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ISOZYME VARIABILITY IN *Helianthus argophyllus*. ITS APPLICATION IN CROSSES WITH CULTIVATED SUNFLOWER

Carrera A.D.*,[1] Poverene M.M.[1] and Rodríguez R.H.[2]

[1] *Department of Agronomy, Universidad Nacional del Sur and CERZOS-CONICET. 8000 Bahía Blanca, Argentina.*
[2] *EEA INTA Balcarce, 7620 Balcarce, Argentina*

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SUMMARY

Isozyme variation was studied in an *Helianthus argophyllus* population, two inbred lines of sunflower, *H. annuus*, an interspecific line and within progenies of crosses between those materials. Nine isozyme systems were resolved on starch gels. Fifteen loci were studied, 11 of them being polymorphic. Both species shared alleles at all loci; four alleles were present only in *H. argophyllus*. Intrapopulation variability in the wild species was analyzed and genetic distance among materials resembled their origin and genetic relationships. Isozyme markers allowed evaluation of uniformity within inbred lines and identification of hybrid seed among progenies of all crosses.

Key words: *Helianthus argophyllus*, *H. annuus*, sunflower, interspecific cross, isozyme analysis.

INTRODUCTION

Helianthus argophyllus T.& G. is an annual, diploid, open pollinated species, placed in section *Helianthus* of the genus, along with the cultivated sunflower, *H. annuus*. Methods of classification based upon morphological, biochemical and molecular data, suggest that *H. argophyllus* is the most closely related species to *H. annuus* within the section (Schilling and Heiser, 1981; Spring and Schilling, 1989; Rieseberg, 1991; Raymond et al., 1994). Wild *Helianthus* species offer opportunities to increase genetic variability in the domesticated sunflower through interspecific hybridization (Škorić, 1993). *H. argophyllus* has been recommended as the most likely source of drought resistance due to its pubescent leaves, low transpiration rate, powerful taproot, and its easy hybridization with *H. annuus* (Laferrière, 1986). New sources of fertility restoration genes have been

* Corresponding author

found in this wild species (Seiler and Jan, 1994) and it has been frequently used in sunflower breeding programs (Seiler, 1988). For example, Impira INTA, a very popular Argentinian open pollinated sunflower variety, originated from an interspecific cross between *H. argophyllus* and *H. annuus*, displaying resistance to *Puccinia helianthii* and drought tolerance (Bauer, 1991).

Conventional plant breeding procedures can be improved by using modern techniques as molecular and isozyme markers. Previous studies have shown a large amount of isozyme polymorphism in hybrids, inbred lines, open pollinated varieties, and in some wild species of *Helianthus* (Dry and Burdon, 1986; Rieseberg and Seiler, 1990; Carrera and Poverene, 1991, 1995; Tersac, 1994)

This paper deals with the determination of isozyme variability in an accession of *H. argophyllus* and its application to the analysis of interspecific crosses with *H. annuus*, and describes genetic markers which allow identification of the hybrids at seed stage.

MATERIALS AND METHODS

Plants

An accession of *Helianthus argophyllus*, two inbred lines of *H. annuus*, an interspecific line, and two hybrids were analyzed (Table 1). B611 is a mantainer line, while A580 is a cytoplasmic male-sterile line. The interspecific germplasm line designated ARG-1575-1 (Notice of Release, North Dakota Agricultural Experiment Station, 1989) was obtained from a cross between cmsHA89 and a *H. argophyllus* population collected in Florida, USA; progeny was then backcrossed twice to cmsHA89, sibmated and selfed. Standard sunflower breeding techniques were used to obtain F_1 seeds. Hybrid origin of seeds obtained from B611 plants was confirmed through a morphological genetic marker, having purple stem of seedlings.

Table 1: Genetic materials of *H. annuus* and *H. argophyllus* examined for isozyme variation

Designation	Description
B 611	Inbred line HA301 x HA302 / HA89 x HA290
A580	Inbred line HA301 x HA302 / HA89 x HA290
H. argophyllus	Accession PI 494571 (USA code)
ARG-1575-1	Interspecific *H. annuus - H. argophyllus* line
H1	F_1 (B611 x *H. argophyllus)*
H2	F_1 (A580 x ARG-1575-1)

