CYTOGENETICAL CHARACTERISTICS OF THE INTERSPECIFIC HYBRIDS

Helianthus annuus L.× *Helianthus resinosus* Small

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INTRODUCTION

The hexaploid species of Helianthus are of interest in the hybridization of cultivated sunflower as source of genes determining important biological features such as resistance, the content of certain valuable fatty acids, protein etc. On the other hand, the cytogenetic study of hybrids may contribute to explain the origin of the hexaploid species.

The object of such hybridization has so far been the hexaploid species *H. tuberosus* and *H. rigidus,* this species manifesting complete incompatibility with *H. annuus* (Georgieva-Todorova, 1976). In this connection it is interesting to study the possibility of hybridization of cultivated sunflower with *H. resinosus*.

This paper presents the first results of the cytogenetic study of hybrids between the two species.

MATERIALS AND METHODS

The mother plants used were from our cytoplasmic male sterile form (Georgieva-Todorova, 1976). The pollinator was a population of *H. resinosus,* and in some cases pollination was carried out by a mixture of pollen from *H. resinosus*+pollen from species with different levels of ploidy : *H. nuttallii, H. argirophyllus* ($2n = 34$), *H. rigidus* ($2n = 102$) etc.

The cytogenetic study of hybrids embraces the characterization of their most important morphological characters, pollen viability, seed set as well as meiosis in the initial species and hybrids (degree of conjugation on the basis of the type of the chromosome association in diakinesis, the character of the individual meiotic phases, etc.).

RESULTS AND DISCUSSION

H. resinosus ($2n = 102$) is a perennial species included in section *Gigantei.* The species is well differentiated, with strictly manifested roproductive barriers, despite the fact that it is to be encountered in the same areas as many other wild species of *Helianthus.* Under our conditions, develops quite well. The roots are long, some upward, becoming woody ; rhisomes short, thick to slender ; stems stout, 1—3 m tall (Heiser, et al., 1967). The leaves mostly alternate or at least alternate above, from narrowly lanceolate to broadly ovate ; disks 2—3 cm in diameter. *H. resinosus* is resistant to *Plasmopara halstedii, Puccinia helianthi,* etc. The data obtained from a study of the gamete compatibility and of the possibilities of obtaining developed seeds in F_0 (Table 1) indicate that the number of seeds in one inflorescence is low ; it usually varies between 3 and 10% when only *H. resinosus* participates as pollinator and between 10 and 20% when pollination is done with mixed pollen.

Table 1

Crossability of *Heliantus annuus* **($2n=34$) with** *Helianthus resinosus* **($2n=102$)**

Year	Total no. of crosses	With *H. resinosus*	Number of seeds per inflorescence (%)		With *H. resinosus*+ pollen of wild species	Number of seeds per inflorescence (%)		
			0—3	3—10		0—3	3—10	10—20
1980	15	5	5	0	10	3	5	2
1981	28	3	1	2	25	12	8	5
1982	20	10	5	5	10	10	0	0
1983	26	16	10	6	10	5	3	2

It is known that crossability is improved when pollen from species of the same genera is added to the male parent (Georgieva, 1976). The number of seeds in F_0 of the cross *H. annuus*×*H. resinosus* increased following the addition of pollen from the species *H. nuttallii* ($2n = 34$), *H. argophyllus* ($2n = 34$) and *H. rigidus* ($2n = 102$). It is difficult to us to explain the reason of improving the seed setting in F_0 (in certain cases — 20% when hybridization was carried out with mixed pollen). It may be assumed that pollen of different species contains different enzymes, some of which are favourable to the germination of pollen from *H. resinosus,* as well as to the growth of the pollen tubes and fertilization of a larger number of egg-cells. It should be mentioned in this respect that there exists markedly expressed interspecific incompatibility between *H. annuus* and the species referred to above (Georgieva-Todorova, 1976).

The results of the development of the F_1 hybrid plants are presented in Table 2, which shows that hybrids were obtained in almost all progenies, with the exception of one, but none of them have shown complete germination of the seeds. For instance in progeny 57/82 from 550 sown seeds germinated 86 i.e.15.64%, and only 8.73% of the total number developed completely. In some progenies like 56/82 neither of the seeds germinated. This shows, as we have established in other hybrid combinations, that the interspecific incompatibility in the crossing of genetically distant forms may be manifested at different stages of ontogenesis.

