Seed morphology and oil composition of wild *Helianthus annuus* from Argentina

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ABSTRACT
Wild *Helianthus annuus* naturalized in Argentina constitutes a potential genetic resource for use in sunflower crop breeding. Seed morphology, oil content and fatty acid composition of nine stable Argentine wild populations were characterized and compared to 17 wild accessions from the US. The achenes were harvested from an experimental field at Bahia Blanca (S 38º41', W 62º14') during February, in three successive summers. Seed dimensions of Argentine accessions were within the range of US accessions, but showed less variability. The lower mottling and higher frequency of stripes in Argentinean populations would be an indication of crop introgression. The oil content, fatty acid composition and iodine value did not differentiate from the wild species origins. None of the Argentine populations showed a fatty acid composition similar or better than the improved mutant lines reported by other authors. All measured seeds traits showed significant differences, pointing to the existence of high variability in this new wild germplasm from Argentina.

Key words: achene – fatty acid – ferality – genetic resources – oil quality – sunflower.

INTRODUCTION
The quality of sunflower oil which contributes to about 80% of the total value of the crop has received considerable breeding efforts in the last 30 years (Fick and Miller, 1997). The main use of sunflower oil is as a salad and cooking oil, being also used as a major ingredient in some vegetal butter and shortening products, but it and for industrial non edible purposes in paints, varnishes, plastics, soap, and detergent (Seiler 2007). Sunflower oil has a high potential as a source for biodiesel production to satisfy the demand for renewable energy (Vannozzi, 2006).

Oil physical and chemical properties determine its end-use, with the fatty acid composition and iodine value being indicative of the oil characteristics. Traditionally, sunflower has been considered as having a polyunsaturated oil because of its high content of linoleic acid, but breeding selection, sometimes helped by chemical mutagenesis, has produced several lines with altered fatty acid composition (Fernandez-Martinez et al., 2006). Low saturated fatty acid content oils are chosen for edible purposes, high oleic mono-unsaturated acid oils are used for high temperature processes (as frying or bio-lubricants), whereas high saturated acid oils are preferentially used for margarine production, because they reduce the need for hydrogenation (Jan and Seiler, 2007).

The wild *Helianthus annuus* naturalized in Argentina grows as extended populations in a wide area across the boundary between humid and sub-humid regions (Poverene et al., 2002). Wild and weedy relatives of crops are genetically much more diverse than cultivated lineages and constitute a genetic resource that has not been fully exploited (Maxted et al., 2006). Wild *Helianthus* species provide a resource for improving oil quality in cultivated sunflower (Thompson et al., 1981) and a potential source of altered fatty acid composition (Seiler, 2004, 2007). The potential of wild sunflower naturalized in Argentina as genetic resource for oil quality improvement is unknown.

The objective of this work was to characterize wild *Helianthus annuus* from Argentina as a potential source for sunflower crop oil composition improvement.

MATERIALS AND METHODS
The wild germplasm was represented by nine stable populations from the diverse agro-ecological conditions where it grows in Argentina (Cantamutto et al., 2008). The accessions were from Rio Cuarto (RCU) S 33º09', W 64º20', Juarez Celman (JCE) S 33º40', W 63º28', Colonia Barón (BAR) S 36º10', W 63º53', Rancul (RAN) S 35º04', W 64º46', Adolfo Alsina (AAL) S 37º16', W 62º59`, Carhué (CHU) S 37º16', W 62º55', Diamante (DIA) S 32º03', W 60º38', Media Agua (MAG) S 31º57', W 68º27', and Las

Malvinas (LMA) S 34º47', W 68º15'. The accessions were collected by M. Poverene and M. Cantamutto in 2002-2003 during exploration trips, regenerated in the experimental field in Bahía Blanca (S 38º41', W 62º14') during the summer of 2004 and stored in the Sunflower Germplasm Active Bank at INTA Manfredi Experimental Station (Córdoba, Argentina) as code numbers 832 to 840.

Wild germplasm from North America (US) represented by 17 populations provided by the USDA-ARS GRIN germplasm system was studied for comparison. States of origin and passport numbers were: Arizona PI 468571, California PI 468580, Colorado PI 468621, Illinois PI 435540, Indiana PI 468633, Iowa PI 597901, Kansas PI 586851, Montana PI 586821, Nebraska PI 586867, Nevada PI 468596, New Mexico PI 468537, North Dakota PI 586807, Oklahoma PI 468483, South Dakota PI 586835, Texas PI 468504, Utah PI 468607, and Wyoming PI 586824 (for more information see www.ars-grin.gov/cgi-in/npgs/acc/display.pl?1080516).