Electrophoresis

Fifteen to twenty seeds from each lot of parent lines and F_1s were analyzed by starch gel electrophoresis. Seeds were soaked in water during 24 h at room temperature. The sample buffer was 0.1M tris-hydrochloric acid pH 7.5 with the addition of 25 µl of mercaptoethanol per 25 ml of buffer. One seed of *H. argophyllus* without its hull was crushed in 50 µl of sample buffer. A third part of an achene of the sunflower lines and F_1 progenies was crushed in 100 µl of the same buffer. The following enzymes were assayed: acid phosphatase (ACP), alcohol dehydrogenase (ADH), esterase (EST), glutamate dehydrogenase (GDH), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phospho-glucoisomerase (PGI), and 6-phosphogluconate dehydrogenase (PGD). Enzymes were resolved in 12% starch gel; the buffer systems and staining methods are described in Carrera and Poverene (1995) following the basic techniques by Soltis et al., (1983). Zymograms were interpreted according to the number of loci and alleles reported in Torres (1983), Kahler and Lay (1985), Rieseberg and Soltis (1989), and Carrera and Poverene (1991, 1995). Loci were designated sequentially, the most anodal migrating isozyme being 1, except for alcohol dehydrogenase which maintained the nomenclature given by Torres (1983). Allelic variants were identified by consecutive lower-case letters, from anode to cathode.

Data analysis

Standard measures of genetic variation, percentage of polymorphic loci (P), mean heterozygosity (H), and mean number of alleles per locus (A), were computed for *H. argophyllus*. Genetic distance values (Nei, 1978) were calculated for the parental populations using the computer program BIOSYS-1 (Swofford and Selander, 1989). A dendrogram based on Nei's genetic distance was constructed according to the UGPMA method.

RESULTS

Genetic variability

Nine enzymes and 15 loci were surveyed electrophoretically. Four loci, Adh-1, Pgi-1, Idh-1 and Idh-2, displayed bands of identical mobility and were considered monomorphic. The remaining 11 loci were polymorphic. Allele frequencies are given in Table 2. Population diversity in *H. argophyllus* was quantified giving the following values: A = 1.7; P = 66.7; and H = 0.209. Lines B611 and ARG-1575-1 showed constant zymograms for all loci, while A580 presented allelic variants for Est-1 and Mdh-1 loci.

Genetic differentiation

H. argophyllus and the cultivated sunflower shared alleles at all isozyme loci. From 18 alleles found in *H. annuus*, 17 were also present in *H. argophyllus*. Four alleles, Adh-2b, Gdh-1b, Lap-1d, and Lap-2b, that were found in *H. argophyllus*, were not previously described in any other strain from our sunflower collection, which comprises germplasm from institutional banks (INTA, National Institute of Agricultural Technology of Argentina and Universities) and from seed companies (Carrera and Poverene, 1991, 1995). Adh-2 is monomorphic for most cultivated sunflower genotypes (Torres, 1983; Carrera and Poverene, 1991, 1995). Cluster analysis based on Nei's genetic distance showed the lower distance values for the inbred lines B611 and A580 (Figure 1). These lines, along with ARG -1575-1 were clearly separated from *H. argophyllus*. Line ARG-1575-1 showed monomorphic zymograms resembling the most common sunflower patterns and was placed closer to cultivated sunflower than from its wild parent *H. argophyllus*. All the alleles present in ARG-1575-1 were also found in A580, except for Pgd-3a.

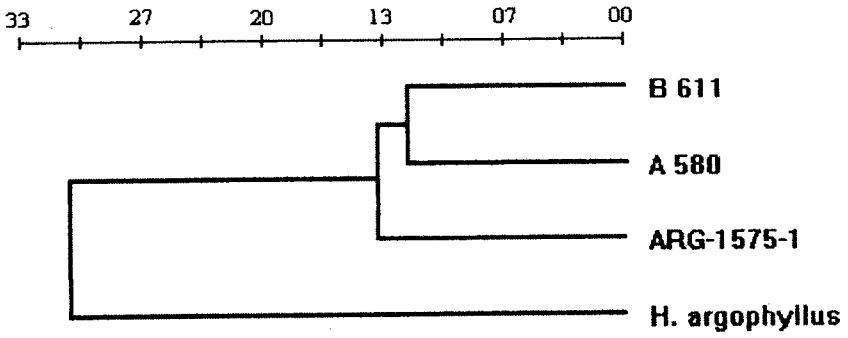

Figure 1. Dendrogram of isozyme diversity based on Nei distance and UPGMA method.