Table 2

Results of the investigations of the seed germination of *H. annuus* × *H. resinosus* and the development of hybrid plants

Year	No. of progenies	Seeds sown	Germinated seeds		Hybrid plants developed	
			No.	%	No.	%
1981	65	500	146	29.20	143	28.60
	65 a	2	1	50.00	1	50.00
	65 b	7	6	85.71	6	85.71
1982	25	137	20	14.60	20	14.60
	32	58	10	17.24	8	13.79
	48	275	61	22.18	60	21.82
	49	8	5	62.50	4	50.00
	50	32	7	21.88	6	18.75
	51	138	43	31.16	41	29.71
	52	8	1	12.50	1	12.50
	53	19	10	52.63	10	52.63
	54	12	4	35.33	2	16.67
	55	5	2	40.00	2	40.00
	56	9	0	0	0	0
	57	550	86	15.64	48	8.73
	58	10	2	20.00	2	20.00
	59	22	6	27.27	1	4.55
	60	120	11	9.17	9	7.50
	65	24	7	29.17	4	16.67
	66	29	11	37.93	11	37.93
	68	24	4	16.67	1	4.17
	69	2	2	100.00	2	100.00
	70	182	48	26.37	47	25.82

The F_1 hybrids proved to be phenotypically interesting (Table 3). It should be pointed out that the first hybrids from this combination were of an intermediary type, though phenotypically much more similar to the cultivated parent with shorter stems lightly ramified, ripening 10—15 days earlier, but with reduced viability of pollen and lower plant fertility. They were resistant to *Plasmopara halstedii* under field conditions.

Almost all hybrid progenies in 1982 and 1983 were phenotypically entirely of the type of *H. resinosus*. Among the numerous interspecific hybrids of *Helianthus,* which we have obtained until now, such a high percentage of hybrids manifesting the type of the wild parent (80.33%) has not been obtained in any of the combinations in which the cultivated species was used as mother. Since there is no information in the literature about hybrids obtained between these species, it is not possible to explain the reason for the stronger phenotype manifestation of the wild parent. However a similar phenomenon, though not so marked, has been observed in the hybridization of *H. annuus* with another hexaploid species (*H. tuberosus)* and with the tetraploid species *H. decapetalus* and *H. hirsutus*. It is interesting to note that this phenomenon is observed in the combination in which the wild parent is highly polyploid and participate as father. To all probability, the number of the conjugated homologous chromosomes from the two types is significant at the recombination of the genetic material of the higher-ploid parent with that of the lower-ploid one, something which is reflected also in the phenotype of the hybrids. The viability of pollen was strongly reduced in all hybrid plants, and 14.4% of the hybrids had totally sterile pollen. The highest is the percentage of plants with pollen viability between 40 and 50%. The fertility of the hybrids is likewise low but these hybrids are of higher fertility, compared with the hybrids obtained by us between other species.

The hybrids keep their phenotype in F_2 (the plants from the wild parent prevail over the others). In Table 4 we show some data for the phenotype variety of the hybrids in F_2. The table gives an idea of the type of inheritance as well as of the fertility of the plants. The phenotype segregation of different progenies are not the same. The parent type dominates in F_2 in most of them. In some progenies as for example in 403/83 from the F_1 plants of the paternal type in F_2 we have observed plants of mother type (from 18 plants — 16 of mother type and 2 of intermediate type) or in the F_1 progenies of the intermediate type in F_2, there have been observed plants of the three types : mother, intermediate and paternal. Pollen viability of hybrids varies in F_2, but as a rule, the plants with higher pollen viability dominate. The fertility is reduced as in other *He-*

Table 3

Characteristics of the F_1 hybrids *Helianthus annuus* **($2n=34$)** × *Helianthus resinosus* **($2n=102$)**

No. of progenies	Character of hybrids		No. of plants with pollen viability %						No. of hybrids with plant fertility (%)				
	Intermediate	Paternal	0	0—30	30—40	40—50	50—60	60—70	0	0—2	2—5	5—10	10—25
4	0	1	1	—	—	—	—	—	1	—	—	—	—
25	3	26	3	3	4	7	4	1	3	6	7	3	2
30	0	1	1	—	—	—	—	—	—	—	1		
32	3	6	1	0	0	5	4	2	1	4	2	—	1
33	1	—	0	—	—	—	—	—	—	—	—	—	—
34/1	—	1	0	—	—	—	—	—	1	—	—	—	—
35	—	1	0	—	—	—	—	—	1	—	—	—	—
50	—	1	—	—	0	—	—	—	—	0	—	—	—
53	—	1	—	—	1	—	—	—	—	1	—	—	—
55	—	1	—	—	—	1	—	—	1	—	—	—	—
58	3	—	—	—	—	—	1	—	—	—	—	1	—
63	3	6	1	3	1	1	0	1	1	5	2	—	—
64	—	2	1	1	—	—	—	—	1	—	1	—	—
68	1	—	—	1	—	—	—	—	1	—	—	—	—
70	1	2	1	—	—	2	—	—	1	1	1	—	—

