

Disease Tolerance in *Helianthus petiolaris*: A Genetic Resource for Sunflower Breeding

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Abstract: Argentina is a major sunflower producer in the world, with crop acreage of 2–2.7 million ha in the last four years. Sunflower crop yield is often influenced by sanitary constraints, mainly fungal pathogens. *Helianthus petiolaris* is a wild species native to North America established in central Argentina displays a high tolerance to a number of fungal diseases and insects. Controlled crosses of this species with sunflower demonstrated that *H. petiolaris* constitutes a valuable genetic variability source for sunflower breeding to improve tolerance to rust (*Puccinia helianthi*), white rust (*Albugo tragopogonis*), verticillium wilt (*Verticillium dahliae*), powdery mildew (*Erysiphe* sp.) and the sunflower moth (*Rachiplusia nu*). This places *H. petiolaris* in an outstanding position as a genetic resource since different important traits could be transferred to the crop through interspecific hybridization.

Key words: Disease tolerance, *Helianthus petiolaris*, Interspecific hybrids, Powdery mildew, Rust, Sunflower moth, Verticillium wilt.

Argentina is one of the three main sunflower (*Helianthus annuus* var. *macrocarpus*) producers over world, along with Russia and Ukraine, with acreage of 2–2.7 million ha in the last four years (MAGyP, 2009). Our country contributed 16% of total world production in tons in 2008, but less in 2009 because of adverse climatic and economic conditions. Sunflower is the fourth major oil crop following soybean, palm, and rapeseed, and has the highest quality among edible oils. Market is around 2.8 million tons per year, Argentina contributing 32%.

Helianthus petiolaris Nutt. is a wild species native to North America that is grown in central Argentina (Cabrera, 1963; Covas, 1966) covering at present nearly five million ha (Poverene et al., 2008). Also known as prairie sunflower, it is an annual, diploid ($2n=2x=34$), and self-incompatible species. Its origin and dispersal in Argentina are uncertain. *H. petiolaris* was first discovered in eastern La Pampa province (Covas, 1966). It readily adapted to semiarid environments and sandy soils, spreading over four provinces, La Pampa, western Buenos Aires, eastern San Luis, and southern Cordoba (Poverene et al., 2002; Cantamutto et al., 2008) and still continues its expansion. It is frequently observed along the roadside, dirt roads and fallow land, in patches on disturbed soils. On cultivated land the prairie sunflower grows in borders and occasionally it invades the crop.

The size of *H. petiolaris* populations varies from 100 to

more than 50,000 plants, with a density of up to 25 plants m⁻², although some sites reach 80 plants m⁻², with a patchy distribution (Poverene et al., 2008). At present, their spreading overlaps the sunflower crop area and as in the northern hemisphere, both species share the flowering time and pollination agents, mainly bees and bumblebees that favor pollen flow and natural hybridization (Rieseberg et al., 1999; Poverene et al., 2004; Gutierrez et al., 2010). Crop genes persist for several generations in wild *Helianthus* populations (Whitton et al., 1997; Linder et al., 1998) depending on the fitness of the populations that acquire them (Alexander et al., 2001; Cummings et al., 2002) and environmental conditions of their habitat (Mercer et al., 2007).

Sunflower production in Argentina has been frequently influenced by sanitary constraints that have affected crops in a direct way, as yield loss, or indirectly as sowing retraction due to harvest uncertainty. The main cause of losses and uncertainties is fungal diseases and their relative importance varies annually according to biological factors (i.e., resistance degree of the sown varieties, prevalent races, pathogen inoculum pressure) and environmental factors (climate and management) (Pereyra and Escande, 1994). Since sunflower is susceptible to several diseases, genetic resistance is the best strategy to confer crop protection.

Wild annual *Helianthus* is an important source of resistance for breeding (Luciano, 1964). In fact, *H. petiolaris*

Table 1. *H. petiolaris* (HP) and open-pollinated crop-wild hybrid progenies (OP) used in this study.

<i>H. petiolaris</i>	OP progenies	Province	County	Location
HP 0502	1	La Pampa	Catriló	S 36°33.9', W 63°39.8'
HP 0802	2	La Pampa	Atreucó	S 37°07.6', W 63°39.7'
HP 0902	1	La Pampa	Capital	S 36°20.4', W 64°13.5'
HP 1002	1	La Pampa	Capital	S 36°21.6', W 64°16.7'
HP 3202	1	San Luis	G. Pedernera	S 33°51.5', W 65°19.4'

Numbers correspond to the sites where the seeds were collected. Two hybrids from HP 0802 population gave one OP progeny each.