Interspecific crosses

Five loci, Acp-1, Est-1, Lap-1, Lap-2 and Pgi-2, were useful for the analysis of progenies from the cross B611 x *H. argophyllus* and allowed identification of F_1 hybrids. All F_1 seeds had the maternal allele but differed according to the allele received from the pollinator plant. Heterozygous zymograms showing alleles from both parents identified interspecific hybrid seeds. Homozygous individuals for alleles shared by the two parents could not be identified as hybrid seeds. Loci Adh-2, Gdh-1, Gdh-2, Mdh-3 and Pgd-3 showed two alleles each in *H. argophyllus*, but only one was found in F_1 progenies, the same allele present in the female parent *H. annuus* (Table 2, Figure 2).

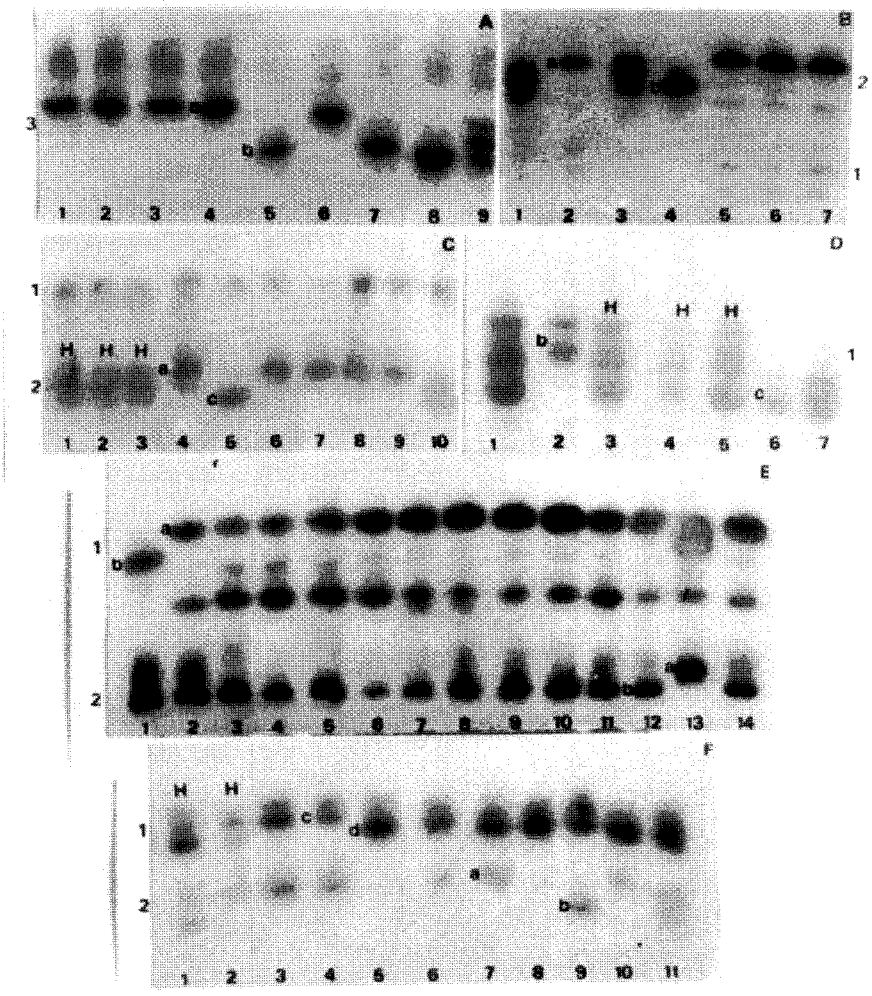

Figure 2 - Zymograms from the cross B611 x H. argophyllus. Loci are numbered in margins. H identifies F_1-hybrids.
A. PGD: lanes 1-2=B611, 3-4=F_1, 5-9= H. argophyllus.
B. ADH: lanes 1-4=H. argophyllus, 5-6=F_1, 7=B611.
C. PGI: lanes 1-4=F_1, 5-6,10= H. argophyllus, 7-9=B611.
D. EST: lanes 1-2= H. argophyllus, 3-6=F_1, 7=B611.
E. GDH: lanes 1-2,9-14= H. argophyllus, 3-5=B611, 6-8=F_1.
F. LAP: lanes 1-2=F_1, 3-4=B611, 5-11= H. argophyllus.

Table 2: Allele frequencies in 15 isozyme loci. Materials are designated as in Table 1 (arg= *H. argophyllus*). N= mean sample size per locus.