Table 4

Characteristics of the F_2 hybrids *Helianthus annuus* **($2n=34$)** × *Helianthus resinosus* **($2n=102$)**

No. of progenies	Type of hybrids in F_1	Type of hybrids in F_2			Number of plants with viable pollen						Number of plants with seed fertility of			
		female	intermediate	male	0 %	1—5 %	5—10 %	20—30 %	30—50 %	50—100 %	0 %	0—5 %	5—10 %	10—20 %
402	male	0	5	5	0	2	2	1	0	0	3	2	5	0
403	male	16	2	0	0	0	0	2	10	6	0	2	5	10
434	male	0	3	2	0	0	0	0	0	5	2	3	0	0
444	intermediate	6	4	2	0	0	0	0	1	11	4	2	6	0
446	male	0	0	1	0	0	0	0	1	0				
473	intermediate	5	2	1	0	1	2	5	0	0	0	—	—	—
477	intermediate	0	2	3	2 plants without pollen			2	1	0	2	2	4	0
478	male	0	5	5	2 plants without pollen									
					1	1	2	4	0	0	2	2	4	2
482	female	10	2	0	1	1	0	4	2	0	0	2	5	5

lianthus interspecific hybrids. The seeds are small, of the male parent type. It is characteristic that the progenies are with a comparatively high percentage of seeds on one plant, which makes it possible to use more material in the following generations.

On the basis of the data about pollen viability and fertility of the plants, used as criteria for genetic relationship and compared with those obtained from our earlier studies with the utilization of other hexaploid species, it may be assumed that *H. resinosus* is more closely related to *H. annuus* than any other species studied until now, included *H. tuberosus* which is assumed to have also one genome from *H. annuus* in its genome structure. This conclusion is supported also by our cytological studies of the hybrid *H. annuus*×*H. resinosus*. Table 5 presents data on the course of meiosis in this hybrid ; the chromosome associations are mainly bivalents although there are individual cells with quadrivalents, trivalents, and univalents. In almost all cells there are univalent chromosomes in MI. It is interesting to note that the number of bivalents in all hybrids investigated varies between 28 and 36 with 1 to 6 univalents, the most frequent case being $33_{II}+6_{I}$. Almost all meiotic phases take place with disturbances of the type of univa-

Table 5

Meiosis in F_1 hybrids *Helianthus annuus* **(2n=34)** × *Helianthus resinosus* **(2n=102)**

No. of plants	Diakinesis chromosome configurations	Metaphase I		Anaphase I		Telophase II		Pollen viability (%)	Fertility %
		total no. of cells	disturbances (%)	total no. of cells	disturbances (%)	total no. of cells	disturbances %		
25/82—2	$35_{\text{II}}+5_{\text{I}}$	283	100	33	27·27	25	20.00	64.70	0.70
	$34_{\text{II}}+4_{\text{I}}$	The number of non included univalents in MI							
	$33_{\text{II}}+6_{\text{I}}$								
	$33_{\text{II}}+6_{\text{I}}$								
25/82—8	$33_{\text{II}}+6_{\text{I}}$	2, 3, 4,	5, 6, 7						
	$28_{\text{II}}+1_{\text{IV}}+3_{\text{I}}$								
	$35_{\text{II}}+1_{\text{I}}$								
	$32_{\text{II}}+1_{\text{III}}+5_{\text{I}}$								
	$33_{\text{II}}+2_{\text{I}}$								
25/82—14	$32_{\text{II}}+4_{\text{I}}$	275		116	62.93	161	8.07	50.1	12.97
	$31_{\text{II}}+3_{\text{I}}$								
	$32_{\text{II}}+4_{\text{I}}$								
	36_{II}								
	$32_{\text{II}}+1_{\text{III}}+5_{\text{I}}$								
	$32_{\text{II}}+5_{\text{I}}$								
	$26_{\text{II}}+5_{\text{I}}$								
	$28_{\text{IV}}+3_{\text{I}}$								
	$31_{\text{II}}+1_{\text{IV}}+2_{\text{I}}$								
	$31_{\text{I}}+1_{\text{I}}$								
	$31_{\text{II}}+2_{\text{I}}$								
	$29_{\text{II}}+1_{\text{IV}}+4_{\text{I}}$	152	100			35	127	64.50	15.50

lents in metaphase I, lagging chromosomes, and chromosomes bridges in anaphases I and II, lagging chromosomes in telophase II etc. Interesting too, is the already mentioned fact that there is a larger percentage of a higher number of bivalents, this being an indication of the existence of homology between the chromosomes of *H. resinosus* and those of *H. annuus.*