Seedlings were grown in a greenhouse for one month and then transplanted by accessions in the experimental field at 1.9 plants/m^2 density during three successive summers (2003-2006). Drip-irrigation was applied to satisfy plant water demands. To regenerate the populations, heads of 20-30 individuals of each accession were bagged prior to open and sib-pollinated by hand during flowering. Bulk seed of mature heads were collected before achene shattering during the last week of February for sibbed and open pollination heads to minimized flowering date effects (Seiler, 1983).

A sample of 30 completely developed achenes from both pollination systems was used for seed description. Seed length, width, and thickness were measured using 10X magnification. The individual seed fresh weight was estimated by the total mass of the achenes. Qualitative traits, shape, pubescence, stripe presence, pericarp colour and mottling were individually determined and computed as frequencies. Argentine qualitative traits were determined using the original seed. Oil composition, fatty acid content and iodine value were evaluated at the EEA INTA Manfredi laboratory by AOCS (2007) approved methods (Ai 3-75, Ce 1-62 and Tg 1a-64) on a 10 g sample of seeds harvested from the experimental field under two pollination systems. Methyl esters of fatty acids were analyzed by Gas Chromatograph Hewlett Packard 6890 with a fire lionization detector and a capillary column HP-INNOWax (Crosslinked Polyethylene Glycol), of 0.32 mm x 30 m x 0.5 mm thick film. Each population was grown for at least two years.

To compare all the accessions, the ANOVA considered country, populations nested in countries, and year as variation sources. For seed qualitative traits of Argentine wild accessions, population and year were considered as sources of variability for the ANOVA. The oil content and fatty acid composition of Argentine accessions were analyzed for open-pollinated and sib-pollinated seed and the pollination system was considered as a source of variability for the ANOVA. LS means were calculated for each parameter and pair-compared using a linear combination of the model using the GLM procedure of SAS (2002). The linear regression between metric parameters was calculated and compared using an ANOVA (Quinn and Keough, 2005). Box-plot graphics were obtained with the InfoStat package (InfoStat, 2002).

RESULTS AND DISCUSSION

Argentine seed dimensions possessed about a half of the variability observed in the sample of US wild sunflowers, with no differences in the relationships between width, length, thick and weight, and were within the extreme values observed in the US populations (Fig. 1). Achene weight, length and width of accessions from both hemispheres corresponded to the expected values for wild and weedy populations (Heiser, 1978; Seiler, 1997).

The frequency of sparse pubescence and grey pericarp was not able to discriminate the between the groups, but stripes and mottling frequency differentiated both wild species origins (Fig. 2). The ranges of all qualitative traits overlapped for the Argentine and US wild origins (Fig. 2). A possible crop introgression in Argentine populations was suggested by their lower mottling (Fig. 2.b) and higher stripes frequency (Fig. 2.d) compared to the US accessions.

Though not included for botanical classification by Heiser (1978), mottling could be considered a wild trait. Stripes are typical of confectionary sunflower (Jan and Seiler, 2007) and characterized the first Argentine varieties (Bertero and Vazquez, 2003). If introgression happened during the colonization process, a strong selection pressure for small seed size would be expected (Alexander et al., 2001) but not for pericarp traits, that seem to be neutral. This could explain the absence of complete separation using seed dimensions, being larger in Argentine wild accessions but within the range of acceptable sizes for wild sunflower (Heiser, 1978). Hybridization with cultivated sunflower, also suggested by a phenotypic study of a number of plant traits (unpublished data), likely took place during the invasive process as a result of the intense gene flow documented in Argentina landscape (Ureta et al., 2008). The introgression process was probably followed by a strong selection for small seed.

