilus* population was 29.4%. The linoleic acid concentration approached 75%, much higher than the 55% expected from a semi-arid environment. The combined saturated palmitic and stearic fatty acids in *H. pumilus* averaged 10.2%, close to cultivated sunflower, which has an average of 11.5%.
- The higher concentrations of linoleic acid indicate that *H. pumilus* could be a potential source of genes for increasing linoleic acid concentration in traditional sunflower oil grown in southern latitudes. The low oil content of this species can be increased by backcrossing to a high oil line. Further research will be needed to determine the inheritance of the fatty acid and oil content traits.

Key words: Genetics resources- oil content- oil quality- wild species

INTRODUCTION

The genus *Helianthus* consists of 52 species and 19 subspecies with 14 annual and 38 perennial species (Schilling, 2006). The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from the wild species, which have provided a continuous source of agronomic and economic traits for cultivated sunflower (Seiler, 1992). Recent emphasis on the concentration and fatty acid composition of sunflower oil has increased interest in using wild species in breeding programs; however, introgression of low oil concentration and quality from the wild species into cultivated sunflower has limited their use in applied breeding programs.

Helianthus pumilus (Dwarf sunflower) is an endemic perennial species adapted to dry, rocky soils of central and southeastern Wyoming to central Colorado at 4,000 to 6,000 feet elevation (Heiser el al., 1969). Based on its occurrence in dry rocky habitats, it frequently has been characterized as drought tolerant with high oil content potential, and thus is a candidate for improving the cultivated sunflower germplasm (Seiler, 1992). Interspecific hybridization of diploid perennial *H. pumilus* and cultivated sunflower has been previously reported by Krauter et al. (1991) using embryo culture and by Nikolova et al. (2004) using traditional breeding methods.

Unfortunately, due to the difficulties in regenerating the earliest collected populations, the USDA-Agricultural Research Service sunflower germplasm collection has not been able to meet the demand for achenes of this species for almost 25 years. The objective of the study was to undertake an exploration of Colorado and Wyoming, USA to collect achenes from the entire distributional range of *H. pumilus* and assess the potential of the populations for improving oil content and quality in cultivated sunflower.

MATERIALS AND METHODS

A sunflower exploration for *H. pumilus* took place from August 7 to August 20, 2005. The exploration covered 5150 km in two states, Colorado and Wyoming. Achenes were collected from 10 to 100 plants within each population and bulked into a single sample. Herbarium specimens were deposited in the USDA-ARS wild *Helianthus* herbarium at Fargo, ND. The achene samples were deposited at the USDA-ARS North Central Regional Plant Introduction Station, National Plant Germplasm System, Ames, Iowa, where they are maintained and distributed.

Each sample represented an isolated, open-pollinated segregating population. Achenes were stored at 5°C and low humidity (<20%) until analyzed for oil and fatty acids. Two portions (7-ml) of each achene sample were cleaned to remove empty achenes, and analyzed for oil concentration (expressed as % on a dry weight basis) by nuclear magnetic resonance (Granlund and Zimmerman, 1975). Fatty acid composition was determined for each collected population with enough seed using a 10-20 mg sample from 10 pulverized achenes. The extracted oil was converted to methyl esters using an organic-catalyzed transesterification (Vick and Jan, 2007). The sample was injected into a Hewlett-Packard 5890 gas chromatograph containing a DB-23 capillary column (25 m x 0.25 mm, J. & W. Scientific). A fatty acid standard 21A (NU-CHEK-PREP, INC.) was used that contained the following acids: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), and arachidic (20:0). Fatty acid peaks were identified by comparing the fatty acid methyl ester peaks and retention time of the standard with the sample peaks. Fatty acid and oil concentrations were determined from two samples per population.