Locus		B611	H1	*arg*	A580	H2	ARG[1]	Locus		B611	H1	*arg*	A580	H2	ARG[1]
Acp-1	a	0.00	0.31	0.75	0.00	0.00	0.00	Lap-1	c	1.00	0.50	0.20	1.00	1.00	1.00
	d	1.00	0.69	0.25	1.00	1.00	1.00		d	0.00	0.50	0.80	0.00	0.00	0.00
Adh-1	b	1.00	1.00	1.00	1.00	1.00	1.00	Lap-2	a	1.00	0.77	0.67	1.00	1.00	1.00
Adh-2	a	1.00	1.00	0.34	1.00	1.00	1.00		b	0.00	0.23	0.33	0.00	0.00	0.00
	b	0.00	0.00	0.66	0.00	0.00	0.00	Mdh-1	a	1.00	1.00	1.00	0.20	0.00	0.00
Est-1	b	0.00	0.29	0.61	0.19	1.00	1.00		b	0.00	0.00	0.00	0.80	1.00	1.00
	c	1.00	0.71	0.39	0.81	0.00	0.00	Mdh-3	a	0.00	0.00	0.55	0.00	0.00	0.00
Gdh-1	a	1.00	1.00	0.88	1.00	1.00	1.00		b	1.00	1.00	0.45	1.00	1.00	1.00
	b	0.00	0.00	0.12	0.00	0.00	0.00	Pgd-3	a	1.00	1.00	0.76	0.00	0.50	0.50
Gdh-2	a	0.00	0.00	0.31	0.00	0.00	0.00		b	0.00	0.00	0.24	1.00	0.50	0.50
	b	1.00	1.00	0.69	1.00	1.00	1.00	Pgi-1	a	1.00	1.00	1.00	1.00	0.00	0.00
Idh-1	a	1.00	1.00	1.00	1.00	0.00	0.00	Pgi-2	a	1.00	0.87	0.40	1.00	1.00	1.00
Idh-2	a	1.00	1.00	1.00	1.00	1.00	1.00		c	0.00	0.13	0.60	0.00	0.00	0.00
N		15.6	15.8	21.3	16.0	15.7	15.8								

[1] ARG-1575-1

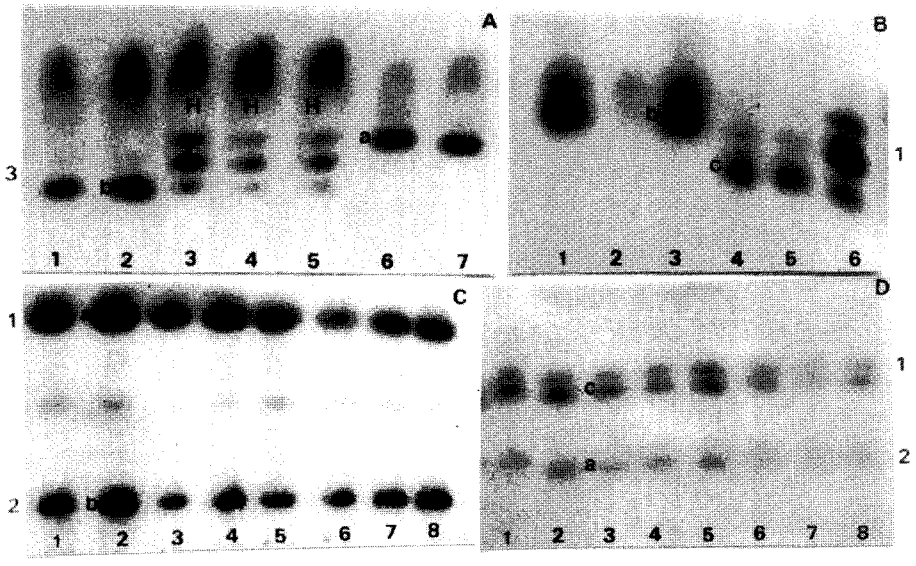

Figure 3 - Zymograms from the cross A580 x ARG-1575-1. Loci are numbered in margins. H identifies F_1 hybrids.
A. PGD: lanes 1-2=A580, 3-5=F_1, 6-7= ARG-1575-1.
B. EST: lane 1= ARG-1575-1, lanes 2-3=F_1, 4-6= A580.
C. GDH and D. LAP: lanes 1-2=A580, 3-5=F_1, 6-8= ARG-1575-1.

