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THE ARGENTINE WILD *Helianthus annuus* L. GENETIC RESOURCE

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SUMMARY

Wild *Helianthus annuus* naturalized in Argentina could be a valuable germplasm source for cultivated sunflower. Five wild populations collected in different environments and with different phenotype expression were evaluated as a genetic resource in a common garden study. The populations showed normal meiosis and produced a good seed set when their plants pollinated the male-sterile inbred line A09 (*cms* PET1). The wild populations restored more than 80% fertility of the HA89 (*cms* PET1) and A10 (*cms* PET1) inbred lines in the F_1 generation. The fertility of one male sterile source from Mendoza was restored (>95%) by the maintainer line B10. The fatty acid composition differentiated a population from Entre Ríos with a high saturated fatty acid content (>107 g kg^{-1}). Another population from La Pampa showed a high level (>50%) of resistance to the Sunflower Chlorotic Mottle Virus (SuCMoV). No wild populations showed tolerance to imazaphyr sprayed at 2× doses (×=80 g i.a. ha^{-1}). A population collected in the coolest environment showed high tolerance to low temperature (15/5°C, neutral day) in the initial grow stages (<3 expanded leaves). A population collected in a dry and hot habitat showed the highest germination (>80%) under water stress (-0.4 MPa) imposed by polyethylene glycol 6000. This population and a second one from southern Buenos Aires showed the lowest leaf temperature increase (<10%) and the highest foliar specific density under artificial drought during the R4 to R6 reproductive stages. It was concluded that wild *H. annuus* naturalized in central Argentina can supply some useful traits for sunflower breeding.

Key words: breeding, germplasm, naturalization, stress, sunflower, tolerance

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INTRODUCTION

Genetic diversity contributes to long term preservation of cultivated species by allowing them to rapidly adapt to changes in their environment (Ramanatha Rao and Hodgkin, 2002). Genetic diversity of a crop combines all the sexually compatible species, including their wild and weedy relatives (Maxted *et al.,* 2006). Typically, wild relatives of crop plants are genetically much more diverse than cultivated lineages and constitute a genetic resource useful to increase the germplasm biodiversity (Harlan, 1992). For sunflower, the extant diversity in wild and weedy relatives is of interest, because it can provide genes useful to overcome biotic and abiotic stresses (Thompson *et al.,* 1981; Škorić, 1992; Faure *et al.,* 2002).

The wild-weedy relative sunflower complex has demonstrated its utility as a genetic resource for the crop. Resistance genes obtained from wild sunflowers (Baez and Mácola, 1954) were incorporated into cultivated sunflower and the first black rust resistant varieties in Argentina were developed. Several inbred lines derived from these genotypes have been used to produce modern commercial hybrids (Bertero de Romano and Vazquez, 2003). In another example of successful transference of traits to the crop, herbicide tolerance from the wild ancestor of *H. annuus* was used to develop imidazolinone tolerant sunflower (Al-Khatib *et al.,* 1998; Kolkman *et al.,* 2004).

Early wild resource explorations were performed in the USA by Murray Kinman and Aurelio Luciano in 1963 (Seiler and Rieseberg, 1997). In Argentina, Cialzeta and Antonelli (1971) and, later, Monge Navarro (1987) considered the naturalized wild *Helianthus* spp. populations as a valuable germplasm source for sunflower improvement.

Naturalized *H. annuus* is distributed across the central area of Argentina between 31° 20´ and 37° 31´ latitude (Poverene *et al.,* 2002). It seems that the founder effect did not limit wild *H. annuus* biodiversity in the colonized environment, because 60 years after the introduction of these sunflowers a high phenotypic variability is still present. The observed biodiversity could have originated from the intense gene flow between wild sunflowers and the cultivated sunflower (Ureta *et al.,* 2008) or from introgression with *H. petiolaris* (Gutierrez *et al.,* 2009). The biodiversity present in Argentine wild *H. annuus* represents nearly two-thirds of that observed in wild populations from the USA (Cantamutto *et al.,* 2010).

The whole genetic value of the naturalized Argentine wild sunflowers is unknown. The aim of this work was to explore the existence of useful traits originating from cultivated sunflowers in the wild populations of *Helianthus annuus* naturalized in the central area of Argentina and to assess their potential utility in increasing germplasm variability in the species.

MATERIALS AND METHODS

***Germplasm selection*:** A naturalized wild *Helianthus* exploration initiated in 2000 (Poverene *et al.*, 2002), guided the selection of representative populations to be studied. Environmental variables of the original habitat and phenotypic characterization of the accessions in a common garden study were assessed according to a previously described methodology (Cantamutto *et al.*, 2008; Presotto *et al.*, 2009). Representative wild *H. annuus* accessions were selected using Principal Component Analysis (PCA) under two basic guidelines: 1- to represent the most different original habitat and 2- to represent the most different phenotypes.

***Reproductive barriers*:** Meiocytes were observed from anthers of five plants of each wild accession grown at the experimental field during the summers of 2008-09 in lactopropionic orcein (Dyer, 1979). Between 192 and 303 meiocytes were observed in each of the five wild populations.

