High infraspecific diversity of wild sunflowers (*Helianthus annuus* L.) naturally developed in central Argentina

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**A R T I C L E  I N F O**

Article history:
Received 15 December 2008
Accepted 10 May 2009

Keywords:
Ferality
Crop-related weed
Non-native weed
Genetic resource
De-domestication

**A B S T R A C T**

The sunflower’s wild relative *Helianthus annuus* L. is a non-native invader in several regions of the world. It was introduced as an experimental forage plant in central Argentina six decades ago where it probably escaped and developed extended populations coexisting with the sunflower crop. If the invasive taxon was diffused without modifications, it would be expected to have phenotypic similarities with its parental sources. Nine populations representative of different geographic regions of central Argentina were compared with 17 populations from the USA (center of origin) in a common garden study at Bahía Blanca, Argentina using 47 phenotypic traits. The nine invasive wild populations were differentiated among themselves and from the native populations by plant form and life cycle traits, oil composition, inflorescence, and achene morphology. Populations from both continents shared traits related to domesticated sunflower, such as bract width over 0.8 cm, but the frequency of this trait was higher in populations from Argentina. The high variability of wild *H. annuus* populations from Argentina did not reveal any founder effects. The diversity found in the invasive populations reflected about three-fourths of the phenotypic variability of those from the center of origin, even though the environmental conditions of the Argentine habitats represented only half of the variability present in the North American habitats. The current findings demonstrated that the invasive wild sunflower populations have developed within few decades a high degree of variability, which could be a source of a novel biodiversity useful as a genetic resource for sunflower crop improvement.

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1. Introduction

Due to genetic and evolutionary implications, species under cultivation and their wild relative complexes have been extensively researched by the historians of domestication and agriculture (Harlan, 1992). The release of modern crop varieties, including new traits obtained by mutagenesis or bioengineering, has changed the focus on the wild-crop relatives study because gene flow could promote the development of new and more complex weeds (Gressel, 2005). In this area of research, the wild and weedy crop relatives are also generating interest due to their potential value as a genetic resource.

It is generally accepted that the sunflower crop, *Helianthus annuus* var. *macrocarpus* L. (Asteraceae), was domesticated around 4000 years ago in North America, the center of origin of the genus, where wild native populations are still found today (Harmer et al., 2004). Russian breeders significantly improved the crop species during the 19th century using selected germplasm introduced into Europe as ornamentals, creating oil seed varieties that were successfully adopted by farmers’ worldwide (Heiser, 1954; Schilling, 2006; Seiler and Rieseberg, 1997). The negative consequence of the global spread of the *Helianthus* genus was the diffusion of some wild populations of *H. annuus* that behave as a non-native invader in several regions of the world (Bertilone et al., 2005; Dry and Burdon, 1986).

In Argentina, the sunflower crop was introduced in the 1930s through domestic populations brought by European immigrants (Bertero de Romano and Vazquez, 2003). The first report of wild sunflower introductions was one decade later, when it was found as an experimental forage crop (Bauer, 1991). It is unknown how
in the following 60 years the wild sunflower spread throughout central Argentina and colonized well-defined agro-ecological habitats (Cantamutto et al., 2008). At present, the wild populations are mostly located west of the more suitable area for the sunflower crop (de la Vega and Chapman, 2006).

The coexistence of the crop and wild sunflowers makes it difficult to disregard the existence of eco-fertility in the diffusion processes (Gressel, 2005; Poverene et al., 2004; Ureta et al., 2008). The use of wild sunflower as a genetic resource, ornamental, or as a contaminant of crop and forage seeds could be some other possible ways of intentional or unintentional introductions into different places in central Argentina.

In the invaded habitats, the populations show different life cycles, height, leaf shape, head morphology (Poverene et al., 2002). These could be attributed to phenotypic plasticity in response to environmental and ecological constraints of the different growing places (Richards et al., 2006). In addition, genetic differences between populations may exist. A common garden study minimizing environmental effects (Bender et al., 2002; Sugiyama, 2003) could be used to estimate the real genetic variability of phenotypic traits between populations (Rawashdeh et al., 2007; Soleimani et al., 2007).

