

INTERGENERIC HYBRIDS IN *Compositae* (*Asteraceae*), I. HYBRIDIZATION BETWEEN CULTIVATED SUNFLOWER *H. annuus* L. AND *Compositae* GENERA

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SUMMARY

Intergeneric hybridization between the cultivated sunflower *H. annuus* L. and 19 related genera of *Compositae* (*Asteraceae*) was investigated. Putative hybrids were obtained from twelve cross combinations. The general cross compatibility level and morphological characteristics of F₁s were examined. In spite of the genetic distance between *H. annuus* and the wild species of genera from the *Compositae*, intergeneric hybridization appears promising as a breeding method for transferring wild germplasm for agronomic purposes. The data presented provide knowledge about the genetic properties of the species and will be useful for future improvement of the cultivated sunflower.

Key words: *Compositae, Helianthus, Heliantheae, Silphium, Matricaria, Cichorium, Chrysanthemum, Gailardia, Telekia, Inula, Carduus, Cosmos, Grindelia, Arctium, Onopordum, Verbesina, Simsia, Tithonia, Aster, Bidens, Cirsium, Calendula, breeding, sunflower intergeneric hybrids*

INTRODUCTION

The genus *Helianthus* has been used extensively for interspecific hybridization by several investigators (Heiser, 1951; Jackson *et al.*, 1956; Thompson *et al.*, 1981; Georgieva-Todorova, 1984). The genetic diversity of the cultivated sunflower is rather limited because of the small number of germplasms used by breeders, and because of the use of the cytoplasmic male sterility (CMS) hybrid breeding system for seed production. Breeding efforts are needed to broaden the genetic variability of cultivated sunflower. Attempts were made to cross *Helianthus* species with closely related genera of *Tithonia* and *Viguera* (Heiser *et al.*, 1969) and species

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from genera distributed in Eastern and Central Europe - *Onopordum acanthium* and *Carthamus tinctorius* (Morosov, 1947; Georgieva-Todorova, 1971). There are few reports of intergeneric hybridization between the cultivated sunflower *Helianthus annuus* L. and species of related genera of *Compositae*.

The use of wild species or genera as parental material is not easy because of the inherent problems such as lack of cross-compatibility, sterility of hybrids, production of non-germinating seeds with undeveloped endosperm, and the linkage of desirable and undesirable traits (D. Kostoff, 1941-1943). The transfer of useful agronomic traits by means of intergeneric hybrids usually involves complex and time-consuming techniques but the potential exists for transferring traits such as specific disease or pest resistance, tolerance to environmental extremes such as salinity, cold and drought, quality characters such as protein fractions and altered levels of amino acids (Table 1).

Table 1: Potential agronomic traits identified in wild species of *Compositae* genera

Species	Trait	Reference
<i>Tithonia rotundifolia</i>	Resistant to downy mildew (<i>Plasmopara halstedii</i>) Tolerant to <i>Phomopsis helianthi</i> <i>Rf</i> -genes	Shindrova, P. and Encheva, V. (unpublished data) Christov, M. and Panajotov, I., 1991
<i>Verbesina helianthoides</i>	Resistant to downy mildew <i>Rf</i> -genes	Christov, M. (unpublished data)
<i>Verbesina alata</i>	<i>Rf</i> -genes	
<i>Verbesina encelioides</i>	<i>Rf</i> -genes	
<i>Telekia speciosa</i>	Resistant to downy mildew <i>Rf</i> -genes	Christov, M. (unpublished data)
<i>Bidens tripartita</i> <i>Arctium lappa</i> <i>Cirsium lanceolatum</i>	High lysine content	Ivanov, P. et al., 1994
<i>Grindelia speciosa</i> <i>Onopordum acanthium</i> <i>Carduus acanthoides</i> <i>Inula helenium</i>	<i>Rf</i> -genes	Christov, M. (unpublished data)
<i>Arctium lappa</i>	Tocopherol (as a source of natural antioxidants)	Ivanov, St. and Aitzetmuller, K., 1994

The species crosses within the tribe *Heliantheae* need to be studied for chromosome affinity between species and within genera, from which phylogenetic affinities and the genetic control of chromosomes can be determined. A research programme of incorporating alien genes into the sunflower genome by intergeneric crossings started in 1985, at the Institute of Wheat and Sunflower "Dobroudja". This paper presents preliminary data on the hybridization between the cultivated sunflower and some species of *Compositae* genera.