To explore the limitations of hybridization with domestic sunflower, ten plants of the inbred male-sterile line A09 (*cms* PET1) were bagged at the R4 stage (Schneiter and Miller, 1981) and hand pollinated during the R5.2-R5.8 stages. A composite mixture of fresh pollen of ten heads randomly taken into a sample of 60 plants of Argentinewild *H. annuus* accessions from AAL and DIA was applied under three pollination frequencies: every day, every two days, and every three days. The same procedure using pollen of the commercial hybrid DK4000 was performed as control. To estimate seed set, filled and empty achenes were counted per head and expressed as percent over total. Data were analyzed by ANOVA and means were compared according to Tukey ($p=0.05$).

***Male sterility and fertility restorers genes*:** The presence of restorers genes was estimated as the frequency of fertile plants over 15 F_1 ($10<n<32$) crosses using the wild Argentine populations as pollen donors and the *cms* inbred lines A10 (*cms* PET-1), HA89 (*cms* PET-1), and HA89 (*cms* RES1) as females. To produce the F_1 generation, a mixture of fresh pollen was obtained from 10 to 30 individuals of each wild population and applied to heads of 5 to 10 plants of the male-sterile inbred lines, bagged at the R4 stage. Fertility was analyzed by ANOVA, considering the male-sterile inbred lines as treatments and wild accessions as replicates, under a randomized complete block design. Means were compared according to Tukey ($p=0.05$).

Male-sterile source from Mendoza province (Poverene *et al.*, 2006) was characterized in ten F_1 generations obtained by controlled crosses with six restorers inbred lines (R49, R307, R432, RMAX1, RHA274, RPET2), three maintainer inbred lines (HA89B, B09, B10), and fertile plants of the same wild population. F_1 plants were considered male-fertile when they produced abundant pollen, while plants without anthers or visible pollen were classified as male-sterile. Individuals with an intermediate anther extrusion and poor pollen release were classified as intermediate. Values were reported as percentage of total plants observed in a cross

(14<n<115) at the experimental field during the 2005-2009 growing seasons. Data analysis was performed by PCA.

***Oil quality*:** Oil content and fatty acid composition (FA) were determined in achenes harvested under common garden conditions, as described previously (Cantamutto *et al.*, 2010). Overall biodiversity in oil composition between accessions was explored by PCA.

***Virus resistance*:** Virus resistance to the Sunflower chlorotic mottle virus (SuCMoV) was assessed. Evaluation was performed on samples of 14-84 individuals from each population grown under glasshouse conditions and artificially inoculated at the V4-V6 growth stage. A SuCMoV isolate maintained on sunflower plants in the greenhouse was used as the inoculum source. Infected leaves were ground in 0.01M Na_2HPO_4/NaH_2PO_4, pH 7 containing 0.1% Na_2SO_3 and silicon carbide 600 mesh added as abrasive (0.25 g/10 ml slurry). Inoculum was applied with a high-pressure airbrush apparatus (Lenardon *et al.*, 2005).

Two weeks after inoculation, plants which showed no disease symptoms were classified as resistant. Incidence values are percentage of infected plants from total inoculated plants from each population. Data were analyzed as ANOVA under a randomized complete block design with tree replicates. Statistical differences among means were determined by the Tukey test (p=0.05).

***Herbicide tolerance*:** Tolerance to herbicides of the imidazolinone family was explored under greenhouse conditions. Plants of wild accessions were grown on plots up to the V4-V6 growth stage at 20-25°C and then sprayed with 0 (control), 0.5, 2.0 and 8.0 × of imazaphyr (×=80 g a.i. ha^{-1}). Two weeks after application, herbicide response evaluation included survival and aerial and underground dry matter accumulation (drying the fresh plant material at 40-45°C). A qualitative scale (0 = no symptoms, 0.25 = slight damage, 0.75 = severe damage, 1 = dead) was also applied to describe the plant reaction to herbicide application. ANOVA was performed considering a factorial treatment arrangement (accession and herbicide doses) under a block design with four replicates of 4-6 individual per experimental unit.

***Low temperature tolerance*:** Two groups of samples were collected from Adolfo Alsina (AAL). One was comprised of plants that had emerged early in the season, while the other was made up of younger plants that emerged later in the season. At the collection time, both groups of fruits were at the same maturity stage, corresponding nearly to the same flowering period. To estimate low temperature tolerance, pre-refrigerated achenes (one week, 5°C) were germinated at room temperature (20-22°C). At tap root emergence, achenes were sown in a cell tray with substrate and maintained under 15/5°C (day/night) constant temperature and a neutral photoperiod. Wild accessions of two other contrasting environments were used as controls. Every two days, growth and development were estimated using a non destructive method. The scale considered cotyledon and leaf development, height and foliar surface growth (0 = no growth or development; 4 = maximum

growth or development). Accessions were compared by means of an integrated index considering growth and development during 30 days after emergence (80% of the value) and height and foliar area (20%). Values were analyzed by ANOVA considering individuals as replicates (24>n>84).