We compared the plant morphology, life cycle, and seed oil composition of naturalized *Helianthus annuus* populations from Argentina with populations from the North American center of origin in a common garden study. If the populations from Argentina showed well-defined similarities with populations of wild sunflowers from the center of origin, this would suggest that introductions served as the most probable origin of the Argentine populations. If not, a new biodiversity in the *H. annuus* species must have been developed under the natural conditions of the invaded agro-ecosystem. This fact would indicate an ongoing microevolution giving the Argentine populations the potential value as a new genetic resource for the sunflower crop.

2. Materials and methods

2.1. Plant germplasm sources

Germplasm from Argentina consisted of wild *Helianthus annuus* populations collected in nine locations representative of the geographical different invaded habitats (Cantamutto et al., 2008). The provinces of Córdoba, La Pampa and Buenos Aires contributed two populations each; Rio Cuarto (RCU) (S 33°09’, W 64°20’), Juarez Celman (JCE) (S 33°40’, W 63°28’), Colonia Barón (BAR) (S 36°10’, W 63°53’), Rancul (RAN) (S 35°04’, W64°46’), Adolfo Alsea (AAL) (S 37°16’, W 62°59’), and Carhué (CHU) (S 37°16’, W 62°55’), respectively. Entre Ríos, San Juan and Mendoza provinces contributed one population each; Diamante (DIA) (S 32°03’, W 60°38’), Media Agua (MAG) (S 31°57’, W 68°27’) and Las Malvinas (LMA) (S 34°47’, W 68°15’), respectively.

Wild germplasm from North America was represented by 17 populations. States of origin and passport numbers were: Arizona (AZ) PI 468571, California (CA) PI 468580, Colorado (CO) PI 468621, Illinois (IL) PI 435540, Indiana (IN) PI 468633, Iowa (IA) PI 597901, Kansas (KS) PI 586851, Montana (MT) PI 586821, Nebraska (NE) PI 586867 Nevada (NV) PI 468596, New Mexico (NM) PI 468537, North Dakota (ND) PI 586807, Oklahoma (OK) PI 468483, South Dakota (SD) PI 586835, Texas (TX) PI 468504, Utah (UT) PI 468607, and Wyoming (WY) PI 586824 (for more information see www.ars-grin.gov/cgi-in/npgs/acc/display.pl?1080516). American germplasms are coded in the following by two, Argentine ones by three characters.

Geographic coordinates and altitude of collection sites for all populations were obtained from USDA (2008) and the Argentine passport data. Mean temperature of the hottest and coolest month and the average annual rainfall of the nearest locality, were obtained from www.worldclimate.com and de Fina (1992) for USA and Argentine populations, respectively.

2.2. Plant material studied in the common garden

Seed dormancy was overcome by maintaining seeds in germination paper in a wet chamber at 5 °C for 1 week (ISTA, 2004). Seedlings were grown for 30 days in the greenhouse at 20–25 °C in 28 × 54 cm² 200-cell plastic trays and then transplanted in rows 2.10 m long with 0.25 m between plants for an equivalent population of 19.047 plants ha⁻¹. Plots at the Agronomy Department, Universidad Nacional del Sur, Bahia Blanca, Argentina (S 38°41’, W 62°14’) were drip irrigated for optimal plant growth. Data were collected from 238 and 248 individuals from Argentina and USA, respectively, during the 2004–2006 summer seasons in experimental plots including 9–24 populations each year. Original seeds were used for the first year, while re-generated achenes were used in subsequent years. Observations, population regeneration, and achene sampling were made on more than 20 individuals per population, except for four USA populations with poor germination which had only 14–18 individuals. Controlled pollinations for seed regeneration were made by hand on heads of sibbed individuals covered with polyamide bags until the end of flowering. Bulked seed lots of open-pollinated mature heads were collected before shattering and used to determine morphological and oil chemical

