

## INTERSPECIFIC HYBRIDIZATION BETWEEN *H. annuus* L. AND *H. praecox* ssp. *hirtus* ENGLEMAN & GRAY

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### SUMMARY

Reciprocal interspecific crosses were made between sunflower (*H. annuus* L.,  $2n=34$ ) and the annual species *H. praecox* ssp. *hirtus* Engleman & Gray ( $2n=34$ ) using conventional breeding methods.  $F_1$ ,  $F_2$  and  $F_3$  hybrids and one backcross generation were obtained. The cross compatibility between *H. annuus* and *H. praecox* ssp. *hirtus* showed differences, depending on the direction of the cross. The manifestation of the parental characters in the hybrid material was studied, as well as the resistance of the  $F_1$ ,  $F_2$  and  $BC_1$  generations to *Plasmopara helianthi*, *Phomopsis helianthi* and *Orobanche cumana*. The seed oil content and fatty-acid composition of the oil in the hybrid progenies were also evaluated. Initial sunflower hybrid material with linoleic type of the seed oil and resistance to downy mildew and broomrape was produced for future breeding programs.

**Key words:** *Helianthus praecox* ssp. *hirtus*, *Helianthus annuus*, interspecific hybridization.

### INTRODUCTION

*Helianthus praecox* is an annual species in genus *Helianthus*, belonging to section *Annui* according to the classification of Shilling and Heiser (1981). There are three subspecies of *H. praecox*, which are geographically isolated. *Helianthus praecox* Engleman & Gray ssp. *hirtus* Heiser is rare and occurs only around Carizo Springs, Texas (Rogers, Thompson & Seiler, 1982). *Helianthus praecox* is adapted to a wide diversity of habitats and possesses considerable variability for most economic and agronomic characteristics (Seiler, 1988). As a wild species, *H. praecox* differs greatly from cultivated sunflower, as far as its qualitative and quantitative characteristics are concerned.

This paper reports the results of the hybridization between *H. praecox* ssp. *hirtus* and cultivated sunflower and the genetic variability of the interspecific hybrids.

## MATERIALS AND METHODS

The investigation was carried out from 1993 to 1996.

***Helianthus praecox* ssp. *hirtus* Engleman & Gray** - Seeds of *H. praecox* ssp. *hirtus*, originating from two different accessions - GT-E-027 and GT-E-148, registered and maintained in the collection of wild *Helianthus* species at IWS "Dobroudja", were sown in the greenhouse and were planted in the field at the two leaf stage. Inflorescence were isolated and emasculated manually and pollinated with pollen from *H. annuus*.

***Helianthus annuus* L.** - Six inbred lines (1607, 2607, 1234, 3064, HA-300 and HA-821) and variety Peredovik were included in the investigation. Both A and B lines were used. The plants of the B lines were treated with 0.003% solution of gibberellic acid at the beginning of the button stage. The inflorescence were isolated prior to anthesis and pollinated with pollen from both accessions of *H. praecox* ssp. *hirtus*. Conventional breeding methods were used. Reciprocal crosses were made. F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> generation hybrids and BC<sub>1</sub> generations were obtained.

Phenotypic, phenologic and reproductive traits were used as criteria for defining the "hybrid status" of the crosses. Self pollination, sib pollination and open pollination were performed for obtaining progenies. The morphology of the F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub> and F<sub>3</sub> plants was observed for 18 characters. The most important of them were: plant height; head diameter; leaf length; leaf width; number of I class branches and length of the longest branch (Table 2). The others were: petiole length, stem thickness, head thickness, number of bract leaves, length and width of bract leaves, number of ray florets, length and width of ray florets, seed size, colour of the stigma and seed set. Seed set was determined according to the formula:

$$\text{Seed set} = \frac{\text{Number of filled seeds per 1 head}}{\text{Total number of disk florets per 1 head}} \times 100 (\%)$$

Seed oil content was determined according to Rushkovski's method and the fatty acid composition, using the gas chromatography method (Ivanov & Nikolova, 1994). The inoculation for *Phomopsis* resistance was made in button stage to the beginning of flowering stage according to the method of Tourvielle *et al.* (1988). Twenty to thirty plants were inoculated in an artificial infection plot. The evaluation for resistance to the pathogen was carried out 14 days after the inoculation using a rating scale (Csengeri, 1990): 0 - healthy plants; 1 - a spot on the stem of the sunflower plant, limited to the petiole; 2 - a spot with 5 cm size; 3 - an annular spot, surrounding the stem of the plant; 4 - broken and withered plants. Evaluation of the material for resistance to downy mildew and broomrape was done according to the methods of Panchenko (1965, 1975). Twenty plants were tested for mildew resistance and thirty plants - for broomrape resistance in the greenhouse.