***Drought tolerance*:** Germination under osmotic stress was evaluated using a gradient of polyethylene glycol 6000 (PEG) (Blum, 2009). Four samples of 25 achenes per accession, kept at 5°C during one week, were located over paper moistened with PEG solutions adjusted to 0 (control), -0.2, -0.4 (PEG 0.4), -0.6, -0.8, -1.0 and -1.5 MPa of osmotic potential (ψ), renewed every two days. Total germination per experimental unit, measured when cotyledons formed a 45° angle, was recorded at 18 days and expressed as percentage with respect to control (GER). An integrate index (PEG INT) was calculated as follows:

PEG INT = Σ (GER * $|\psi|$) for ψ = -0.2, -0.4, -0.6, -0.8, -1.0 and -1.5 MPa

Morpho-physiological response to water stress at flowering was evaluated in a field experiment with two irrigation treatments on three replicates of ten plants for each wild accession. The experimental unit consisted of a row 3 m long, flanked by two rows of photosensitive sorghum (non flowering). In a limited irrigation treatment, rain was excluded by black polyethylene mulch covering the inter-row space. Water sufficiency was provided by frequent (2-3 days intervals) drip irrigation periods up to satisfy the potential evapo-transpiration during the R4-R7 growth period (40 days). In this treatment, the available total water (drip irrigation plus natural rain) in the period was 236 mm. A drought treatment consisted in drip application of one third of this level (78 mm).

On individual plants, final height (HEI), total leaf number (LNU), total head number (NUCAP), and head diameter (CADIA) on a first order branch were recorded. Foliar surface (TOSUR) was estimated as a product between width and leaf length at the middle plant section and LNU. Total reproductive surface (SUP) was estimated as an arithmetical product between NUCAP and individual head surface.

Leaf temperature (LT), leaf temperature depression with respect to air temperature (DT), and relative water content (RWC) were measured (10<n<15) on sunny days at noon (Blum, 2009). Chlorophyll content was estimated by a Chlorophyll Metter (SPAD-502, Konica Minolta®) in SPAD units (SPAD). Foliar specific density (FSD) was estimated according to Coleman (2008).

Morphological and physiological traits (preceded by D in figure 10) were calculated as a change with respect to water sufficiency treatment, and expressed as percentage.

RESULTS AND DISCUSSION

***Germplasm selection*:** The five wild *H. annuus* populations selected for this study (Table 1) represent a wide biodiversity of Argentine sunflowers not only by their original habitat but also in their morphological and life traits. The macro- and micro-environmental variables of the populations' habitats (Cantamutto *et al.*,

2008) were different (Figure 1) and ten phenotypic traits allowed their differentiation by means of PCA with 79% of variance retained by the two first axes (Figure 2).

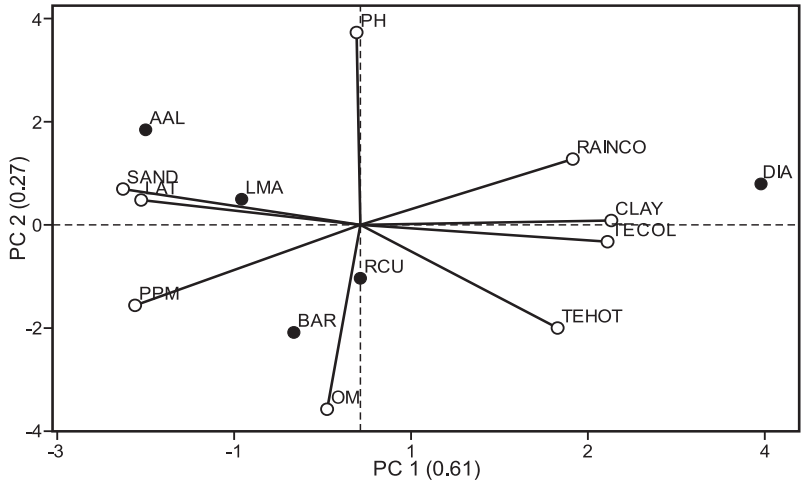

Figure 1: Differentiation of wild Helianthus annuus from Argentina according to the abiotic parameters of the collection site.
(LAT=Latitude; THOT=mean temperature of the hottest month; TCOL=mean temperature of the coolest month; RAINCO=annual rainfall plus irrigation; CLAY=clay soil percentage; SAND=sand soil percentage; PPM=soil available phosphorus; OM=organic matter content; PH=soil pH). See wild population nomenclature in Table 1.