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>Description</th>
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<tbody>
<tr>
<td>CIC</td>
<td>days from transplant to end of flowering</td>
</tr>
<tr>
<td>DIB</td>
<td>days from transplant to 10% of flowering</td>
</tr>
<tr>
<td>HNU</td>
<td>head number</td>
</tr>
<tr>
<td>LLG</td>
<td>leaf length (cm)</td>
</tr>
<tr>
<td>LNU</td>
<td>leaf number</td>
</tr>
<tr>
<td>LSI</td>
<td>leaf width x length (cm²)</td>
</tr>
<tr>
<td>PHT</td>
<td>plant height at begin of flowering (cm)</td>
</tr>
<tr>
<td>LWI</td>
<td>leaf width (%)</td>
</tr>
<tr>
<td>PLG</td>
<td>petiole length (cm)</td>
</tr>
<tr>
<td>SDI</td>
<td>stem diameter at mid-height (cm)</td>
</tr>
<tr>
<td>AEF</td>
<td>anthocyanin stigma frequency</td>
</tr>
<tr>
<td>AFP</td>
<td>anthocyanin pale frequency</td>
</tr>
<tr>
<td>BIN</td>
<td>bract length: width relationship</td>
</tr>
<tr>
<td>HDI</td>
<td>head disk diameter (cm)</td>
</tr>
<tr>
<td>HLF</td>
<td>heads with leaf on the back frequency</td>
</tr>
<tr>
<td>RDF</td>
<td>red disk frequency</td>
</tr>
<tr>
<td>BSF</td>
<td>black stripes frequency</td>
</tr>
<tr>
<td>GPF</td>
<td>grey pericarp frequency</td>
</tr>
<tr>
<td>OSF</td>
<td>ovoid shape achene frequency</td>
</tr>
<tr>
<td>SFW</td>
<td>achene fresh matter weight (mg)</td>
</tr>
<tr>
<td>STH</td>
<td>achene thickness (mm)</td>
</tr>
<tr>
<td>SWT</td>
<td>achene width (mm)</td>
</tr>
<tr>
<td>C181</td>
<td>oleic acid content (%)</td>
</tr>
<tr>
<td>C182</td>
<td>linoleic acid content (%)</td>
</tr>
<tr>
<td>C220</td>
<td>behenic acid content (%)</td>
</tr>
<tr>
<td>C240</td>
<td>lignoceric acid content (%)</td>
</tr>
<tr>
<td>C832</td>
<td>linoleic/oleic relationship</td>
</tr>
<tr>
<td>YOI</td>
<td>iodine index</td>
</tr>
</tbody>
</table>
composition. The harvest was done during the last week of February to minimize the flowering date effects (Seiler, 1983).

2.3. Phenotypic traits

General observations of plant structure and branching were performed according to Hockett and Knowles (1970). Forty-one morphological, four phenological, and 12 chemical descriptors from the USDA (2008) Germplasm Resources Information Network (GRIN) were used to characterize the wild populations from Argentina and North America under common garden conditions. Quantitative traits of individual plants included: final plant height (cm), stem diameter at mid-height (cm), head position-angle (deg), leaf number and total head number, leaf width (cm) and length (cm), petiole length (cm) and their relationships determined on first-order leaves at the flowering stage. The categorical traits of presence of a main head, cuneate leaf base, cordate leaf shape, flat leaf surface, dentate leaf margin and stem anthocyanins were observed on individual plants and computed as frequency of each population per year. At the population level, life cycle was computed as days from transplant to beginning of flowering (10% of plants in bloom), beginning to mid (10–50% of plant in bloom), mid to end (50–100% of plant in bloom), and total cycle duration (transplant to 90% of fully dry plants).

Quantitative traits measured on first-order heads included: ray flower number, ray width (cm), ray length (cm), bract number, length (cm), width (cm), their relationships, and head diameter (cm). The presence of leaves on head back, pale anthocyanin, stigma anthocyanin, and red disk flowers were reported as frequencies for each population for each year. The quantitative traits were measured on 30 achenes included achene length (mm), width (mm), thickness (mm) using 10× magnification on individual achenes, achene length × width (mm^2) were determined and also average fresh achene weight (g). Categorical achene traits—ovoid shape, pubescence, stripes, lateral stripes, black stripes, grey pericarp, motting and anthocyanin presence—were computed as frequencies for each population.

Oil concentration (g/kg) and fatty acid composition, and iodine value were evaluated according to AOCS (2008) approved methods (Ai 3–75, Ce 1–62 and Tg 1a–64). Methyl esters of fatty acids were analyzed in a Hewlett Packard 6890 gas chromatograph with a flame ionization detector and a capillary column grapher with a flame ionization detector and a capillary column. The latitude and altitude of the wild species distribution in Argentina had significantly lower values than those of provenances from the northern hemisphere (Table 1). Mean temperatures of the coolest month of the Argentine and the USA wild sunflower habitats were significantly different, but there were no differences between those origins in the warmest month’s mean temperature and average rainfall. In spite of this, all the habitats of the Argentine populations were within the range of environmental conditions of USA wild sunflower habitats (Table 1).

