HELIANTHUS PETIOLARIS IN ARGENTINA AND ITS SPONTANEOUS HYBRIDIZATION WITH CULTIVATED SUNFLOWER

Mónica Poverene, Alicia Carrera, Miguel Cantamutto, Soledad Ureta, Carla Delucchi, Virgina Alonso Roldán, and **Jessica Basualdo**
Departamento de Agronomía, Universidad Nacional del Sur, CERZOS-CONICET, 8000 Bahía Blanca, Argentina
E-mail: poverene@criba.edu.ar

Abstract

Helianthus petiolaris has been naturalized in central Argentina and its geographical distribution overlaps the sunflower crop region. Intermediate forms between both species are often found in wild populations. To study variability and occurrence of natural hybrids, 26 representative populations growing up to 100 m away from the sunflower crop in three provinces were sampled and a common garden experiment was conducted. Highly significant differences were found among populations for morphological quantitative traits, and there was also variation for qualitative traits and phenology. Hybrid origin of intermediate plants found in 10 out of 26 populations was assessed through morphology, phenology and fertility. Hybrid plants were rather different and all were at least partially fertile. These results have biological implications concerning gene flow and introgression, and practical ones regarding the possibility of releasing genetically modified sunflower cultivars.

Introduction

Helianthus petiolaris Nutt. (Asteraceae) is a diploid species (x=17) native to North America and well known as a source of cytoplasmic male sterility. It was introduced in Argentina about 50 years ago, presumably as contaminants in forage seed lots, and found favourable conditions in the semiarid environment and sandy soils of the central region of the country. At present, it is widespread in La Pampa, San Luis, western Buenos Aires and southern Cordoba provinces, between 33º14’ and 38º48 S (Poverene et al., 2002). Populations are patchily distributed along roadsides, wire fences, and in the external rows of crops, mainly sunflower, but soon disappear when the soil is ploughed. Plants grow from November to April. Morphological descriptions match *H. petiolaris* ssp. *petiolaris*, according to Heiser (1961). However, phenotypic variability is found within and among populations. Genotypic variation was also found through isozyme markers analysis (Poverene et al., 2003).

The extensive overlapping with sunflower crop regions, the coincidence of life cycles and common pollinator insects facilitate interspecific crosses between *H. petiolaris* and sunflower, *H. annuus* var. *macrocarpus* L. Although both species differ in chromosome constitution, only seven out of 17 chromosomes are collinear in both species, and there are important barriers to hybridization (Rieseberg et al., 1995), hybrids have been found for many years in Argentina (Covas and Vargas López, 1970; Ferreira, 1980). Hybridization and introgression between *H. petiolaris* and *H. annuus* have been extensively studied in their centre of origin, where they have originated at least three new homoploid species (Rieseberg et al., 1995, 1999a,b; Rieseberg and Linder, 1999; Buerkle and Rieseberg, 2001).

We found intermediate forms between *H. petiolaris* and *H. annuus* in several of the 150 sites in Argentina where *H. petiolaris* accessions were collected over three years. According to the site where they were found, it was possible to infer the direction of pollen flow, from cultivated to wild plants and the reciprocal (Cantamutto et al., 2003). Seeds of 32 intermediate plants were sown in the experimental field of the Agronomy Department and progeny tests demonstrated segregation of phenotypic characters of both species (Poverene et al., 2003).

The aim of this work was to study *H. petiolaris* distribution and variability in Argentina, and occurrence and characterization of natural hybrids from crosses with cultivated sunflower. Gene flow between both species and its consequence is of concern because this may imply a secondary centre of genetic variability for these *Helianthus* species in the Southern hemisphere. Moreover, sunflower is one of the most important oil crops in Argentina and there is an interest in releasing genetically modified cultivars, which are at present under evaluation, and information about potential environmental impact will be demanded.