Data were analyzed by a combined analysis, named Descriptive Statistics and t-Test, using MS Excel software.

RESULTS AND DISCUSSION

**Cross Compatibility**

The results of hybridization between *H. annuus* and *H. praecox* ssp. *hirtus* are presented in Table 1. Both accessions of *H. praecox* - E-027 and E-148 hybridized successfully with cultivated sunflower in both directions, resulting in 43 F<sub>1</sub> (*H. praecox* x *H. annuus*) and 194 F<sub>1</sub> (*H. annuus* x *H. praecox*) plants altogether for the two years of investigation. Hybrid plants were produced in first, second and third generations. When the wild species was used as female parent, the cross compatibility was slightly lower - 60.65 % (averaged over two years), but significantly different at P (0.01 level of probability from the cross compatibility value of the reciprocal crosses (63.39 %). A conclusion could be made that the wild species hybridizes easier with *H. annuus* when it is the pollinator in the crosses. Nevertheless, we noticed a good cross compatibility between *H. praecox* ssp. *hirtus* and *H. annuus*, though the data varied over the years.

Table 1: Cross compatibility between *H.praecox* ssp. *hirtus* (E-027 and E-148) and *H.annuus*

Hybrid combination	Inflorescence pollinated			Seeds produced			Hybrid plants	
	Total Nr.	With seed set Nr.	%	Total Nr.	Aver. Nr. per head	Seed set %	Nr.	% according to seed obtained
<b>1994</b>								
E-027 x H.an.	40	29	72.5	215	7.41	4.84	22	10.23
E-148 x H.an.	40	34	85.0	541	15.91	8.29	21	3.88
H.an. x E-027	24	14	58.3	330	23.57	2.17	49	14.85
H.an. x E-148	24	17	70.8	704	41.41	2.54	70	9.94
<b>1995</b>								
E-027 x H.an.	31	7	22.6	43	6.14	4.01	0	0
E-148 x H.an.	33	17	51.5	53	3.12	1.62	0	0
H.an. x E-027	20	14	70.0	665	47.5	2.4	41	11.28
H.an. x E-148	23	15	65.2	294	19.6	1.03	34	11.56
<b>Average for 1994 and 1995</b>								
H.pr. x H.an.			60.7 ± 5.29			4.69		3.53
H.an. x H.pr.			63.4** ± 6.02			2.04		13.34

t stat = 3.15 > t crit. = 2.05 at P ≤ 0.01

The seed set also varied with years and cross combinations, but averaged over the two years it was higher in the crosses of wild species x cultivated sunflower - 4.69 % and lower in the reciprocal crosses - 2.04 %. The putative hybrid seed viability was very low in the crosses of *H. praecox* x *H. annuus* - 3.53 % and higher in the reciprocal crosses - 13.34 %.

**Hybridization**

First generation hybrids were produced in almost all cross combinations. Morphological data about the reciprocal F<sub>1</sub>'s are presented in Table 2. The phenotype of

Table 2: Morphology of some characters of F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub> and BC<sub>1</sub> plants