MATERIALS AND METHODS

Variety Peredovik was maintained through sib-pollination and five pure lines were used as female plants: HA-89, 3004, 1607, 1721 and 6054. In order to obtain crosses between populations of the male parents and sunflower, the cytoplasmic male sterile form of the female plants was used. The pure lines HA-89, 3004, 1607 and 1721 were crossed onto CMS PET-1 (Leclerq, 1969) or ARG-3 cytoplasm (Christov, 1990). Chemical castration with gibberellic acid (GA3) was performed in order to obtain cytoplasmic male sterile plants.

The florets of the female inflorescences of variety Peredovik were emasculated. Stigmas of the emasculated and male sterile inflorescences were pollinated three times with fresh pollen. Chromosome number and ploidy level of pollinators are shown in Table 2.

Table 2: Chromosome number of male parents used for crossing

Plant	2n
<i>Helianthus annuus</i> L.	34
<i>Silphium perfoliatum</i> L.	14
<i>Matricaria chamomilla</i> L.	18
<i>Cichorium intybus</i> L.	18
<i>Chrysanthemum leucanthemum</i> L.	18
<i>Telekia speciosa</i> (Schreb.) Baumg.	20
<i>Inula helenium</i> L.	20
<i>Carduus acanthoides</i> L.	22
<i>Cosmos bipinnatus</i> Cav.	24
<i>Grindelia speciosa</i> Willd.	24
<i>Calendula officinalis</i> L.	28
<i>Arctium lappa</i> L.	32
<i>Gaillardia speciosa</i> (hybrida) Fouger.	34
<i>Onopordum acanthium</i> L.	34
<i>Verbesina alata</i> L.	34
<i>Verbesina helianthoides</i> Michaux	34
<i>Verbesina encelioides</i> Benth et Hook	34
<i>Simsia foetida</i> Cav. (Blake)	34
<i>Tithonia rotundifolia</i> Blake	34
<i>Bidens tripartita</i> L.	48
<i>Cirsium lanceolatum</i> Scop.	68
<i>Aster speciosa</i> Tourn.	72

Hybrid seeds were sown in the greenhouse after maturity. At the two-leaf stage, the plants were planted in the field. Some of the plants were self-pollinated, and others open pollinated. Seeds from second and third hybrid generations were produced after selfing.

Standard methods were used to test fertility, morphological characters and cytological observations. Statistical parameters of mean value (\bar{x}), variation coefficient (cv), student t-test (t) and an indicator of inheritance (d/a), where:

$$d = F_1 - MP,$$

$$a = (P_1 - P_2)/2,$$

$$MP = (P_1 + P_2)/2$$

{ P_1 -mean value of female parent;

P_2 -mean value of male parent;

F_1 -mean value of hybrids}

(Mather, 1949) were determined.

RESULTS AND DISCUSSION

The cross compatibility level of the species within the tribe *Helianteae* with the cultivated sunflower is given in Table 3. Crosses between *H. annuus* and species from twelve genera were successful, i.e., hybrid plants reached maturity.