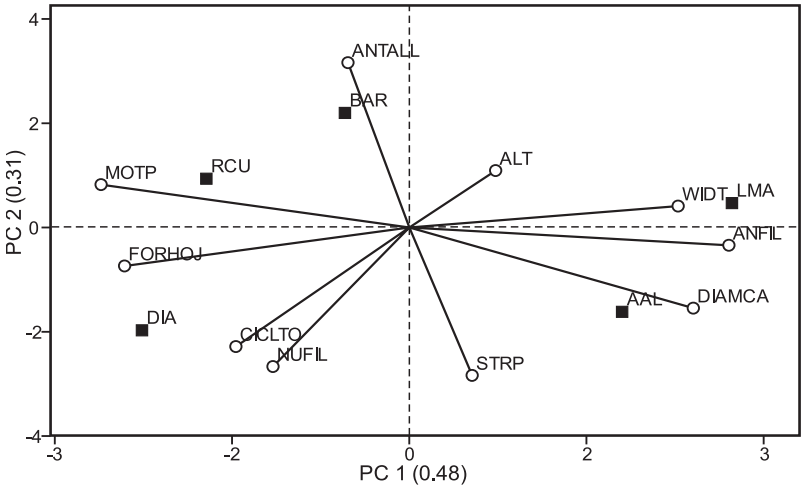

Figure 2: Differentiation of wild Helianthus annuus from Argentina according to the phenotype in a common garden study.
(ANTALL=stem antocyanin frequency; ALT=plant height; ANFIL=bract width; CICLTO=total cycle; DIAMCA=head diameter; FORHOJ=leaf shape; MOTPR=mottled fruit frequency; NUFIL=bract number; STRP=stripes fruit frequency; WIDT=fruit width. See wild population nomenclature in Table 1.

Table 1: General habitat description of the Argentine wild *Helianthus annuus* populations characterized as genetic resource

Population	BAG	Province	Eco-region	PET	Crop area
AAL	839	Buenos Aires	Pampa	yes	yes
BAR	838	La Pampa	Espinal	yes	yes
DIA	834	Entre Ríos	Espinal	no	no
LMA	835	Mendoza	Monte	no	no
RCU	832	Córdoba	Espinal	no	yes

BAG=code number of INTA Sunflower Active Bank; Eco-region according to Burkart (1999); PET=coexistence with H. petiolaris naturalized populations; Crop area = sunflower extensive production area

***Reproductive barriers*:** The five wild *H. annuus* populations from Argentina did not present any reproductive barrier under common garden conditions. The diakinesis stage, found in 15.9% of the observed cells, did not show any chromosome abnormalities. This implies that it is unlikely that any introgression events with *H. petiolaris* have occurred. If this had happened, we would have probably detected some meiotic irregularities at diakinesis (Rieseberg *et al.*, 1995).

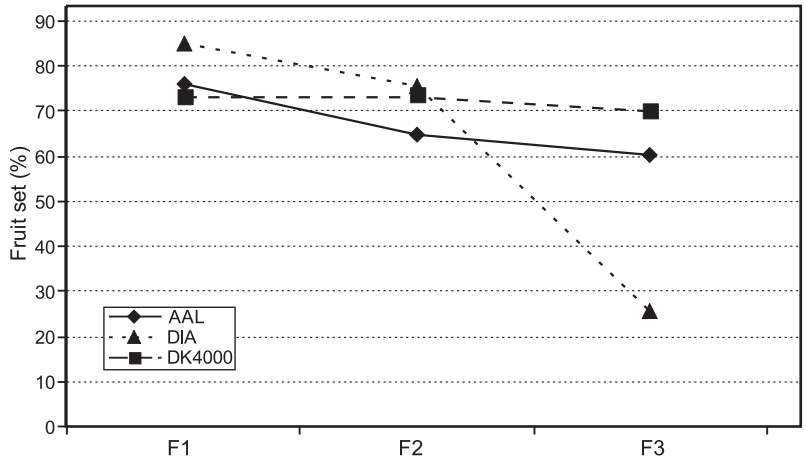

Figure 3: Fruit set as percentage of filled achenes relative to total fruit, obtained in A09 male-sterile inbred line pollinated with fresh pollen of two wild Argentine H. annuus (AAL and DIA, see Table 1) under three pollination intervals: every one, two or three days. A commercial hybrid, DK4000, was used as control.

The two wild *H. annuus* Argentine accessions from AAL and DIA produced similar fruit set as the commercial hybrid DK4000 of inbred line A09 (*cms* PET1) after daily pollination (Figure 3). The accession from DIA reduced its pollination effectiveness by one third when pollen application interval was increased up to three days. AAL did not show such reduction, probably due to an adaptation to drier conditions. DIA was collected in a more humid climate with an annual mean rainfall of over 900 mm, whereas the AAL habitat has less than 700 mm (Figure 1).

***Male sterility and fertility restorers*:** The five wild populations from Argentina showed more than 80% of male fertility restoration of HA89 (*cms* PET1) and A10 (*cms* PET1) (Figure 4). All pollen sources failed to restore HA89 (*cms* RES1), as was expected according to Echeverría *et al.* (2003).