Material	Plant height (cm)		Head diameter (cm)		Leaf length (cm)		Leaf width (cm)		Nr. of pr. branches		Length of the longest branch (cm)	
	M ± x σ / Range	M ± x σ / Range	M ± x σ / Range	M ± x σ / Range	M ± x σ / Range	M ± x σ / Range	M ± x σ / Range	M ± x σ / Range	M ± x σ / Range	M ± x σ / Range	M ± x σ / Range	
F <sub>1</sub>	H. an. x E-027	171.1 ± 14.86 51.48/108 - 245	9.7 ± 1.08 3.74/6 - 19	16.7 ± 1.87 6.49/9 - 26	15.5 ± 2.36 8.19/5 - 27	13.7 ± 1.78 6.15/5 - 25	97.2 ± 12.10 41.92/30 - 146					
	H. an. x E-148	166.8 ± 9.46 39.0/120 - 235	8.74 ± 0.73 2.99/5 - 15	17.4 ± 1.78 7.35/8 - 37	15.1 ± 1.67 6.87/5 - 30	11.29 ± 1.26 5.18/4 - 22	98.0 ± 10.72 44.18/36 - 150					
F <sub>2</sub>	E-027 x H. an.	110.0 ± 3.51 11.64/90 - 126	4.9 ± 0.31 1.04/3 - 6.5	18.6 ± 1.75 5.80/9 - 27	18.4 ± 1.88 6.23/8 - 27	13 ± 0.38 1.26/11 - 15	145.7 ± 4.73 15.70/126 - 181					
	E-148 x H. an.	111.0 ± 4.80 17.31/76 - 135	4.3 ± 0.24 0.86/2.1 - 5.5	22.6 ± 0.96 3.46/17.5 - 28	22.3 ± 0.93 3.35/15.5 - 27	8.6 ± 0.67 2.43/3 - 12	130.8 ± 4.23 15.26/102 - 148					
F <sub>3</sub>	H. an. x E-027	178.0 ± 8.54 41.82/75 - 290	13.1 ± 0.73 3.58/7 - 23	16.4 ± 0.95 4.65/6 - 24	16.2 ± 0.96 4.72/5 - 24	9.4 ± 1.84 8.44/1 - 31	53.2 ± 11.61 50.60/6 - 180					
	H. an. x E-148	158.8 ± 10.41 46.56/70 - 220	11.3 ± 0.79 3.55/5 - 18	13.9 ± 1.02 4.58/5 - 22	12.9 ± 1.10 4.93/5 - 20	8.3 ± 0.89 3.99/1 - 15	72.3 ± 12.17 54.45/7 - 182					
BC <sub>1</sub>	E-148 x H. an.	122.5 ± 2.50 3.54/120 - 125	8.5 ± 1.5 2.12/7 - 10	15.5 ± 0.50 0.71/15 - 16	9.5 ± 0.50 0.71/9 - 10	29.0 ± 1.0 1.41/28 - 30	102.5 ± 17.50 24.75/85 - 120					
	H. an. x E-027	163.8 ± 7.70 37.74/110 - 225	12.0 ± 0.64 3.13/6 - 19	16.8 ± 1.22 5.98/9 - 40	17.6 ± 1.56 7.62/10 - 44	9.3 ± 1.15 5.65/1 - 19	68.7 ± 7.72 37.80/17 - 156					
P	H. an. x E-148	128.9 ± 11.21 41.93/65 - 200	10.0 ± 0.57 2.15/7 - 15	17.4 ± 1.53 5.72/8 - 27	16.5 ± 1.80 6.73/7 - 28	8.4 ± 1.48 5.56/1 - 21	80.4 ± 9.07 33.93/38 - 156					
	E-027 x H. an.	175.8 ± 21.35 52.29/140 - 280	12.3 ± 1.52 3.72/8 - 18	21.8 ± 2.90 7.11/15 - 34	26.0 ± 3.47 8.51/17 - 42	24.0 ± 2.0 4.89/16 - 30	86.0 ± 15.90 38.94/33 - 135					
P	H. an. x E-027) x H. an.	167.5 ± 36.31 72.63/105 - 265	9.5 ± 2.36 4.73/6 - 16	15.0 ± 0.82 1.63/13 - 17	10.5 ± 2.10 4.20/6 - 16	11.0 ± 1.29 2.58/8 - 14	179.3 ± 16.19 32.38/131 - 200					
	(H. an. x E-148) x H. an.	112.5 ± 12.50 17.68/100 - 125	9.5 ± 2.50 3.54/7 - 12	17.0 ± 5.0 7.07/12 - 22	14.5 ± 6.50 9.19/8 - 21	10.0 ± 0 0/10 - 10	158.0 ± 24.0 33.94/134 - 182					
P	E-027	137.7	2.5	6.3	5.4	12	130.0					
	E-148	112.0	2.5	6.8	5.1	11	112.0					
P	inbred 2607	119.0	17.4	20.4	19.8	0	0					
	inbred 1234	113.0	19.6	28.4	24.8	0	0					