Table 2. Mean values of morphological and phenological traits in populations of two biotypes (HP and OP) registered in the experimental field and cultivated sunflower, *Helianthus annuus* (HA). Population numbers correspond to different geographical origins.

Populations	days to blooming ¹	days to senescence ²	plant height (cm)	number of branch	seeds weight per plant (g)	1000-kernel weight (g)
HP 0502	49	125	116.2	125.6	58.53	4.2
HP 0802	49	123	113.2	125.7	31.60	2.6
HP 0902	51	127	107.6	123.3	43.31	3.8
HP 1002	47	121	111.6	126	40.56	3.1
HP 3202	48	122	96.6	122.1	38.43	2.9
OP 0502	49	115	147.6	96.3	10.88	6.8
OP 0802-1	41	102	151.8	107.3	24.02	10.7
OP 0802-2	42	104	154.9	107	31.87	11.1
OP 0902	45	103	137	97.8	20.58	6.3
OP 1002	40	102	139.1	110.6	17.02	9.2
OP 3202	43	104	146.5	100.7	33.83	9.6
HA ³	70	110	170	0	60	70
Among biotypes ⁴	*	**	**	**	*	**
Within biotypes ⁴	ns	ns	ns	ns	ns	ns

¹ Beginning of opening of the first head (R4 in the scale of Schneiter and Miller (1981)).

² Physiological maturity (R9 in the scale of Schneiter and Miller (1981) of the last opened head.

³ Ideotype of a commercial sunflower (Jan and Seiler (2007)).

⁴ Significance according to Kruskal Wallis test (**, $p \leq 0.01$; *, $p \leq 0.05$; ns, non significant).

was the donor of the male sterility source that made possible the production of sunflower commercial hybrids (Leclercq, 1969) and could have some other interesting traits (Covas and Vargas López, 1970; Ferreira, 1980). Cáceres et al. (2006, 2007) found genetic resistance to *Sclerotinia sclerotiorum* and *Phomopsis helianthii* in Argentinean germplasm.

From 2004 to 2007, we observed in the experimental field at Bahia Blanca (S 38°41'38", W 62°14'53") that most sunflower (*H. annuus* var. *macrocarpus*) plants were severely affected by pathogens and insects while *H. petiolaris* plants were hardly ever affected. If this wild species had resistance genes against main crop adversities, this would be expressed in wild-crop offspring. The goal of this work was to evaluate five *H. petiolaris* accessions and their progenies following crop hybridization, under natural infection of the main crop adversities in our locality.

Materials and Methods

Five *H. petiolaris* (HP) accessions were collected in four different localities of La Pampa and one of San Luis province, growing close (<100 m) to sunflower crops (Table 1). A seed sample of each accession was sown in the experimental field. Among ca. 840 plants, six natural F1 wild-crop hybrids were identified, each accession produced one hybrid, but one from La Pampa produced two hybrids, (Gutierrez et al., 2010). Open-pollinated (OP) progenies of these interspecific hybrids were grown in summer in 2007. Seeds were placed in plastic trays on wet paper and kept at 4°C for one week. Then they were sown (1 November 2007) and grown with appropriate watering and temperature, until the seedlings had 4–6 leaves. At that stage they were transplanted into the experimental field (12 December 2007) along with seedlings obtained from their respective *H. petiolaris* population. Hence, each