Materials and Methods

Between 2000 and 2003 we made a number of trips to study geographical distribution of *H. petiolaris* populations in the country. Collection of plant specimens and seed allowed us to gather 150 accessions. In 2003, a common garden experiment in the experimental field of the Agronomy Department comprised 26 accessions from different sites of La Pampa, San Luis and Buenos Aires provinces. These populations were growing up to 100 m away from sunflower crops at the time of collection. Bulked samples of seed were collected from wild heads exposed to pollen flow from the crop in each site. A subset of seeds was grown in the greenhouse and transplanted to field plots, in a completely randomized design with two replications. Plots were of 20 m length, with plants spaced 0.20 m at each side of the drip tape, and distance between rows was 1.5 m. A plot of cultivated sunflower (Dekalb 3900) was sown at the same time.

Phenotypic variation was studied through morphology and phenology. Data on leaf size and shape, head and disc diameter, bract (phyllary) width, days to flowering, and life cycle length, were collected on six representative plants of each plot, three leaves and three heads of each plant. Data were subjected to ANOVA and Kruskal-Wallis nonparametric analysis (because of deviations from a Gaussian distribution of some traits), principal component analysis and cluster analysis based on mean linkage and mean Euclidean distance. Qualitative traits such as disc flower and seed color, leaf appearance (undulate/flat) and margin (entire/serrated), plant height, branching pattern and pathogen symptoms were also recorded.

The same morphological and phenological data were used to characterize presumed hybrid plants, from pollination by cultivated sunflower. Fertility was estimated through pollen viability (stained as in Alexander, 1969) and seed set. Data from each hybrid were compared with data from three *H. petiolaris* plants of the same accession and with three plants of cultivated sunflower. Mean comparisons (Hochberg test), principal component analysis and a hybrid index (Grant, 1989) for some traits (leaf margin and appearance, disc flower color) were calculated.

Results and Discussion

Phenotypic Variation. *Helianthus petiolaris* populations varied for qualitative traits, in height and leaf appearance. Tall plants (more than 1 m height) were more frequent (16 of 26) than short plants (less than 1 m). Undulate leaves were more frequent (18/26) than flat ones. One population segregated for red and yellow disc flowers; the remaining presented purple red disc flowers only. Seeds varied in color, being reddish brown or gray, sometimes yellowish, but always hairy and maculated. Most populations (22 of 26) showed more than one seed color. Populations did not show any visual symptoms of disease, although some of them showed powdery mildew at the end of life cycle.

All the quantitative characters showed highly significant differences among populations with ANOVA ($p<0.0089$) and Kruskal-Wallis tests ($p<0.0045$). Principal component analysis did not reveal any agreement between morphological variation and geographical location of populations. The first component explained 45% of variance and was correlated with phyllary width, leaf length and shape. The third component explained 14% of variance and was correlated with leaf width and head diameter (Figure 1).

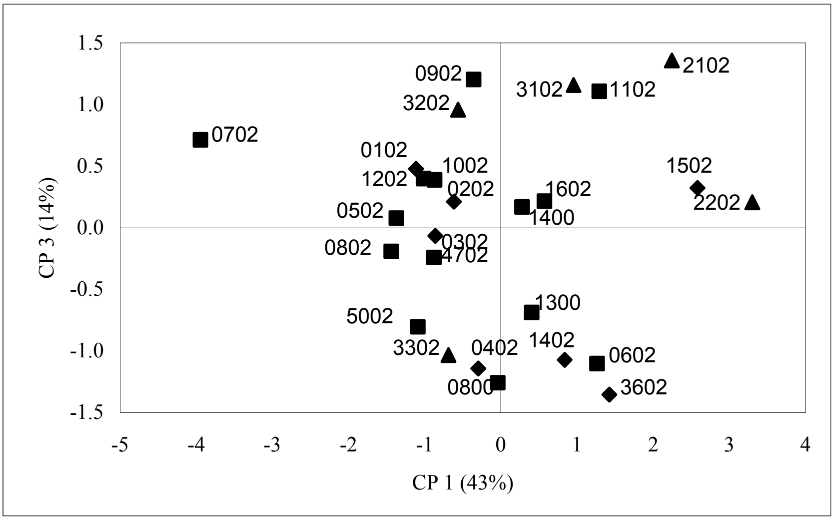

Figure 1. Principal component analysis of 26 populations of *H. petiolaris* from Buenos Aires (squares), La Pampa (rhombs) and San Luis (triangles) provinces. Numbers correspond to accessions in our collection.