M = Mean; x = Standard Error; σ = Standard Deviation; Range = Min - Max.

the  $F_1$  plants was intermediate with many features common to the wild parent: the branched type with at least I and II classes of branches, some of them longer than the stem, the leaf blade, the anthocyanin coloration of the stem, petioles and disk florets. A very typical trait of the wild species was the long peduncle of the hybrid plants. A large variation could be observed even in this generation for plant height, the length of the longest branch and other traits.

A great diversity of phenotypes was produced in the  $F_2$ ,  $F_3$  and  $BC_1$  generations. The plant height differed greatly and varied from 65 to 290 cm in the different progenies (Table 2). Most of the plants were densely branched (1 - 31 branches per plant) with varying types of branching; with head diameter from 5 to 23 cm. Some of them had anthocyanin colored inflorescence, some had yellow disk florets.

Differences could also be observed in flowering and vegetation period. Both accessions of *H. praecox* ssp. *hirtus* reach an early maturity and this influenced the vegetation period of the hybrids, with plants from the different hybrid generations varied according to this character.

#### Oil content and fatty acid composition

Data about the seed oil content and fatty acid composition of some  $F_2$ ' are presented in Table 3. The seed oil content of the two accessions of *H. praecox* ssp. *hirtus* is generally low. It was a little higher in the  $F_2$  material, compared with accession E-148, but much lower than that of cultivated sunflower. Most of the hybrids had good linoleic acid contents and higher palmitic acid contents, compared with cultivated sunflower's fatty acid composition and will be selected in future breeding programs.

Table 3: Oil content and fatty acid composition of the oil of  $F_2$  material

Material	Oil in the kernel %	Linoleic acid %	Oleic acid %	Stearic acid %	Palmitic acid %
Hybrid material					
E-027 x 1234	51.6	66.2	22.4	4.2	7.2
E-148 x 1607	52.4	64.2	23.7	4.3	7.9
1607A x E-027	53.2	63.5	24.4	5.6	6.5
1607A x E-148	50.5	62.3	23.8	5.7	8.2
1234A x E-148	50.2	62.6	26.2	6.0	5.2
3064A x E-027	48.1	64.9	22.3	4.9	7.9
$M \pm x$	$51 \pm 0.74$	$63.9 \pm 0.59$	$23.8 \pm 0.59$	$5.1 \pm 0.31$	$7.15 \pm 0.46$
$\sigma$	1.81	1.47	1.44	0.76	1.14
<i>Helianthus praecox</i> ssp. <i>hirtus</i>					
E-027	53.5	54.9	27.5	5.9	11.7
E-148	41.6	58.8	25.4	5.8	10.0
<i>Helianthus annuus</i>					
var. Peredovik	60.4	62.7	27.6	4.7	5.0

### Disease resistance

The results from the testing of the F<sub>2</sub> and F<sub>3</sub> material for resistance to mildew, broomrape and *Phomopsis* are included in Table 4. Resistance to *Phomopsis helianthi* was observed in both accessions of *H. praecox* ssp. *hirtus* and in the F<sub>2</sub> and F<sub>3</sub> progenies of two different hybrid combinations of accession E-148: 1607 A x E-148 and 2607 A x E-148. The second one was also partially resistant to *Orobanche cumana* (50 %). Partial resistance to both diseases, *Plasmopara helianthi* and *Orobanche cumana* was observed in few combinations: 1607 A x E-027; 1607 A x E-148 and 3064 A x E-148, ranging from 21.1 to 46.4 % in mildew resistance and from 10 to 60 % in broomrape resistance. Though 100% resistance to *Plasmopara helianthi* and *Orobanche cumana*, was not observed in the hybrid material, the results of the F<sub>2</sub> and F<sub>3</sub> material showed that a further selection would be possible. Resistant plants could eventually be selected, for resistance to mildew and broomrape is controlled by dominant genes (Christov *et al.*, 1992).