RESULTS

The exploration was successful in collecting 45 representative populations of *H. pumilus* from its entire distributional range with 29 populations collected in Colorado and 20 from Wyoming. Figures 1 and 2 show the typical habitats where the species was collected. Thirty-nine of the 45 populations collected were analyzed for oil content and fatty acid composition since some populations did not have adequate achenes for analysis, therefore only 21 populations from Colorado, and 18 from Wyoming were analyzed (Tables 1 and 2).

The *H. pumilus* populations from Colorado had an average oil content of 24.9% (Table 1), while populations from Wyoming averaged 25.2% (Table 2). The overall average oil content was 25.1%, considerably lower than cultivated sunflower, which averages 45.0%. The highest oil content was observed in a population from Laramie County, Wyoming (site 2506) with 29.4%.

The average linoleic acid concentration approached 75.0%, much higher than the 55% expected from a semi-arid environment (Tables 1 and 2). The linoleic acid concentration averaged 74.8% for Colorado and 75.4% for Wyoming. The corresponding oleic aid concentration averaged 12.7% for Colorado and 12.3% for Wyoming, with an overall average of 12.5%.

The combined saturated palmitic and stearic fatty acid concentrations in the *H. pumilus* populations averaged 10.2%, with a 10.3% average for Colorado, and 10% for Wyoming (Tables 1 and 2). This is approximately equal to the average of 11.5% in cultivated sunflower oil.

Fig. 1. *Helianthus pumilus* in a roadside ditch near the Colorado and Wyoming border, site 2499, with Chimney Rock in the background, Albany County, Wyoming.

Fig. 2. *Helianthus pumilus* plants in a gravelly roadside ditch at site 2498, Albany County, Wyoming. Insert shows the texture of the soil.

Table 1. Location, elevation, oil content, and fatty acid composition of *Helianthus pumilus* populations collected from August 7-20, 2005 in Colorado.

Site	Alt. (ft.)	Nearest Feature	County	Oil Content	Fatty Acid Concentration			
				(%)	Palmitic (%)	Stearic (%)	Linoleic (%)	Oleic (%)
2473	8000	Monument	El Paso	24.2	6.5	4.4	76.0	11.0
2474	7080-7280	USAF Academy	El Paso	27.0	6.8	3.1	70.0	17.7
2475	7280	USAF Academy	El Paso	23.0	7.0	3.8	73.1	13.9
2476	6540	Manitou Springs	El Paso	25.7	7.2	3.7	73.7	13.1
2478	6120-6240	Cañon City	Fremont	20.5	6.9	3.9	73.4	13.3
2480	8900	Westcliffe	Custer	24.1	6.0	3.0	76.8	12.1
2484	6750-6800	Texas Creek	Fremont	20.8	7.2	6.2	69.5	14.7
2486	7800	Bailey	Park	22.5	6.4	3.6	75.4	11.5
2487	7810	Idaho Springs	Clear Creek	25.7	6.6	3.6	75.8	11.6
2488	7800-8000	Golden Gate Canyon Park	Gilpin	25.0	6.6	4.0	75.2	11.8
2489	8350	Nederland	Boulder	25.5	6.0	4.2	77.0	10.6
2490	8300	St. Vrain Canyon	Boulder	23.4	5.9	3.1	73.8	15.1
2491	6900	Bellvue	Larimer	25.6	6.2	3.5	77.3	11.0
2492	5800-6000	Cache Poudre Canyon	Larimer	26.8	6.5	3.6	74.6	13.2
2493	6600	Cache Poudre Canyon	Larimer	27.9	6.7	3.6	77.0	10.5
2495	5400	Livermore	Larimer	27.7	6.4	3.1	76.0	12.8
2496	5950-6160	Livermore	Larimer	24.5	7.0	3.7	76.6	10.5
2497	7100	Virginia Dale	Larimer	26.7	6.5	3.7	76.2	11.5
2519	5460	Nunn	Weld	27.4	6.7	3.5	73.6	13.9
2520	8000	Monument	El Paso	22.2	6.0	3.2	78.3	10.5
2521	7000	Parker	Douglas	26.1	6.8	3.2	72.5	15.4
			Mean	24.9	6.6	3.7	74.8	12.7

Table 2. Location, elevation, oil content, and fatty acid composition of *Helianthus pumilus* populations collected August 13-18, 2005 in Wyoming.