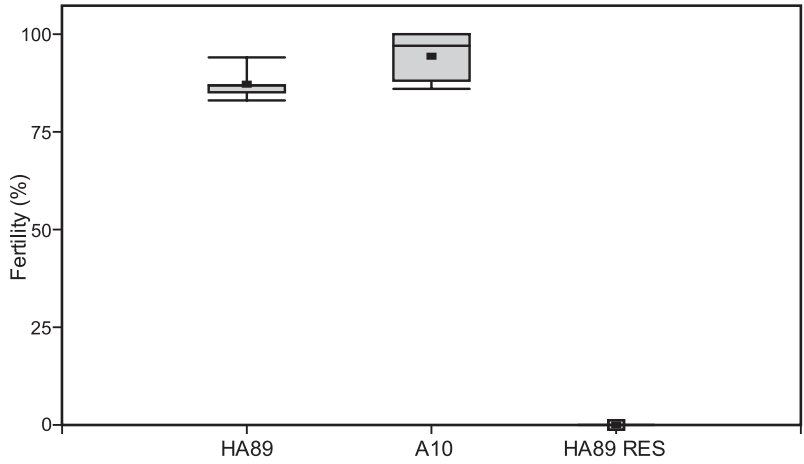

Figure 4: Fertility in F_1 generation of crosses between the male-sterile inbred lines cms PET1 HA89, cms PET1 A10 and cms RES HA89 (HA89 RES) with five wild Argentine populations (Table 1).

The male sterile source found in LMA (Poverene *et al.*, 2006) was restored by the maintainer (B) lines B10, HA89 B and B09 for the PET1 cytoplasm. The latter produced 96% fertile progeny in the F_1 generation (Figure 5).

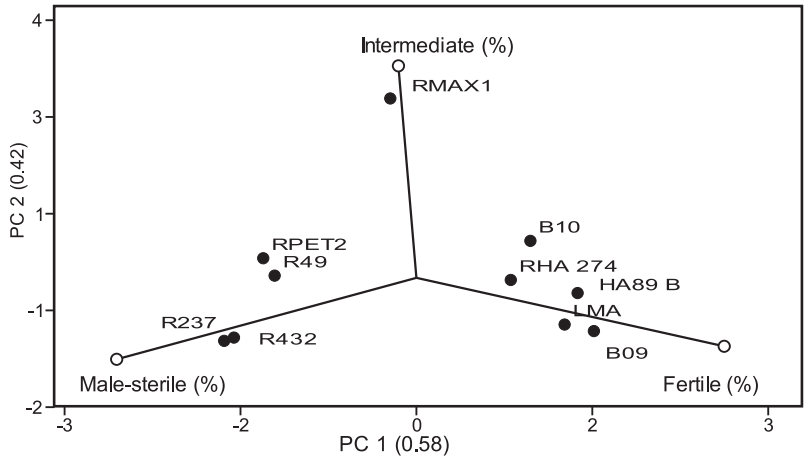

Figure 5: Fertility in F_1 generation of crosses between the male-sterile strain from Mendoza province with six restorer inbred lines, three maintainer inbred lines and normal individuals of the same wild population. Fertile plants showed anthers and pollen was visible, while male-sterile plants did not show anthers or pollen. Intermediate plants showed low anther extrusion and pollen release.

Among the restorer lines, RHA274 showed the best performance but produced only 66% of male fertile individuals at the same generation level. The other restorers (RPET2, R49, R432 and R237) failed to produce complete fertile progeny in the F_1 generation. Normal plants of the same population showed 88% of male fertility restoration. Isolation of the restorer genes into LMA was not obtained, because one out of four restorer wild plants produced only two progenies under controlled self-pollination.

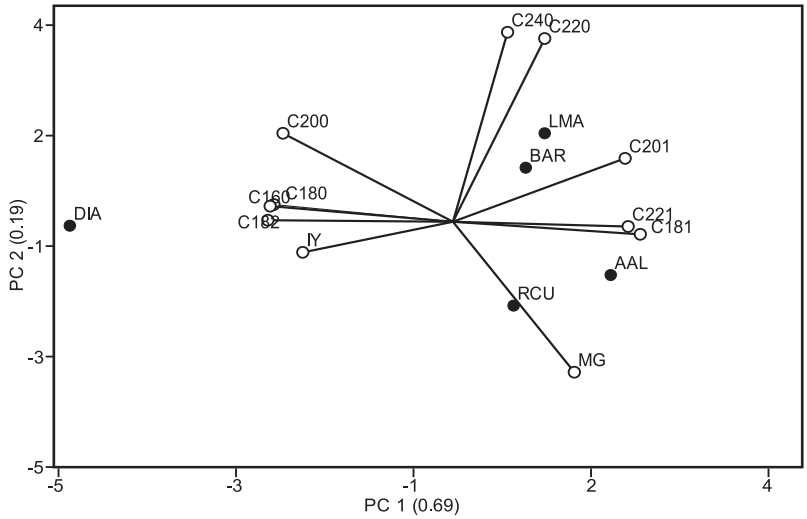

Figure 6: Overall diversity in oil composition of wild H. annuus populations naturalized in Argentina (Table 1) revealed by PCA analysis under a common garden study. (MG=oil content; IY=iodine value. Fatty acid was represented by the letter C followed by three-digit numbers, the first two being the carbon atoms number. The third digit indicated the unsaturated unions).