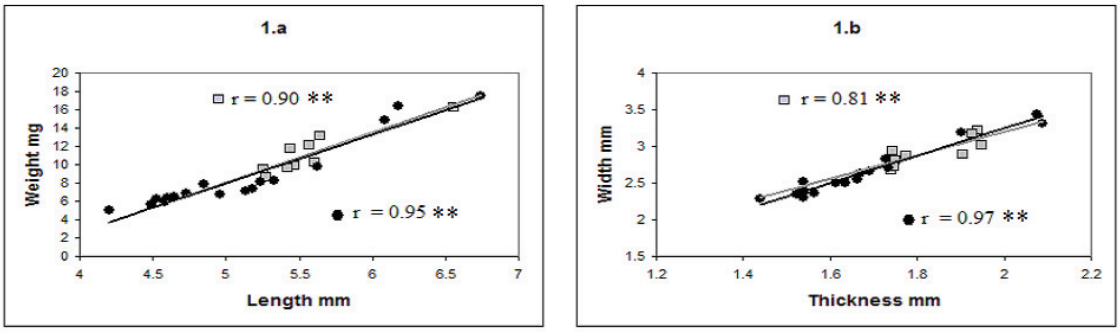

Fig. 1. Morphological relationships in wild *Helianthus annuus* seeds grown for three years in an experimental field. Argentine (grey squares) and North American (black circles) populations showed no differences in linear correlation between parameters.

Within the Argentine accessions, an ANOVA showed that populations differed for all the analyzed morphological traits (Table 1). Year effect was evident only in seed weight and length, probably due to differences in climatic conditions during grain filling. The significant effect of year on pericarp colour could be due to differences in achene size making it difficult to clearly visualize this trait in small seeds. The Argentine accession, CHU had the smallest seed dimensions, significantly different from LMA and MAG, which had the largest achenes (Table 1). The CHU accession also had a higher ovoid shape and grey pericarp frequencies. RCU, RAN, and JCE showed mottling in all seeds, significantly different from LMA, MAG and AAL with low mottled seed frequency. Considering all the traits together, RCU, BAR and CHU seemed to be a pure wild strains as opposed to LMA, AAL and MAG which showed introgressed crop-related traits (big seeds, presence of stripes, low mottling). These findings agree with the hypothesis that Rio Cuarto was as an entry point of wild *Helianthus annuus* before 1950s (Bauer, 1991) from where the invasive process progressed (unpublished data).

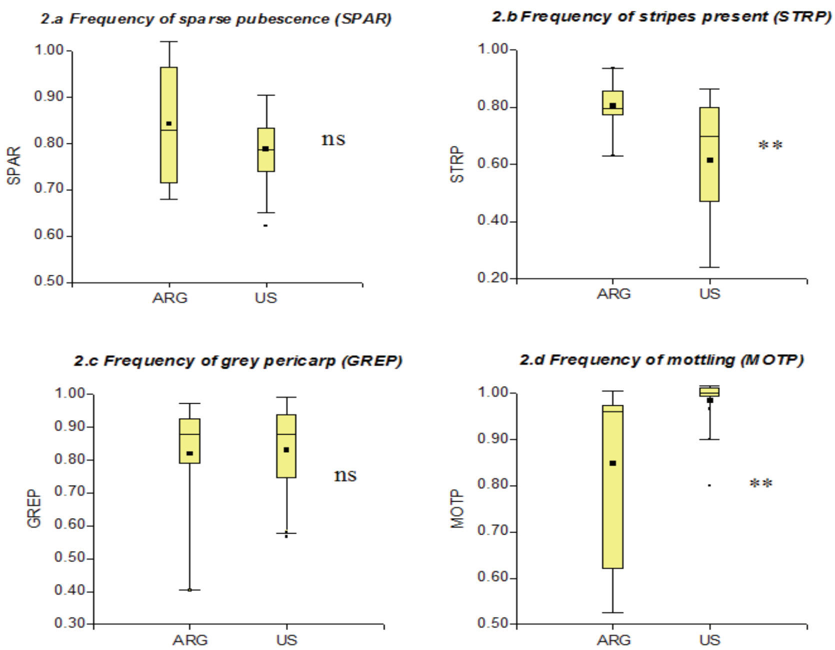

Fig. 2. Seed morphological descriptors of 26 wild sunflower populations from Argentina and the US grown for three years in an experimental field. Box-plots show the LS means distribution, ANOVA differences between both sources are indicated in each case. Year effect was not significant for all traits

Table 1. Morphological seed traits of nine wild *Helianthus annuus* populations from Argentina.