Table 3: Crossability between *H. annuus* and some species of *Compositae* genera

Hybrid combination	Pollinated inflorescences with seeds				Hybrid plants	
	total number	number	% heads with seeds	number of seeds	total number	% plant obtained
HA-89 ms PET-1 x <i>Silphium perfoliatum</i>	9	1	11.1	249	22	8.8
HA-89 ms ARG-3 x <i>Silphium perfoliatum</i>	1	1	100.0	13	4	30.8
HA-89 ms PET-1 x <i>Matricaria chamomilla</i>	16	1	6.2	73	17	42.5
L 3004 ms PET-1 x <i>Matricaria chamomilla</i>	11	1	11.0	1	0	0.0
L 1721 ms ARG-3 x <i>Cichorium intybus</i>	9	1	11.0	1	0	0.0
HA-821 ms ARG-3 x <i>Chrysanthemum leucanthemum</i>	11	1	9.0	2	0	0.0
L 6054 GA3 ms x <i>Gaillardia speciosa</i>	2	1	50.0	3	3	100.0
L 3004 ms PET-1 x <i>Gaillardia speciosa</i>	2	1	50.0	3	3	100.
L 2418 ms PET-1 x <i>Gaillardia speciosa</i>	1	1	100.0	16	14	87.5
L 1036 ms PET-1 x <i>Gaillardia speciosa</i>	2	1	50.0	6	5	83.3
HA-89 ms ARG-3 x <i>Telekia speciosa</i>	24	5	20.8	171	44	25.7
L 6054 GA3 ms x <i>Telekia speciosa</i>	2	1	50.0	12	7	33.3
L 1607 ms PET-1 x <i>Telekia speciosa</i>	4	0	0.0	0	0	0.0
L 3004 ms ARG-3 x <i>Carduus acanthoides</i>	6	2	33.3	3	2	66.7
L 3004 ms PET-1 x <i>Inula helenium</i>	14	0	0	0	0	0
HA-89 ms ARG-1 x <i>Carduus acanthoides</i>	12	2	6.7	293	46	15.7
L 1607 ms PET-1 x <i>Carduus acanthoides</i>	4	0	0.0	0	0	0.0
L 3004 GA3 ms x <i>Carduus acanthoides</i>	6	2	0.0	3	2	0.0
L 3004 ms PET-1 x <i>Cosmos bipinnatus</i>	6	0	0.0	0	0	0.0
HA 89 ms PET-1 x <i>Cosmos bipinnatus</i>	11	0	0.0	0	0	0.0
L 1721 ms PET-1 x <i>Cosmos bipinnatus</i>	4	0	0.0	0	0	0.0
L 1721 ms ARG-3 x <i>Cosmos bipinnatus</i>	6	1	16.6	1	0	0.0
HA-89 ms ARG-3 x <i>Grindelia speciosa</i>	21	1	4.8	16	2	12.5
L 3004 ms PET-1 x <i>Arctium lappa</i>	4	0	0.0	0	0	0.0
HA-89 ms PET-1 x <i>Arctium lappa</i>	2	0	0.0	0	0	0.0
L 3969 ms PET-1 x <i>Arctium lappa</i>	2	0	0.0	0	0	0.0
L 3004 ms PET-1 x <i>Onopordum acanthium</i>	20	0	0.0	0	0	0.0
L 3004 ms ARG-3 x <i>Onopordum acanthium</i>	4	1	25.0	59	23	39.0
L 1721 ms PET-1 x <i>Onopordum acanthium</i>	2	0	0.0	0	0	0.0
L 3004 ms ARG-3 x <i>Verbesina helianthoides</i>	5	1	20.0	41	21	51.2
L6054 ms GA3 x <i>Verbesina alata</i>	2	1	50.00	5	4	80.0
L 3004 ms PET-1 x <i>Verbesina alata</i>	2	0	0	0	0	0.0
HA-89 ms ARG-3 x <i>Verbesina encelioides</i>	2	0	0	0	0	0.0
L 2164 emasc. x <i>Simsia foetida</i>	7	2	28.58	37	0	0.0
HA-89 ms ARG-3 x <i>Tithonia rotundifolia</i>	2	1	50.0	19	19	100.0
HA per emasc. x <i>Tithonia rotundifolia</i>	2	2	100.0	83	21	25.3
L 1721 emasc. x <i>Tithonia rotundifolia</i>	1	1	100.0	56	28	50.0
L 2235 ms PET-1 x <i>Aster spec.</i>	4	1	25.0	2	2	100.0
HA cv. Per. emasc. x <i>Bidens tripartita</i>	2	1	50.0	13	6	46.2
L 1721 emasc. x <i>Bidens tripartita</i>	1	1	100.0	68	31	45.6
L 3004 ms PET-1 x <i>Cirsium lanceolatum</i>	3	1	33.3	5	0	0.0
L 3004 ms PET-1 x <i>Calendula officinalis</i>	2	0	0.0	0	0	0.0
L 3004 ms ARG-3 x <i>Calendula officinalis</i>	2	0	0.0	0	0	0.0
HA cv. Per. emasc. x <i>Calendula officinalis</i>	4	0	0.0	0	0	0.0