***Oil quality*:** The DIA accession differentiated for its oil composition, with high levels of saturated FA (>107 g kg^{-1}, Figure 6). Taking into account this high value, it could be considered as a promising source for sunflower breeding (Seiler, 2004). Overall, the remainder of the populations showed similar FA composition, the AAL accession being the one that produced the highest oleic content with 218 g kg^{-1} of fat. This concentration represents a standard value and it is of no interest to sunflower breeding (Fernández Martínez *et al.*, 2009).

***Virus resistance*:** All wild *H. annuus* populations from Argentina showed more than 20% resistance when individuals were artificially inoculate with SuCMoV. In contrast, the susceptible control showed a high incidence of systemic infections (Figure 7). Among the Argentine accessions, BAR showed the highest resistance frequency, with more than a half of the individuals not expressing any disease symptoms. The BAR accession might have introgressed with *H. petiolaris*, because it was collected in an area where both wild species coexist (Table 1) and shows morphological evidence of gene flow (Gutierrez *et al.*, 2009).

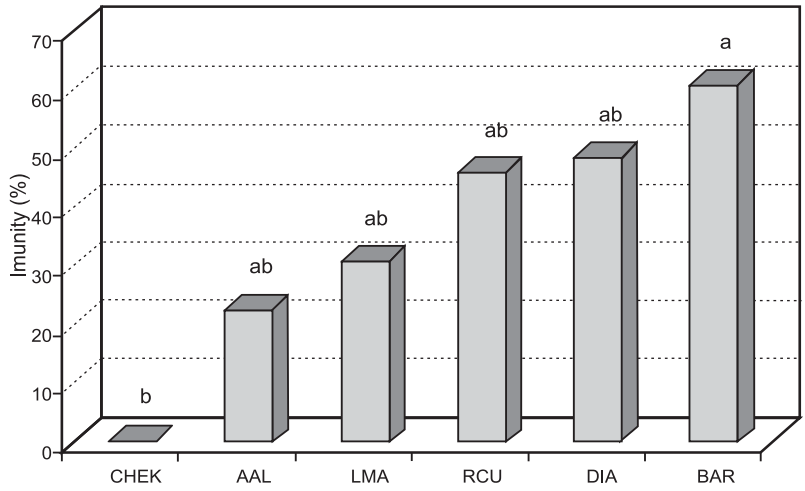

Figure 7: Frequency of plants without virus symptoms after artificial inoculation with SuCMoV observed in the wild H. annuus Argentine accessions (Table 1). Contiflor 17 was used as control.

***Herbicide tolerance*:** Survival, shoot and root dry matter accumulation did not differentiate the five wild *H. annuus* accessions (Table 2). Still, under the 2× imazaphyr dose, the mean survival was over 80%, but at this application level the plants showed severe symptoms of herbicide damage (Figure 8). Half of the recommended imazaphyr doses (0.5 ×) differentiated accessions, DIA and LMA being the more tolerant ones (Figure 8).

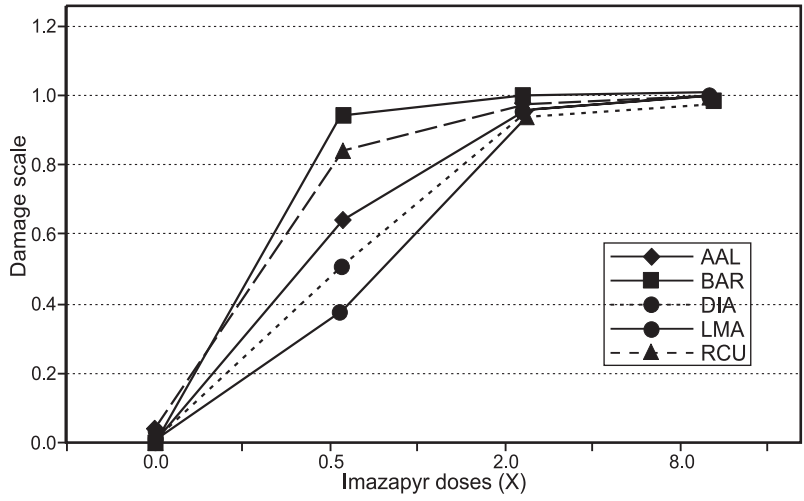

Figure 8: Wild populations' responses to the application of four imazaphyr doses (× = 80 g a.i. ha^{-1}). The damage scale considered 0 = no symptoms; 1 = dead. See nomenclature of populations in Table 1.

This fact does not imply an initial stage of natural selection under agroecosystem forces, because both populations were located in areas with low diffusion of sunflower crop and herbicide use (Table 1). The high damage level observed at 2× doses seems to show a lack of natural tolerance to imidazolinone herbicides in the wild populations studied.

Table 2: Response of five wild Argentine *H. annuus* populations two weeks after the application of four imazaphyr doses (×=80 g a.i. ha^{-1}) at V4-V6 growth stages in greenhouse conditions.