Table 4: Resistance to *Plasmopara helianthi*, *Orobanche cumana* and *Phomopsis helianthi*, found in F<sub>2</sub> and F<sub>3</sub> material

Origin	Generation	<i>Plasmopara helianthi</i> %	<i>Orobanche cumana</i> %	<i>Phomopsis helianthi</i> 0 - 4 Scale
1607A x E-027	F <sub>2</sub> / 1995	40.0	10.0	4
1607A x E-148	F <sub>2</sub> / 1995	46.4	12.5	4
1607A x E-148	F <sub>2</sub> / 1996	0	0	0
2607A x E-148	F <sub>3</sub> / 1997	0	50.0	0
3064A x E-148	F <sub>2</sub> / 1997	21.1	60.0	4
<i>Helianthus praecox</i> ssp. <i>hirtus</i>				
E-027	parent	85	57.1	0
E-148	parent	70	-	0
<i>Helianthus annuus</i>				
<i>H.annuus</i>	parent	0	0	4

### CONCLUSION

*Helianthus praecox* ssp. *hirtus*, accessions GT-E-027 and GT-E-148 showed a good crossability with cultivated sunflower in both directions of hybridization, but better results were obtained from the crosses *H. annuus* x *H. praecox* ssp. *hirtus*. The F<sub>1</sub> hybrid plants had intermediary phenotype. There was a great diversity of phenotypes in F<sub>2</sub>, F<sub>3</sub> and BC<sub>1</sub> material. Most of the hybrid plants had adequate linoleic contents of the oil. Partial resistance to mildew and broomrape and 100 % resistance to *Phomopsis* were observed in some phenotypes.

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**MATERIAL VEGETAL INICIAL OBTENIDO DEL CRUCE INTERESPECÍFICO ENTRE *H. annuus* L. Y *H. praecox* ssp. *hirtus* ENGLEMAN Y GRAY**

## RESUMEN

Se hicieron cruces interespecíficos entre girasol *H. annuus* ( $2n=34$ ) y la especie anual *H. praecox* ssp. *hirtus* Engleman y Gray ( $2n=34$ ). Se utilizaron métodos convencionales. El híbrido  $F_1$  y las generaciones  $F_2$  y retrocruces ( $BC_1F_1$ ) fueron obtenidos. La compatibilidad de cruce entre *H. annuus* y *H. praecox* ssp. *hirtus* fué investigada, mostrando diferencias dependiendo de la dirección del cruce.

La manifestación de los caracteres en el material híbrido fue estudiada, así como la resistencia de la  $F_1$ ,  $F_2$  y  $BC_1F_1$  para *Plasmopara helianthi*, *Phomopsis helianthi* y *Orobancha cumana*. El contenido de aceite y la composición de ácidos grasos del aceite en las descendencias del híbrido fueron evaluadas. El material híbrido inicial con tipo linoléico en el aceite y resistencia a mildiu y jopo fué producido para ser incluido en futuros programas de mejora.

**PREMIÈRES GÉNÉRATIONS DE TOURNESOL ISSU  
D'HYBRIDATION INTERSPÉCIFIQUE ENTRE *H. annuus* L.  
ET *H. praecox* ssp. *hirtus* ENGLEMAN & GRAY**

RÉSUMÉ

Des croisements interspécifiques ont été réalisés entre le tournesol (*H. annuus* L.,  $2n=34$ ) et l'espèce annuelle *H. praecox hirtus* Engleman & Gray ( $2n=34$ ). Les méthodes conventionnelles de sélection ont été utilisées. Les hybrides de première ( $F_1$ ) et de deuxième génération ( $F_2$ ) et le premier back-cross ont été obtenus. L'étude de la compatibilité de croisement entre *H. annuus* et *H. praecox hirtus*, a montré des différences liées au sens de l'hybridation.

L'expression des caractéristiques parentales dans le matériel hybride a été étudiée, de même que la résistance des générations  $F_1$ ,  $F_2$  et  $BC_1$  au *Plasmopara helianthi*, au *Phomopsis helianthi* et à l'*Orobanche cumana*. La teneur en huile et la composition en acides gras de l'huile dans les descendance d'hybrides a été évaluée.

Du matériel hybride de départ, à graines de type linoléique et résistant au mildiou et à l'orobanche a été produit pour contribuer aux programmes de sélection.