The original habitats of Argentine populations grouped in only two of the four clusters formed by the overall environmental conditions analyzed (Fig. 1). Two populations from the irrigated western part of Argentina (MAG and LMA) grouped with five populations from the USA, while the other seven populations of the dry land of central Argentina clustered with only one population from Texas.

3. Results

3.1. Germplasm geographical origin

The latitude and altitude of the wild sunflower species distribution in Argentina had significantly lower values than those of provenances from the northern hemisphere (Table 1). Mean temperatures of the coolest month of the Argentine and the USA wild sunflower habitats were significantly different, but there were no differences between those origins in the warmest month’s mean temperature and average rainfall. In spite of this, all the habitats of the Argentine populations were within the range of environmental conditions of USA wild sunflower habitats (Table 1).

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3.2. Phenotypic comparison

The first two PCA axes differentiated all the wild populations based on plant and life cycle, inflorescence, achene, and oil concentration and composition traits (Fig. 2a–d). The plant and life cycle traits distributed the Argentine populations along three quadrants of the PCA, with the LMA population differing by larger plants, plant height, number and leaf size (Fig. 2a). The Argentine population pairs RCU-JCE, AAL-CHU and RAN-BAR all showed similarities, but were all differentiated from the North American populations (Fig. 2a).

Table 1
Geographic and climatic variables (mean ± S.D.) from the habitats of populations from Argentina and North American grown in the common garden experiment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>North America</th>
<th>Argentina*</th>
</tr>
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<tbody>
<tr>
<td>Latitude (deg)</td>
<td>39.0 ± 5.1 N</td>
<td>34.6 ± 2.1 S*</td>
</tr>
<tr>
<td>Altitude (m.o.s.l.)</td>
<td>722 ± 452</td>
<td>267 ± 202*</td>
</tr>
<tr>
<td>Annual rainfall (mm)</td>
<td>511 ± 296</td>
<td>591 ± 259</td>
</tr>
<tr>
<td>Mean temperature of the hottest month (°C)</td>
<td>25.3 ± 3.2</td>
<td>24.2 ± 1.1ns</td>
</tr>
<tr>
<td>Mean temperature of the coolest month (°C)</td>
<td>−1.3 ± 7.1</td>
<td>8.1 ± 1.4**</td>
</tr>
</tbody>
</table>

ns = not significant.

* Significance of differences according to Kruskal–Wallis test.

* p < 0.05.

** p < 0.01.
Considering the inflorescence traits, the Argentine populations were distributed in all the PCA quadrants, as well as the North American populations, but the DIA population was clearly separated from the rest by the low frequencies of red disks, and pale and stigma anthocyanins (Fig. 2b). Some Argentine populations showed similar inflorescence traits to the North American ones. The populations from RCU and JCE were similar to NE, BAR was similar to NM, and the CHU population showed similarity with UT, MT and OK (Fig. 2b). The achene traits distributed the Argentine populations into all PCA quadrants (Fig. 2c). The LMA population differed by its larger achenes and low frequency of grey pericarp. The populations from RCU and UT were similar (Fig. 2c).

The oil composition differed among the Argentine populations and distributed them in all the PCA quadrants (Fig. 2d). The DIA population differed from the others by its high iodine index and linoleic acid concentration. The Argentine AAL population showed similarity with the North American population from WY, while BAR was similar to IL, and the population from TX was similar to KS (Fig. 2d).

Taken as a whole, the phenotypic traits grouped the populations into four clusters using Ward’s minimum-variance hierarchical
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Eight Argentine populations grouped with the North American populations from NE, IA, IN and IL. The remainder Argentine population, DIA, showed high similarity with the North American populations from NV, CO, UT, CA and A.

3.3. Crop introgression

The wild *Helianthus annuus* populations naturalized in Argentina showed traits indicative of their wild condition. In all populations individuals were predominately completely branched (wild type) with no plants having a single head (cultivated type), or basal or top branching (indicative of commercial restorer lines).

The frequency of plants bearing a main head, yellow disks, or head diameter over 4.5 cm was not different between North American and Argentine populations (Table 2). However, the frequency of plants with phyllaries over 0.8 cm in width was significantly higher among Argentine populations compared to the two North American groups, which did not differ. In the two North American populations from IN and IL, the frequency of involucral bracts over 0.8 cm in width was higher than 70%, the same as in Argentine populations from MAG, AAL, and LMA. North American populations from NE, IO, ND, KS, AZ and the remaining Argentine populations showed 20–50% of plants with phyllaries over 0.8 cm in width.