Doses	Survival (%)	Shoot dm (g)	Root dm (g)
0.0 ×	99 a	84 a	38 a
0.5 ×	98 a	72 bc	27 b
2.0 ×	83 a	55 ab	17 c
8.0 ×	46 b	36 c	12 c
	ANOVA		
Doses	**	**	**
Population	Ns	ns	ns
Doses × Population	ns	ns	ns

Means followed with the same letter do not differ according to Tukey test (p=0.05)

***Low temperature tolerance*:** Both subgroups of the AAL accession showed higher growth and development than DIA did in the low temperature experiment (Figure 9).

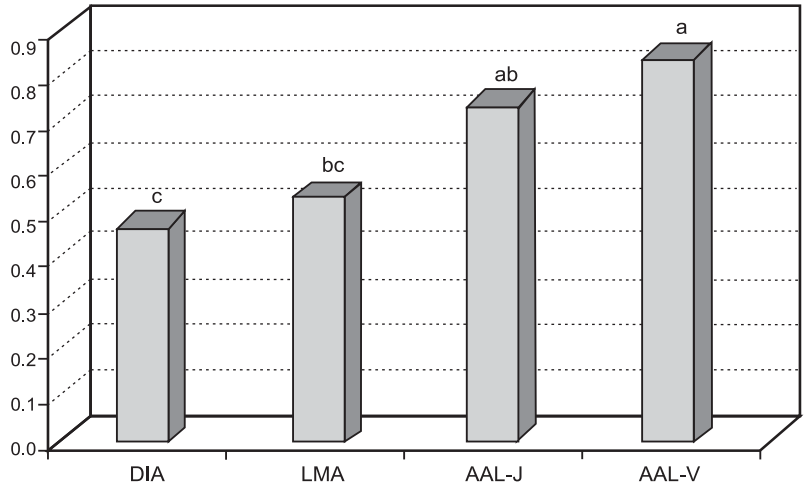

Figure 9: Growth and development of wild H. annuus accessions from Buenos Aires (AAL), Mendoza (LMA) and Entre Ríos (DIA) Argentine provinces under low temperature conditions (15/5°C). The AAL accession was collected in two groups; old (AAL-V) and young (AAL-J) individuals. The scale integrates the growth and development increase during the month after emergence (see text)

Even though there were no differences between the progenies of old and young plants at the collecting site, older plants seemed to produce progeny with better

performance in the low temperature environment. This could be due to natural selection at emergence in its original habitat that allows early growth, detected as older plants at recollection time. Also, the AAL environment was the coldest one, being DIA collected at the hotter environment (Figure 1). LMA was collected in an intermediate climate and also presented intermediate growth and development as response to low temperature.

***Drought tolerance*:** The Argentine wild accessions differed in their response to water limitations measured by some physiological and morphological traits. The RCU population showed lower depression in reproductive surface under drought (Figure 10). RCU also germinated well under water restrictions imposed by PEG, a high foliar specific density and the lowest leaf temperature depression with respect to air temperature under water stress at flowering. AAL, showing a similar response to water limitation, was collected in a dryer habitat (Table 1). This finding seems to confirm the potential utility of wild species as a source of genes for tolerance to abiotic stress in sunflower breeding (Škorić, 2009).

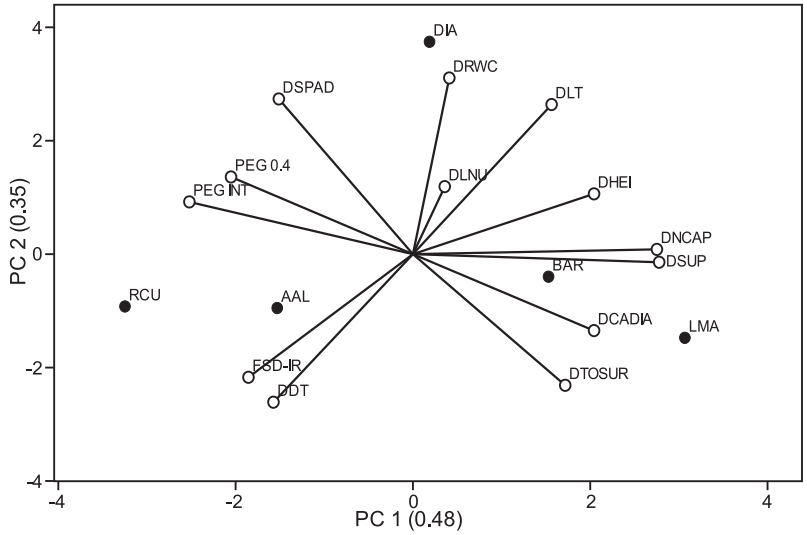

Figure 10:Overall diversity in drought tolerance of wild H. annuus populations from Argentina (Table 1).
(PEG 0.4=germination under -0.4 MPa imposed by Polyetilen Glicol (PEG); PEG INT=integrate value of germination under seven PEG concentrations (see text); FSD-IR=specific density under artificial drought; DSPAD=SPAD depression by drought; DRWC=relative water content depression by drought; DLNU=leaf number per plant reduction by drought; DLT=leaf temperature depression by drought; DHEI=plant height shortened by drought; DNCAP=head number reduction by drought; DSUP=reproductive surface reduction by drought; DCADIA= head diameter reduction by drought, DTOSUR = foliar surface reduction by drought.