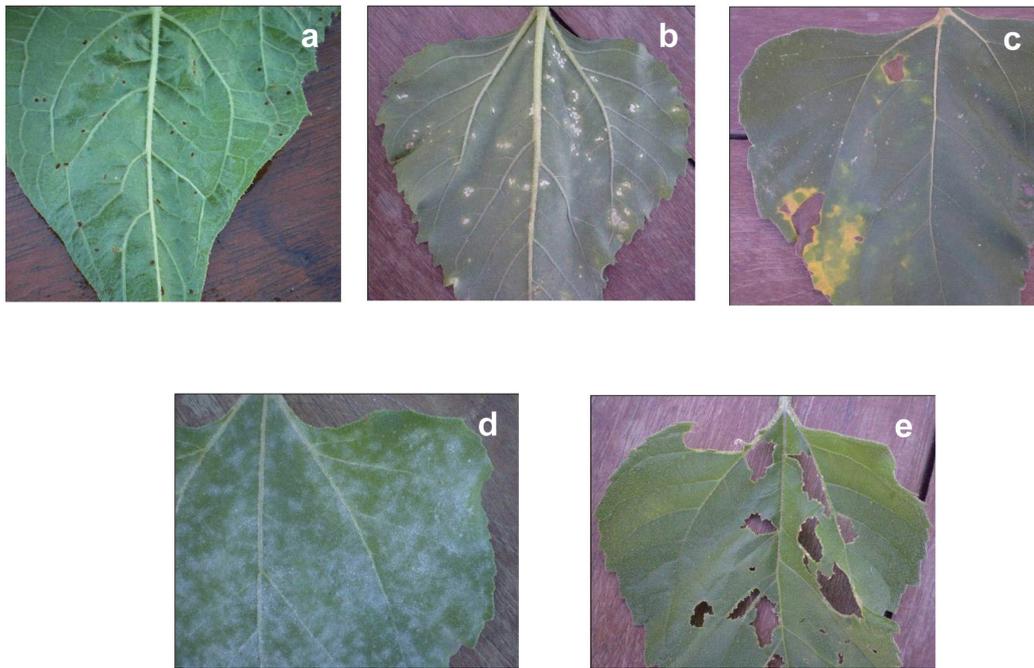


Fig. 1. Adversities observed in the experimental field, a) rust (*Puccinia helianthi*), b) white rust (*Albugo tragopogonis*), c) verticillium wilt (*Verticillium dahliae*), d) powdery mildew (*Erisiphe* sp.), e) sunflower moth (*Rachiphusia nu*).

Table 3. Number of *H. petiolaris* (HP) and open-pollinated first generation hybrid progenies (OP) plants affected by fungus and moth attack.

Adversities	HP (n=360)	OP (n=360)	H	p-value	Significance
Verticillium	0 a	3 a	0.73	0.0745	ns
Rust	0 a	39 b	5.19	0.0017	**
White rust	2 a	56 b	19.20	<0.0001	**
Mildew	0 a	100 b	15.89	<0.0001	**
Sunflower moth	11 a	241 b	24.99	<0.0001	**

Different letters indicate significant differences in the Kruskal-Wallis test ($p \leq 0.05$).

population comprised 20 *H. petiolaris* plants and 20 OP hybrid progenies. They were grouped according to their geographical provenance in 12 m² plots, in a completely randomized design with three replicates.

Morphology and phenology data were registered for every plant, as a part of a wider study addressed to compare fitness of wild and wild-crop plants: days to blooming and senescence, plant height, branch number, and seed weight. Variance among and within biotypes was analyzed by the non-parametric Kruskal-Wallis test. As this study was directed to find differences in damage levels by natural biotic stresses, it began with an ocular inspection of the diseases present on plants during that agricultural year. The presence of symptoms per plant was registered within each group for each adversity. Data were analyzed by the non-parametric Kruskal-Wallis test with InfoStat (2006).

Results

Morphological and phenological traits showed highly significant differences among HP and OP biotypes, but there were no differences within biotypes from different geographical sites, according to Kruskal Wallis test (Table 2).

Diseases found in the experimental field were rust (*Puccinia helianthi*), white rust (*Albugo tragopogonis*), verticillium wilt (*Verticillium dahliae*), powdery mildew (*Erisiphe* sp.) and the sunflower moth (*Rachiphusia nu*) (Fig. 1).

Highly significant differences were found between *H. petiolaris* and the wild-crop hybrid progenies in four out of five observed diseases (Table 3). The wild population was scarcely affected by fungi or moths, while wild-crop hybrid progenies showed differences in damage level for five adversities. The sunflower moth caused the highest damage, displaying some degree of damage in 241 out of

Table 4. Mean number of OP plants in the experimental field affected by different adversities according to their geographical origin (site).

Site	Verticillium	Rust	White rust	Mildew	Sunflower moth
0502	0	0	2	1.67	10.67 ab
0802	0.17	5.5	4.33	9	17.17 bc
0902	0	0	2	2	9.67 ab
1002	0.33	0.33	2.33	4.33	7 a
3202	0.32	1.67	3.67	7.33	18.67 c
p-value	0.686	0.051	0.423	0.157	0.019
Significance	ns	ns	ns	ns	*

Significance level by Kruskal Wallis test (* $p \leq 0.05$, ns non significant. Means following by the same letter are not significantly different).