In the cross A580 x ARG-1575-1 only one locus, Pgd-3 was useful as genetic marker; all F_1 seeds showed an heterozygous pattern, confirming their hybrid origin. Alleles Est-1c and Mdh-1a, present in the maternal line 580, were not found in any F_1 seeds (Table 2, Figure 3).

DISCUSSION

Starch gel electrophoresis was an useful tool for the assessment of genetic variability in *H. argophyllus*, cultivated *H. annuus* and their crosses. Eleven out of 15 isozyme loci resulted polymorphic. The accession PI 494571 showed a considerable intrapopulation variation. Genetic diversity values were rather high when compared to previous reports on other *H. argophyllus* populations native to USA (Rieseberg, 1991), although a nonequivalent set of isoenzymatic loci was used in both studies.

The two inbred lines showed an isozyme uniformity, according to the high level of inbreeding of the analyzed genotypes. This technique allowed confirmation of genetic homogeneity of the inbred lines, except for A580, where two segregating loci were found. It was concluded that A580 has not reached uniformity yet, even after six selfing generations.

The high number of alleles shared by both annual species is probably the result of their close genetic relationship. Four unique alleles found in *H. argophyllus*, Adh-2b, Gdh-1b, Lap-1d, and Lap-2b, are specific genetic markers and would be useful in the analysis of interspecific crosses. The high similarity among B611 and A580 can be addressed to their genetic relationship, because they have been obtained through crossing and selection procedures from the same parent lines (Table 1). Isozyme uniformity in ARG-1575-1 shows that the backcrossing, sibmating, and selfing following the original interspecific cross caused a decrease of genetic variability. Moreover, the specific alleles of *H. argophyllus* were probably lost during the process of crossing and inbreeding. The short genetic distance between ARG-1575-1 and the two inbred lines, can be explained since line HA89, which was the recurrent parent for ARG-1575-1, is also one of the parents of B611 and A580.

The technique used here was very effective for the identification of F_1 seed of hybrid origin, albeit in cases where the parents share numerous alleles, as in A580 and ARG-1575-1. Lack in both F_1s of some alleles that were present in the parent populations was due to drift effects, because of the scanty number of plants selected as pollen donors (i.e., Adh-2, Table 2) or as female parents (i.e., Est-1, Table 2).

The observed isozyme variability was in accordance with the genetic origin and breeding management of the materials. Described polymorphic loci could be useful for the identification of interspecific F_1 seeds between *H. annuus x H. argophyllus*. The electrophoretic technique requires only a small quantity of coty-

ledon tissue; the remainder of the seed can give rise to a plant which could be scored by other criteria. Consequently, breeders engaged in a backcross program could use these alleles as genetic markers of agronomic traits.

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VARIABILIDAD ISOENZIMATICA EN *Helianthus argophyllus*. SU APLICACION EN CRUZAMIENTOS CON GIRASOL CULTIVADO

RESUMEN

Se estudió la variación isoenzimática en una población de *Helianthus argophyllus*, en dos líneas endocriadas de girasol cultivado, *H. annuus*, en una línea interespecífica y en la progenie de dos cruzamientos entre ellas. Nueve sistemas isoenzimáticos se revelaron en geles de almidón. Se estudiaron 15 loci, 11 de los cuales fueron polimórficos. Ambas especies compartieron alelos en todos los loci, aunque cuatro alelos se encontraron sólo en *H. argophyllus*. Se analizó la variabilidad intrapoblacional en la especie silvestre y la distancia genética entre los materiales reflejó su origen y relaciones de parentesco. Los marcadores isoenzimáticos permitieron evaluar la uniformidad de las líneas e identificar semilla híbrida en todos los cruzamientos.

VARIATION ISOENZIMATIQUE DANS *Helianthus argophyllus*. SON APPLICATION DANS CROISEMENTS AVEC LE TOURNESOL CULTIVÉ

RÉSUMÉ

La variation isoenzymatique dans une population de *Helianthus argophyllus*, dans deux lignées de tournesol, *H. annuus*, dans une lignée interspécifique et dans la descendance de croisements entre eux a été étudié. Neuf systèmes enzymatiques on été révélés sur gel d'amidon. Dans 15 loci étudiés, 11 se sont révélés polymorphiques. Les deux espèces ont partagé des allèles dans tous les loci et en plus on a identifié quatre allèles appartenant à *H. argophyllus*. Les distances génétiques entre les génotypes ont été d'accord avec son origine et ses relations de parenté. Les marqueurs enzymatiques ont permis d'évaluer l'uniformité des lignées et d'identifier les hybrides dans tous les croisements.