CONCLUSIONS

The representative sample of the wild populations explored showed normal reproduction. All hybridized easily with domestic sunflower and restored the fertility of *cms* PET1 male sterility inbred lines in the F_1 generation. Some populations showed a fatty acid composition that could represent a source of novel variability for the crop. A wild *Helianthus annuus* population from La Pampa province showed a high frequency of SuCMoV resistance. The population collected in the coolest environment in Buenos Aires province showed tolerance to low temperature during the initial growth stages. Another population from Cordoba stands out for its overall response to drought at reproductive stages. It could be concluded that the Argentine wild *Helianthus annuus* constitutes a promising genetic resource for the sunflower crop.

ACKNOWLEDGEMENTS

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El RECURSO GENÉTICO del *Helianthus annuus* L. SILVESTRE DE ARGENTINA

RESUMEN

El *Helianthus annuus* naturalizado en la Argentina podría ser un germoplasma valioso para el cultivo de girasol. Se evaluaron cinco poblaciones silvestres colectadas en diversos ambientes y con diferente fenotipo, en un estudio de jardín común. Las plantas mostraron meiosis normal y produjeron abundante semilla cuando polinizaron la línea pura androestéril A09 (*cms* PET1). Las plantas silvestres restauraron en más del de 80% la fertilidad de las líneas puras HA89 (*cms* PET1) y A10 (*cms* PET1) en la generación F_1. La fertilidad de una fuente de androesterilidad de la población de Mendoza fue restaurada (> el 95%) por la línea mantenedora B10. Una población de Entre Ríos se diferenció por el alto contenido de ácidos grasos saturados (>107 g kg^{-1}). Otra población de La Pampa mostró alto nivel (>50%) de resistencia al virus del moteado clorótico del girasol (SuCMoV). Las poblaciones silvestres de Argentina no mostraron tolerancia al imazapir aplicado en una dosis 2× (× = 80 g i.a. ha^{-1}). Una población colectada en un ambiente frío de la provincia de Buenos Aires mostró alta tolerancia a bajas temperatura (15/5°C, día neutral) en etapas iniciales (<3 hojas expandidas). Otra población colectada en un hábitat seco y cálido presentó elevada germinación (>80%) bajo estrés hídrico (-0.4 MPa) impuesto mediante polietilenglicol 6000. Esta población y otra de Buenos Aires mostraron bajos aumentos de la temperatura de la hoja (<10%) y elevados niveles de densidad foliar específica bajo sequía artificial durante R4 a R6. Se concluye que el *H. annuus* naturalizado en la región central de Argentina podría proveer algunos rasgos útiles para el mejoramiento del girasol.

LA RESSOURCE GÉNÉTIQUE de l'*Helianthus annuus* L. SAUVAGE d'ARGENTINE

RESUME

Helianthus annuus sauvage naturalisé en Argentine peut être un matériel génétique valable pour le tournesol cultivé. Cinq populations sauvages collectées dans différents milieux et avec différents phénotypes ont été évaluées comme ressource génétique sous l'étude jardin de commun. Les plantes ont montré une méiose normale et elles ont produit une abondante quantité de graine quand elles ont pollinisé la ligne pure mâle-stérile A09 (*cms* PET1). Les plantes sauvages ont reconstitué la fertilité en plus de 80% des lignes pures HA89 (*cms* PET1) et A10 (*cms* PET1) à la génération F_1. La fertilité d'une source de mâle-stérilité de la population de Mendoza a été reconstituée (>95%) par la ligne B10. La composition en acides gras a différencié une population de Entre Ríos, avec un contenu élevé d'acides gras saturés (>107 g kg^{-1}). Autre population de La Pampa a montré un niveau élevé (>50%) de résistance au virus de la marbrure chlorotique du tournesol (SuCMoV). Les populations sauvages d'Argentine n'ont pas montré de tolérance à l'*imazaphyr* pulvérisé à une dose de 2× (× = 80 g /p.a. ha^{-1}). Une population colleté dans un milieu froid de la province de Buenos Aires a montré une tolérance élevée aux basses températures (15/5°C, jour neutre) pendant les étapes initiales (<3 feuilles ouvertes). Autre population ramassée dans un habitat sec et chaud a montré une germination élevée (>80%) sous stress hydrique (- 0.4 MPA) imposé avec

polyethylen glycol 6000. Cette population et une autre de Buenos Aires ont montré des basses augmentations de la température de la feuille (<10%) et haut niveaux de densité foliaire spécifique sous une sécheresse artificielle pendant les étapes R4 à R6. Comme conclusion, l' *H. annuus* sauvage naturalisé dans la région centrale d'Argentine peut fournir quelques traits utiles pour l'amélioration du tournesol.