Wild population[1]	Seed dimensions[2] Weight mg	Length mm	Width mm	Thickness mm	Seed traits frequency[3] Ovoid shape	Sparse pubescence	Stripes	Grey pericarp	Mottling
AAL	11.7 bc[4]	5.6 bc	2.9 bd	1.9 a	0.92 a	0.67 c	0.93 a	0.94 ab	0.64 b
BAR	9.3 d	5.5 bc	2.9 bd	1.7 b	0.98 a	0.74 bc	0.77 b	0.99 a	0.98 a
CHU	8.8 d	5.2 c	2.6 e	1.7 b	0.96 a	0.66 c	0.89 a	0.97 a	0.98 a
DIA	10.2 c	5.5 bc	2.8 ce	1.8 b	0.92 a	1.00 a	0.85 a	0.81 bc	0.97 a
JCE	9.4 d	5.4 bc	2.8 ce	1.8 b	0.90 a	0.69 bc	0.62 c	0.95 ab	1.00 a
LMA	17.4 a	6.7 a	3.3 a	2.0 a	0.90 a	0.96 a	0.79 ab	0.41 c	0.53 b
MAG	13.2 d	5.7 b	3.0 ac	2.0 a	0.87 a	1.00 a	0.85 a	0.75 c	0.64 b
RAN	11.4 bc	5.4 c	3.1 ab	1.9 a	0.63 b	0.92 a	0.71 b	0.83 ac	1.00 a
RCU	9.0 d	5.3 c	2.7 de	1.8 b	0.95 a	0.84 b	0.80 a	0.90 ac	1.00 a
ANOVA									
Population	**	**	**	*	*	**	**	**	**
Year	**	**	ns	ns	ns	ns	ns	*	ns

[1]See text for population codes. [2]Achenes harvested during three years in the experimental field. [3]Original seed accessions and achenes harvest in the experimental field. [4]LSmeans with different letters showed differences at $p < 0.05$

The oil content, fatty acid composition and iodine value did not show differences between the wild species origins (Fig. 3) but showed a year effect in fatty acid composition and iodine value. A higher palmitic acid concentration (Fig. 3.b) and a lower oleic acid concentration (Fig. 3.d) was found in Argentine accessions, with the other chemical parameters within the ranges observed for the US wild populations.

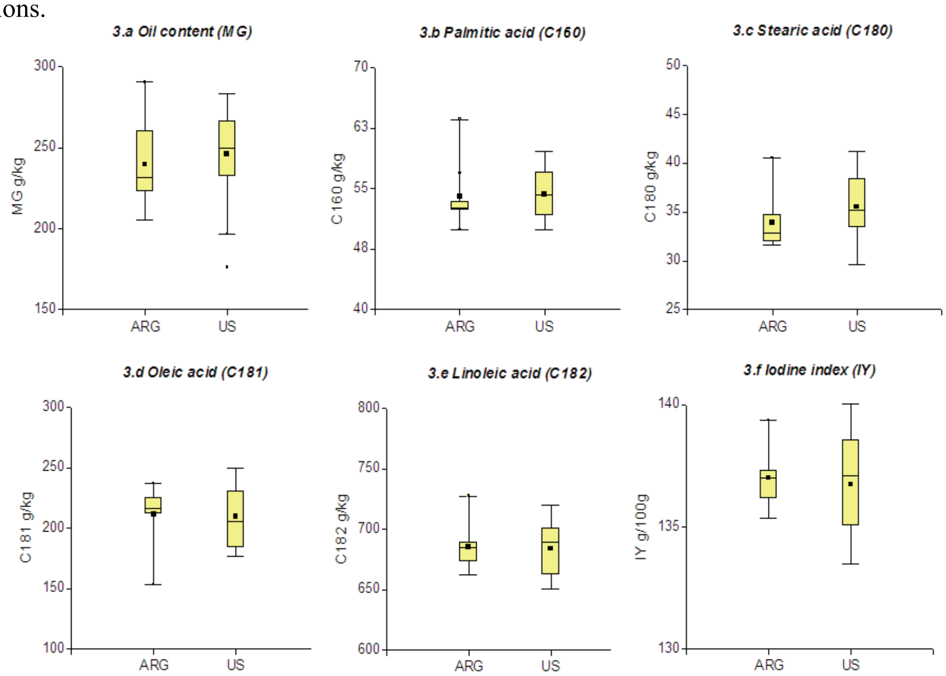

Fig. 3. Oil composition of wild *Helianthus annuus* open pollinated populations from Argentina (ARG) and North America (US) grown in Bahia Blanca, Argentina over three years. No differences were observed between both groups. Box-plots show the LS means distribution of 26 wild populations.