Table 4: Comparative morphology of putative hybrids and parents
a) plant height

Parents and hybrids	Plant height				
	x	t		cv	d/a
HA-89 ms PET-1	100.7			3.33	
<i>Silphium perfoliatum</i>	205.6	39.87***		2.00	
F ₁	85.7	1.75	20.12***	14.86	-1.29
HA-89 ms PET-1	100.7			3.33	
<i>Matricaria chamomilla</i>	24.3	28.00***		18.26	
F ₁	88.82	3.33**	27.22***	13.13	0.68
HA-89 ms ARG-3	100.7			3.33	
<i>Telekia speciosa</i>	84.6	10.22***		4.92	
F ₁	82.6	10.25***	25.68***	8.73	-1.25
L 6054	127.7			11.93	
<i>Telekia speciosa</i>	84.6	19.59***		4.92	
F ₁	97.8	35.95***	4.75***	4.99	-0.39
HA-89 ARG-1	100.7				
<i>Carduus acanthoides</i>	120.9			12.57	
F ₁	99.2	0.0	2.68*	14.51	-1.15
L 3004 ARG-3	114.0			8.37	
<i>Carduus acanthoides</i>	120.9	0.77		12.57	
F ₁	92.5	0.0	0.0		-2.45
HA-89 ARG-3	100.7			3.33	
<i>Grindelia speciosa</i>	61.50	5.02***		25.68	
F ₁	80.0	0.0	0.0		-0.06
L 6054 GA3 ms	127.7			11.93	
<i>Gaillardia speciosa</i>	45.3	92.58***		8.54	
F ₁	93.3	31.82***	27.27***	3.09	0.17
HA-89 ms PET-1	100.7			3.33	
<i>Gaillardia speciosa</i>	45.3	34.68***		8.54	
F ₁	171.7	31.80***	56.16***	4.45	3.56
L 3004 ms ARG-3	114.0			8.37	
<i>Onopordum acanthoides</i>	148.8	3.28***		12.67	
F ₁	91.13	3.93***	4.74***	19.34	-2.31
L 3004 ms ARG-3	114.0			8.37	
<i>Verbesina helianthoides</i>	106.2	2.02		3.63	
F ₁	110.2	0.56	0.64	19.16	-0.14
HA-89 ARG-3	100.7			3.33	
<i>Tithonia rotundifolia</i>	133.1	9.86***		9.49	
F ₁	169.5	64.28***	12.78***	6.04	3.25
HA cv. Per. emasc.	182.7			8.07	
<i>Bidens tripartita</i>	127.4	3.93***		18.87	
F ₁	113.3	5.09***	1.27	22.26	-1.51
L 2235 ms PET-1	145.2			2.34	
<i>Aster speciosa</i>	106.2	34.51***		3.73	
F ₁	117.5	14.97***	4.91***	3.01	-0.42