CONCLUSION

Hybridization between *H. annuus* (2n = 34) and *H. resinosus* (2n = 102) is successful. The hybrids, obtained for the first time, indicate that it is possible te use this species with a view to transferring to sunflower the genetic material conditioning the resistance to diseases. The cytological study has made it possible to draw certain conclusions about the homology of the chromosomes of the two species.

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CARACTÉRISTIQUES CYTOGÉNÉTIQUES DES HYBRIDES INTERSPÉCIFIQUES *HELIANTHUS ANNUUS* L.×*HELIANTHUS RESINOSUS* SMALL

Résumé

On a étudié et décrit l espèce sauvage *H. resinosus* (2n=102). Celle-ci est une source de résistance à l'attaque de plusieurs pathogènes du tournesol, parmi lesquels *Plasmopara halstedii* et *Puccinia helianthi.* Le nombre des semences hybrides (F_0) a considérablement augmenté quand le pollen de cette espèce a été mélangé avec le pollen d'autres espèces de *Helianthus.*

L'incompatibilité interspécifique entre *H. annuus* et *H. resinosus* s'est manifestée lors de différents stades de l'ontogénèse. Les hybrides F_1 ont été phénotypiquement différents, plus de 80% des cas étaient semblables à *H. resinosus,* surtout quand ceci a été utilisé comme géniteur mâle.

La viabilité du pollen des plantes F_1 a été très réduite, et une stérilité totale du pollen a été enregistrée chez 14,4% des plantes. Quoique la fertilité des plantes F_1 a été basse, elle est bien plus élevée que celle des hybrides résultés du croisement d'autres espèces de *Helianthus.* Les types parentaux ont prédominé dans la génération F_2.

Conformément aux données sur la viabilité du pollen et la fertilité des plantes, on peut affirmer que *H. resinosus* est plus apparenté à *H. annuus* que d'autres espèces hexaploïdes, comme *H. tuberosus.* La méiose des hybrides *H. annuus*×*H. resinosus* a indiqué l'existence de l'homologie d'une partie des chromosomes de l'espèce hexaploïde et ceux de *H. annuus,*

L'hybridation entre les deux espèces étant relativement facile à réaliser, il est possible de transférer du matériel génétique important, de l'espèce sauvage à celle cultivée.

CARACTERÍSTICAS CITOGENÉTICAS DE LOS HÍBRIDOS INTERSPECÍFICOS *HELIANTHUS ANNUUS* L.×*HELIANTHUS RESINOSUS*

Resúmen

Está descrita la especie *H. resinosus* (2n =102). Ésta es una fuente de resistencia al ataque de varias enfermedades de girasol, entre las cuales ***Plasmopara halstedii*** y *Puccinia* ***helianthi***. El número de semillas híbridas (F_0) aumentó considerablemente al mezclar del polen de esta especie con polen de otras especies de *Helianthus*.

La incompatibilidad interespecifica entre *H.* ***annuus*** y *H.* ***resinosus*** se manifestó en diferentes estadios de ontogenésis. Los híbridos F_1 fueron diferentes fenotipicamente, màs del **80** por ciento de los casos se parecieron al *H. resinosus*, sobre todo cuando éste se empleó como pariente paterno.

La viabilidad del polen de las plantas F_1, fue muy reducida, y en 14,4% de los individuos se ha señalado una esterilidad total del polen. A pesar de que la fertilidad de las plantas F_1 fue baja, ésta es mucho más alta que la de los híbridos resultados por el cruce de otras especies de ***Helianthus***. Los típos parentales dominaron en la generación F_2.

Partiendo de los datos referentes a la viabilidad del polen y la fertilidad de las plantas se puede afirmar que *H.* ***resinosus*** está emparentada más con *H.* ***annuus*** que otras especies hexaploides, como *H.* ***tuberosus***. La meiosis de los híbridos *H.* ***annuus***× *H.* ***resinosus*** indicó la existencia de la homología entre una parte de los cromosomas de la especie hexaploida y los de *H.* ***annuus***.

La hibridación entre las dos especies siendo relativamente fácil de realizarse, es posible transferar material genético importante desde la especie salvaje hasta la cultivada.