Site	Alt. (ft.)	Nearest Feature	County	Oil Content	Fatty Acid Concentration			
				(%)	Palmitic (%)	Stearic (%)	Linoleic (%)	Oleic (%)
2498	7630	Tie Siding	Albany	21.7	7.5	3.9	77.3	9.3
2499	7680	Chimney Rock	Albany	21.5	6.6	3.3	75.0	12.6
2500	7500	Laramie	Albany	23.0	6.6	4.0	76.2	10.8
2501	7560	Laramie	Albany	22.0	6.7	3.7	75.7	11.1
2502	7600	Laramie	Albany	25.8	5.8	3.6	77.4	10.9
2503	7220	Laramie	Laramie	25.2	6.5	3.7	78.9	8.7
2505	6760	Federal	Laramie	25.6	6.6	3.9	75.7	11.2
2506	6890	Horse Creek	Laramie	29.4	6.3	2.9	76.5	11.9
2508	5415	Chugwater	Platte	25.7	6.1	2.8	76.7	12.0
2509	6050	Wheatland	Albany	28.5	6.8	2.5	76.6	11.7
2510	5080	Wheatland	Platte	28.9	6.5	3.2	72.3	15.4
2511	5160	Hartville	Platte	27.6	6.7	3.1	75.1	12.7
2512	5650	Lusk	Niobrara	22.8	6.9	2.7	76.5	11.6
2513	6200	Esterbrook	Converse	25.7	6.4	2.6	73.4	14.9
2515	4910	Dayton	Sheridan	24.9	6.3	2.9	74.8	13.6
2516	5350	Casper	Natrona	25.1	6.5	2.7	71.4	16.5
2517	4870	Wheatland	Platte	28.3	6.1	3.1	74.0	14.5
2518	5230	Cheyenne	Laramie	23.2	7.1	3.2	75.0	12.4
			Mean	25.2	6.7	3.3	75.4	12.3

DISCUSSION

It had been over 20 years since this species was last collected for the wild sunflower germplasm collection. The highest oil content was observed in a population from Laramie County, Wyoming with 29.4%, 30 higher than previously reported for this species (Seiler, 1994). The low oil content of this species can be increased by backcrossing to a high oil hybrid.

The linoleic fatty acid concentration observed in the *H. pumilus* populations is unusually high for a semi-arid environment. The linoleic acid concentration approached 75.0%, much higher than the 54.0%

expected from its semi-arid environment. Generally, high temperatures during flowering, achene filling, and maturation favor a low linoleic acid concentration and a high oleic acid concentration (Seiler, 1986). This relationship is common to both wild and cultivated sunflower. Generally, the cooler northerly latitudes have higher levels of linoleic acid in the oil and the warmer southerly latitudes have considerably lower linoleic concentrations (De Haro and Fernandez-Martinez, 1991). The higher concentrations of linoleic acid in *H. pumilus* could be a potential source of genes for increasing linoleic acid concentration in traditional sunflower oil when grown in a warm climate. The combined saturated palmitic and stearic fatty acids in *H. pumilus* averaged 10.2%, about equal to traditional cultivated sunflower oil that averages 11.5%.

The majority of the recently collected *H. pumilus* accessions (91%) are available for distribution and accessions with low inventory can be successfully regenerated at the Parlier, CA, a USDA-ARS cooperating location that has an environment favorable for regenerating this species.

Further research will be needed to determine the inheritance of the fatty acids and oil content. Other agronomic traits will need to be monitored during the introgression of these traits into cultivated sunflower. The addition of 45 populations of *H. pumilus* to the wild sunflower germplasm collection will insure their preservation for the future, and will greatly increase the available genetic diversity for improving the cultivated sunflower, keeping it a viable and competitive global oilseed crop.

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