t = Student's t-test; * = p < 0.05; ** = p < 0.01; *** = p < 0.001

Table 4: Comparative morphology of putative hybrids and parents
b) head diameter

Parents and hybrids	Head diameter				
	x	t		cv	d/a
HA-89 ms PET-1	19.9			4.58	
<i>Silphium perfoliatum</i>	1.7	72.96***		6.87	
F ₁	4.6	30.77***	8.88***	21.09	-0.67
HA-89 PET-1	19.9			4.58	
<i>Matricaria chamomilla</i>	0.5	88.27***		9.32	
F ₁	4.3	38.0***	16.70***	19.61	-0.60
HA-89 ms ARG-3	19.9			4.58	
<i>Telekia speciosa</i>	2.7	78.36***		4.28	
F ₁	5.7	8.11***	33.44***	20.49	-0.65
L 6054	17.8			2.28	
<i>Telekia speciosa</i>	2.7	78.36***		4.28	
F ₁	5.09	15.19***	43.56***	31.04	-0.14
HA-89 ARG-1	19.9			4.58	
<i>Carduus acanthoides</i>	0.9	67.71***		17.80	
F ₁	6.3	22.86***	11.64***	30.97	-0.43
L 3004 ARG-3	14.6			27.24	
<i>Carduus acanthoides</i>	0.9	14.13***		17.80	
F ₁	9.5	0.0	0.0		0.25
HA-89 ARG-3	19.9			4.58	
<i>Grindelia speciosa</i>	1.9	56.38***		14.02	
F ₁	3.0	0.0	0.0	2.28	-0.87
L 6054 GA3 ms	17.8				
<i>Gaillardia speciosa</i>	2.6	379.75***		4.32	
F ₁	8.3	45.24***	27.11***	6.93	-0.25
HA-89 PET-1	19.9			4.58	
<i>Gaillardia speciosa</i>	2.6	78.46***		4.32	
F ₁	16.0	3.61***	14.84***	16.54	0.55
L 3004 ARG-3	14.6			27.24	
<i>Onopordum acanthium</i>	1.9	12.66***		14.02	
F ₁	5.9	6.53***	7.32***	35.67	-0.36
L 3004 ARG-3	14.6			27.24	
<i>Verbesina helianthoides</i>	2.6	6.19***		6.21	
F ₁	7.7	4.83***	12.54***	32.15	-0.15
HA-89 ARG-3	19.9			4.58	
<i>Tithonia rotundifolia</i>	1.9	64.29***		20.04	
F ₁	10.4	37.84***	65.69***	9.83	-0.05
HA-89 cv. Per. emasc.	20.0			12.05	
<i>Bidens tripartita</i>	0.8	33.33***		10.73	
F ₁	9.9	9.05***	14.74***	14.40	-0.05
L 2235 ms PET-1	17.0			3.1	
<i>Aster speciosa</i>	0.9	282.83***		4.3	
F ₁	9.5	20.83***	24.00***	7.4	0.02

t = Student's t-test; * = p < 0.05; ** = p < 0.01; *** = p < 0.001

Four cross combination, with *Cosmos bipinnatus*, *Arctium lappa*, *Calendula officinalis* and *Inula helenium*, were unsuccessful. The problems related to interspecific (intergeneric) hybridization resulted in varying numbers of crossings with different levels of incompatibility being observed. There were crosses where seeds were produced, but some did not germinate, while those that did germinate died during the seedling stage. These crosses included the species *Cichorium intybus*, *Chrysanthemum leucanthemum*, *Matricaria chamomilla*, *Simsia foetida*, *Cirsium lanceolatum* and *Cosmos bipinnatus*. It appears that incompatibility barriers due to either death of developing embryo (endosperm deficiency) or incompatibility of pollen and stigma could be overcome by the use of the embryo culture system or other techniques.

It should be noted that with a few exceptions, the female plants of the unsuccessful hybrids used cytoplasmic male sterility of the PET-1 cytoplasm (*H. petiolaris* Nutt.) (Leclercq, 1969). The influence of cytoplasm on crossability needs further investigation. It is believed that in the future it could be predicted in advance which species combinations would be preferred as the female plant in order to produce a viable hybrid.