Lack of concordance with origin was observed in wild populations of *H. annuus* in the centre of origin and addressed to their human-dispersed nature (Arias & Rieseberg, 1995). The lack of a clinal structure in the Argentine *H. petiolaris* populations indicates that random drift has been more important than adaptive processes in differentiation among populations, which are typically patchy and ephemeral. The blurred geographic pattern can also be addressed to multiple events of introduction and dispersion through trucks and trains, which drag heads and seeds along their way. The results agree with seed isozyme variation, previously assayed in 22 populations (in preparation). Cluster analysis formed four main groups; each one contained some geographically close related accessions, but also no related ones. Two groups included those populations where interspecific hybrids were found, suggesting that some populations share traits, however inconspicuous, that could arise from introgression with *H. annuus*. Field collection trips over three years indicate that *H. petiolaris* is progressively spreading. Variability among and within populations would allow adaptation to different environments.

Interspecific Hybridization. Gene flow from cultivated sunflower to *H. petiolaris* and recurrent hybridization events were confirmed when intermediate plants were found in 10 out of the 26 *H. petiolaris* populations in the field. Frequency of intermediate plants per population ranged from 0.005 to 0.02, with mean of 0.013. Their hybrid origin was conclusively demonstrated through morphology and fertility. Intermediate plants were taller than *H. petiolaris* plants of the same accession, less branched with branching mostly above, bigger heads and leaves, wider discs and phyllaries. Pollen stainability and seed set were lower than in *H. petiolaris* plants (Table 1, Figure 2).

Table 1. Some characters in sunflower interspecific hybrids and parent species. Mean data and standard deviation, significance and Hochberg mean comparison.

Character	*H. petiolaris*	Intersp. Hybrids	Cultivated sunflower	Sign.
Leaf length	8.24 ± 1.51(a)	14.59 ± 2.70(b)	22.83 ± 4.44(c)	**
Leaf width	5.26 ± 0.94(a)	12.99 ± 3.08(b)	21.5 ± 6.58(c)	**
Leaf shape l/w	1.59 ± 0.22(b)	1.14 ± 1.33(a)	1.09 ± 0.13(a)	**
Disc diameter	2.41 ± 0.33(a)	4.09 ± 0.97(b)	13.5 ± 1.14(c)	**
Seed set	71.8 ± 5.26(a)	11.67 ± 24.19(b)	-	**
Pollen stainability	82.46 ± 13.24(a)	30.64 ± 18.61(b)	-	**

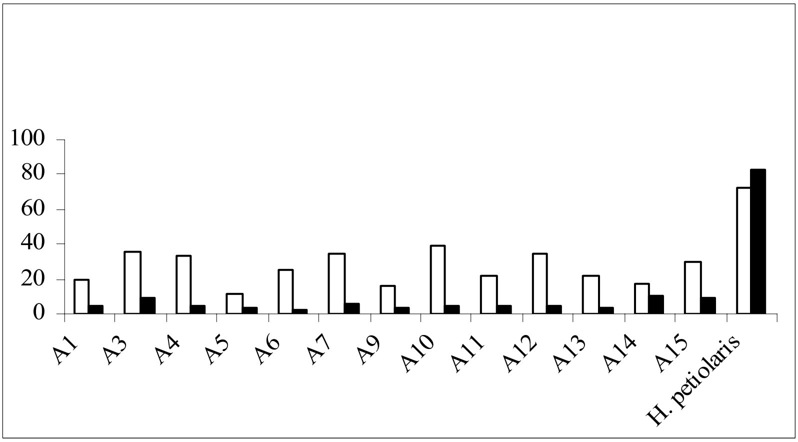

Figure 2. Pollen stainability (white bars) and seed set (black bars) percentage in interspecific hybrids (A1-A15) and *H. petiolaris* (mean data of four populations).