360 plants. *Verticillium* was the less frequent disease, observed in three out of 360 wild-crop offspring plants but in none of the *H. petiolaris* plants.

No significant differences were found in damage levels among geographical origins of wild populations. The same occurred for wild-crop hybrid progenies regarding diseases (Table 4). Differences for the moth attack were observed among sites of origin, being San Luis population the most affected, with 56 harmed individuals over 60 sampled plants. The following were populations 0802 from La Pampa, without differences from the other two localities in the same province, 0502 Catrilo and 0902 Capital. Population 1002 was significantly different from the remaining.

Discussion

Morphological and phenological traits among HP and OP biotypes were addressed to sunflower crop introgression (Gutierrez et al., 2010). However, no differences were found within biotypes from different geographical origins.

Rust (*Puccinia helianthi*) and white rust (*Albugo tragopogonis*) are diseases that mainly affect leaves, *Verticillium dahliae* affects leaves and stems, powdery mildew (*Erysiphe cichoracearum*) also affects leaves and occasionally, stems and bracts. Sunflower moth (*Rachiplusia nu*) feeds on leaves during its larval stage, destroying the parenchyma without damaging the veins. From 2004 to 2007, we observed these diseases and plague in our experimental field; the sunflower crop plants (*H. annuus* var. *macrocarpus*) were heavily attacked and *H. petiolaris* plants very tolerant and seldom affected.

Wild populations have been used as a source for genetic resources for more than 60 years, to incorporate economically valuable traits to crops. In the past 20 years, breeders have made use of wild species for crop improvement and the most reliable traits have been those related with plague and disease resistance, abiotic stress, quality, and male-sterility. Among these, plague and disease resistance have contributed with more than 80% beneficial to crop breeding (Hajjar and Hodgkin, 2007).

Sunflower inbred lines derived from interspecific crosses

with the wild species *Helianthus tuberosus* and *H. argophyllus* showed a satisfactory resistance to *Sclerotinia sclerotiorum* (Degener et al., 1999). Cáceres et al. (2006) found a high level of resistance to the same fungus in some Argentinean *H. petiolaris* accessions, and considered this species as a potential source for disease resistance in leaves and stems in sunflower breeding. Resistance to *Phomopsis helianthi*, a fungus which produces stem canker in sunflower was also found in *H. petiolaris*. This disease produced severe damages in sunflower crops in Uruguay, although serious attacks of this pathogen have not yet been detected in Argentina (Cáceres et al., 2007).

Cytoplasmic male sterility (CMS) has been found in a number of wild species, resulting in a considerable hybrid research. In sunflower, CMS came from *H. petiolaris*, the male sterile cytoplasm donor at present utilized for obtaining high yield commercial hybrids (Leclercq, 1969). All sunflower production in the USA and near 60–70% in the rest of the world is achieved through this kind of hybrids. Disease research in *H. petiolaris* is of concern since CMS manipulation is by now the only way to produce commercial hybrid seed. Large-scale usage of CMS in maize based in a unique source, known as T-cytoplasm, had to be abandoned when plants carrying this cytoplasm were severely affected by corn leaf blight (*Bipolaris maydis* race T) in the early 1970' (Levings and Siedow, 1992). A similar situation affecting sunflower would result in a more severe damage.

This work and previous ones (Covas and Vargas López, 1970; Seiler and Rieseberg, 1997) show that *H. petiolaris* should be considered a valuable source of genetic variability for sunflower breeding, since a number of economic important traits can be transferred to domestic sunflower through intraspecific hybridization. In spite of differences caused by several chromosome rearrangements between the two species (Rieseberg et al., 1995; Burke et al., 2004) interspecific hybrids are at least partially fertile and allow gene transfer to crop. This feature along with the continuous emergence of new crop plague/pathogen strains, and climate or environmental changes constitutes an important challenge for obtaining cultivars adapted to

novel needs and changing conditions. *H. petiolaris* populations are susceptible to gene flow from crop, which could cause exogamic depression, a fitness reduction in the progeny of crosses from widely different populations. The higher incidence of observed adversities in wild-crop hybrids, compared to *H. petiolaris* plants, points to a resilience mechanism that ensures the removal of advanced generation wild-crop hybrids in *H. petiolaris* wild/weedy populations. However, it is necessary to apply conservation strategies to protect the germplasm of valuable species in Argentina.

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