4. Discussion

The range of environmental conditions of the Argentine habitats seems to be narrower than those observed in the USA, representing about half of the total variability analyzed (two of four clusters shown in Fig. 1). This could imply the absence of certain climates in the invaded environment or that central Argentina has not yet been completely colonized. Following this point of view, the valley where most of the certificated sunflower seed production is concentrated in Argentina (located at S 39° 14’ to S 39° 54’ latitude) is at risk of invasion by a wild sunflower (Cantamutto et al., 2007).

The biodiversity measured using 57 phenotypic traits completely separated all populations with more than 70% of the variance retained in the first two components. This is higher than the variability found in French wild invading populations (Serieys et al., 1997). The only recurrent relationship found in the Argentine populations, was between RCU and JCE. These populations showed similarities of plant and life cycle, head traits, but this similarity was not present for achene and oil composition traits (Fig. 2a–d). Both Argentine populations were connected and could represent geographical extremes of a mega-population of ca. 100 km lengths. Gene flow would be enhanced by plant density in this region, avoiding differentiation through fragmentation and genetic drift. No other pair of populations was consistently joined in the PCA for the four groups of traits.
None of the Argentine populations showed consistent similarities to North American ones. In two of the four groups of analyzed traits, only the RCU population showed a close similarity with populations from the North American continent, confirming that it could be possibly the entry point of the wild sunflower into Argentina. Considering plant and life cycle, the population from RCU was similar to NE, but considering the achene morphology it was similar to UT (Fig. 2a and c). UT is the only North American population which showed similarity with the Argentine populations from RCU and CHU, but only in one of four groups of traits.

The biodiversity taken as a whole in the Argentine wild populations represented about three-fourth of that reflected in the USA populations (Fig. 3). This proportion seems to be high considering that they developed under environmental conditions that represent only half of the variability of those from the center of origin (Fig. 1).

Introgressive hybridization between crops and their wild relatives has been the origin of some invasive plants (Abbott et al., 2003; Campbell et al., 2006; Elstrand et al., 1999; Hall et al., 2006; Hancock, 2005; Schmeller et al., 2005) and probably is the origin of the broad variability in wild Argentine sunflowers. The Argentine agro-ecosystem can be considered a favorable environment for gene flow between crop and wild relatives (Poverene et al., 2004) since the naturalized wild annual sunflower populations are sympatric with sunflower crop over an extensive area (Ureta et al., 2008).

Phyllary (bract) width provides robust evidence of introgression with cultivated sunflower in wild populations from Argentina, but this crop trait was also present in some USA populations. Mean phyllary width of LMA, ALL and MAG populations exceeded 0.8 cm, whereas the remaining populations showed some individuals with wider bracts. Among the North American populations, mean phyllary width over 0.8 cm was found in populations from IN and IL. Among them, the North American population from IN and the Argentine LMA, seems to be the more introgressed with cultivated sunflower. Both possessed large plant size, large disks, phyllary widths over 0.8 cm and large achenes.

Considering the biodiversity observed in the center of origin, our findings demonstrated for the first time the existence of high phenotypic variability in the invasive populations of Helianthus annuus in Argentina. It seems that founder effects did not limit wild H. annuus biodiversity in Argentina, because 60 years after their introduction nearly three-fourths of the USA wild germplasm phenotypic variability is still present. Additionally, some traits of Argentine populations seem to be new, because they were absent in the North American populations, such as the oil composition of the DIA population. These results demonstrated for the first time that invasive wild sunflower populations possess the capacity to maintain and develop intense infraspecific variability in new habitats, probably with the help of gene flow with the sunflower crop. Considering the high biodiversity, Argentine wild populations of H. annuus could be considered a novel germplasm with potential utility for sunflower crop improvement.

Acknowledgements

The authors would like to thank the Fundación Carolina, Spain, for a fellowship to M.C., and to National Research Council of Argentina (CONICET) for a fellowship to A.P. We are also grateful to Drs. Ignacio Romagosa and Lluís Torres from Centro UdL-IRTA, Lleida, Spain for assistance with statistical data analyses. This research was supported by grants ANPCYT-PICT-08-9881, UNS-PGI 24A106 and INTA PNCER 1339.

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