The hybrid index showed that leaf margin and appearance and disc flower colour were intermediate between *H. petiolaris* and cultivated sunflower. Hybrid plants were phenotypically very different, 1.20 to 1.80 m height, mostly wide cordate leaves but sometimes smaller and lanceolate, mostly long petioles and sometimes very thick. Half of the hybrids had very large discs and phyllaries and half resembled more the *petiolaris* type. Variation in leaf, disc and phyllary size was the most informative for PC analysis, which placed hybrids between both parental species (Figure 3).

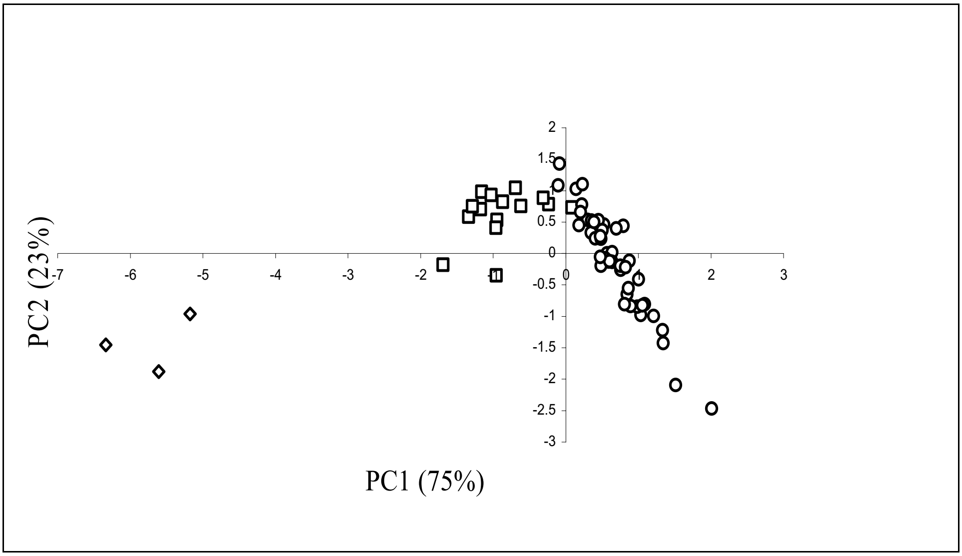

Figure 3. PC analysis of morphological traits in *H. petiolaris* (circles), cultivated sunflower (rhombs) and hybrids (squares).

The life cycle of eight hybrids was intermediate (136 to 205 days) between *H. petiolaris* accessions (146 to 220d) and the cultivated sunflower (<125d), but six hybrids showed a much longer cycle (>220d) being transgressive. New morphological and phenological traits allow ecological differentiation of hybrids from their paternal species, generating favourable conditions for divergence.

In spite of partial chromosomal and genic barriers between both species (Rieseberg et al., 1995), our results confirm that repetitive hybridization and gene flow are frequent in *H. petiolaris* populations growing close to sunflower crops in Argentina. Given that these species have been in contact for at least 50 years under adaptive conditions, new genetic resources for breeding may be found in the wild. Transgenes would probably spread to feral populations if genetically modified cultivars were released, yet we cannot assert that it would have any environmental impact. In order to study a second generation in the summer of 2003-2004, pollen of each hybrid plant was used to pollinate *H. petiolaris* plants of the same population. Seed was collected from these backcrosses and also from the open-pollinated hybrids. Plants from wild populations in Fig.1, hybrids, backcrosses and advanced generations are at present under molecular marker analysis.

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