Argentine populations showed differences between the accessions for all the chemical parameters (Table 2). An oil content variation between 21.4 to 28.2% was typical of wild seeds and was only affected by population variability. The year had a significant effect on palmitic acid content and highly significant effects on oleic, linoleic, linolenic concentration, oleic/linoleic ratio and iodine value. Even though the grain filling of all analyzed achenes correspond to the same month, a variation between 35.4 to 40.5ºC of maximum temperature registered during this period could explain the year effect since the oil content and fatty acid composition are influenced by temperature (Harris et al., 1978). Slight variations in nitrogen availability (Steer and Seiler, 1990), water regime (Flagella et al., 2002) and night minimum temperature (Izquierdo et al., 2006) can have an effect on oleic and linoleic sunflower concentrations and maybe responsible for the observed year effect.

Table 2. Oil content and chemical composition of nine wild *Helianthus annuus* from Argentina. Achenes correspond to grain-filling in February with sibbed and open pollination systems. Data are LS means of three years.

Wild `population[1]	Oil content	Fatty acid composition						Iodine value
		palmitic 16:0	stearic 18:0	oleic 18:1	linoleic 18:2	linolenic 18.3	oleic: linoleic	
	g/kg DM	g/kg						g/100g
AAL	282 a[2]	52 cd	32 cd	218 a	684 c	0.71 d	0.32 a	137 cd
BAR	238 bd	52 cd	33 cd	205 ab	696 bc	0.77 cd	0.29 ab	138 bd
CHU	261 ac	54 bc	31 d	199 ab	701 bc	0.86 ac	0.28 ab	139 ac
DIA	217 d	65 a	42 a	135 c	743 a	1.01 a	0.18 c	141 a
JCE	236 bd	52 cd	32 cd	201 ab	700 bc	0.83 bd	0.29 ab	139 ac
LMA	226 cd	51 cd	31 cd	211 a	690 bc	0.76 cd	0.31 a	138 bd
MAG	228 cd	57 b	34 c	181 b	713 b	0.89 ac	0.25 b	139 ac
RAN	214 d	54 bc	37 b	211 a	681 c	0.87 ac	0.31 a	136 d
RCU	270 ab	50 d	31 d	194 ab	711 b	0.92 ab	0.27 ab	140 ab
Pollination								
Sibbed	242	54	33	180	718	0.84	0.25	140
Open	241	54	34	210	686	0.86	0.31	137
ANOVA								
Population	*	**	**	**	**	*	**	*
Year	ns	*	ns	**	**	**	**	**
Pollination	ns	ns	ns	**	**	ns	**	**
Population x pollination	ns	ns	ns	ns	ns	ns	ns	ns

[1] See text for population code. [2] LSmeans with different letters showed differences at $p < 0.05$

There was a high significant effect of the pollination system on oleic and linoleic concentration, their relationship and the iodine value (Table 2) as expected considering both parent influence. Given the general inverse relationship, sibbed seeds produced lower oleic acid and higher linoleic acid concentration than open pollinated seeds. The 15% gain for oleic acid from open pollination was insufficient to reach the maximum value observed in AAL. The cause of the increased in average oleic content from open pollination could be addressed in future studies.

In general, the fatty acid composition did not show values of interest with respect to those reported for improved mutant lines with altered fatty acid composition (Fernandez-Martinez et al., 2006). None of the Argentine accessions showed less than 39 and 26 g/kg of palmitic and stearic acid content, nor more than 300 g/kg of palmitic acid to be considered low or high in saturated acid content. None of the Argentine accessions showed oleic acid over 860 g/kg or linoleic concentration over 780 g/kg, similar to values of improved mutant lines.

The AAL accession had the highest oleic concentration, but was only different from MAG, RAN and DIA. Among Argentine germplasm, DIA showed the most variability in fatty acid composition, with higher palmitic, stearic, linoleic, linolenic, and iodine values and the lower oleic acid content. This population from Diamante represented a life cycle that is significantly longer than the other North America and Argentine accessions (unpublished data) and could constitute a unique germplasm of potential value.

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