The putative intergeneric hybrids were easily recognized by morphological traits. Identification of hybrid populations was facilitated by the fact that the parental species are morphologically dissimilar and hybrids between them are easily recognized. Morphological characteristics of F₁ hybrid plants indicated the uniformity of the first generation. There were no large variations among the various hybrid populations. The hybrids were usually of intermediate phenotype, but characters of the cultivated sunflower were more pronounced. Individual plants from all crosses were branched with a well-differentiated central stem. The branches were situated mainly in the upper half of the plant in the crosses between *H. annuus* and *Onopordum acanthium*, *Bidens tripartita*, *Silphium perfoliatum*, and *Carduus acanthoides*. The leaves were of the *H. annuus* type, usually well-developed, but smaller than those in the cultivated sunflower. However, in some cases, as in the crosses with *Tithonia rotundifolia* and *Bidens tripartita*, certain abnormalities such as deformed heads or leaves were observed.

It is interesting to note the presence of anthocyanin pigment in all hybrid combinations - in stems, leaves and inflorescences - with differing degrees of color intensity. It is quite possible that complementary genes interaction resulted in the formation of new characters in the first generation. The occurrence of new characters in the first generation of *Nicotiana* interspecific hybrids had been reported by Kostoff (1941-1943).

Sixty-five to ninety-five percent of the plants were male fertile in the crosses where the female parent was male sterile. The inflorescences were similar to those of the cultivated sunflower. After the putative hybrids were established in the field, their hybrid nature was verified by comparing the morphological characteristics of the hybrids with those of the parents.



Figure 1: F_1 (*H. annuus* x *Bidens tripartita*)



Figure 2: F_1 (*H. annuus* x *Tithonia rotundifolia*)

Figure 3: Heads of F_1 (*H. annuus* x *Verbesina helianthoides*)



Figure 4: Leaves of F_1 hybrid *H. annuus* and *Carduus acanthoides*

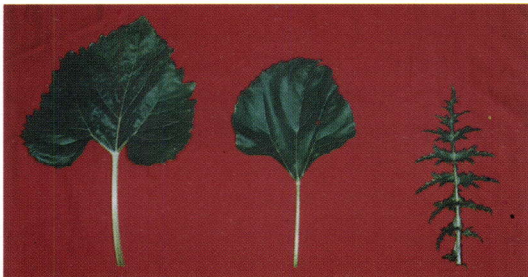


Figure 5: Leaves of F_1 hybrid *H. annuus* and *Carduus acanthoides*

A comparative summary of observations and measurements of the parent species and putative hybrids is given in Table 4 (a, b). The variability of the F₁ generation was expressed by variation coefficient (cv), which ranged from 3.01 to 22.06 for plant height and from 6.93 to 35.67 for head diameter. In general, the variability was higher than that in either parental species, covering 91.7% of the possible range for head diameter and 50% for plant height.

Additional confirmation of hybrids could be obtained later from estimates of pollen fertility and meiotic abnormalities in pollen mother cells (PMC's). Cytologically, the putative F₁ hybrids were not analyzed because of insufficient plant material in many cases. The obtained hybrids will be studied further in order to assess their future behavior. Detailed data on the fertility and cytology of hybrids will be presented in the next report.

CONCLUSION

In spite of the genetic distance between *H. annuus* and the wild species from *Compositae* genera, intergeneric hybridization appears promising as a breeding method for transferring wild germplasm for agronomic purposes. In summary, introgression between the cultivated sunflower and species from *Compositae* has probably occurred in nature. Many species are directly cross compatible with *H. annuus* and we believe many other crosses could be obtained by embryo rescue techniques and breeding methods. The data presented provides some knowledge about the genetic properties of the species and will be useful for future improvement of the cultivated sunflower.

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