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CYTOGENETIC STUDY OF F_1 INTERSPECIFIC HYBRIDS OF THE SECTION *Helianthus*

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SUMMARY

Cytogenetic peculiarities during the passing of meiosis were studied in anthers of 7 interspecific F_1 hybrids obtained by crossing annual wild species (*Helianthus annuus*, *H. praecox*, *H. argophyllus*) with the cultivated sunflower. The hybrids derived from *H. annuus* and *H. argophyllus* were characterized by a low frequency of aberrations (6.5-8.6%), which gives evidence of a good balance of the chromosome complex of these hybrids. The hybrids derived from the crosses between *H. praecox* and the cultivated sunflower were considerably less stable because of a high frequency of aberrations in meiosis (29.6-50.0%).

Key words: ***Helianthus*, interspecific hybrids, microsporogenesis, aberrations**

INTRODUCTION

The main disadvantage of interspecific plant hybrids is low seed yield which does not allow the hybrids to be widely introduced in selection and genetic research. This problem is present in the genus *Helianthus* which has 49 wild species differing in type of development (annual, perennial species) and ploidy level (diploid, tetraploid, hexaploid species) (Georgieva-Todorova, 1990). The group of annual wild species is of specific interest since its members can be easily crossed with the cultivated sunflower (Popov *et al.*, 2005; Gavrilova *et al.*, 2003), allows the genes for valuable selection traits, such as abiotic and biotic resistance (Cerboncini *et al.*, 2002; Besard *et al.*, 1997), new *cms* systems (Jan, 2000) *etc.*, to be introduced from wild species. For a wider use of interspecific hybrids in sunflower breeding, their detailed study is needed including cytological investigations.

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From practical point of view it is of interest to study the meiosis in interspesific sunflower hybrids resulting from crosses between wild and the cultivated species in so far as it allows to select stable genotypes that can be used in breeding programs for creating heterotic hybrids. Thus the cytogenetic study of interspesific hybrids permits to exchange genes between chromosomes of the crossed species.

The aim of the examination was to study cytological peculiarities of the interspesific hybrids obtained by crossing several annual wild species with the cultivated sunflower.

MATERIALS AND METHODS

In this study we used the wild annual diploid species of the genus *Helianthus* (n=17): *H. annuus* L. H-151, *H. annuus* L. ANN-1064, *H. annuus* L. ANN-1366, *H. praecox* Engelm. & Gray, *H. argophyllus* Torrey & Gray (female parent) which were crossed with cultivated sunflower lines X908-B, X1006-B and X1012-B (male parent) to produce interspecific F_1 hybrids. The cultivated inbred lines used for hybridization were selected at the Yurjev Plant Production Institute. These lines are sterility maintainers. Cytoplasmic male-sterile lines of these maintainer lines have been used at the Institute for the development many commercial sunflower hybrids.

Flower buds of interspecific hybrids were collected at the ontogenetic stage R2 (Schneiter *et al.*, 1981) in morning hours (7-10 a.m.) and fixed in a mixture of acetic acid and alcohol (1:3) for 12 hours. The samples were then washed three times with ethanol and stored in 70% ethanol at the temperature of +4°C. Cell staining was carried out according to Shiaf after hot hydrolysis by Feulgen (Pausheva, 1988). Meiosis was studied on temporary squash preparations in 2% acetocarmine drop.

On average 3-4.5 thousand cells were analyzed in 3-5 plants of each hybrid combinations. The pairwise comparison of data was performed using the statistical tests of t-Student and ø-Fisher (Lakin, 1980).

RESULTS AND DISCUSSION

A significant criterion describing the progression of meiosis in interspecific diploid sunflower hybrids is the occurrence of 17 bivalents at diakinesis of pollen meiocytes. In the interspecific hybrids studied, this criterion ranged from 76.2 to 99.0% (Table 1). High frequency of bivalent chromosome pairing in the crosses between the wild forms and the cultivated sunflower was indicated by the occurrence of close bivalents (4.99-6.59), especially in the hybrids derived from *H. praecox* which had this index at the levels of 2.88 and 2.67. The hybrids with *H. annuus* indicated a high level of the synapsis, which differed in the number of chiasmata per bivalent (1.29-1.40) and meiocytes (21.92-23.80) and which significantly exceeded these criteria in comparison with the hybrids involving *H. praecox* (1.16 and 1.17; 19.72 and 19.94, respectively).

Table 1: Nature and frequency of chromosomal aberrations at the stage of diakinesis of meiocytes in interspecific sunflower hybrids

Hybrid combination	Number of meiocytes	Number of chiasmata per meiocytes	Number of chiasmata per bivalent	Number of closed bivalents per meiocyte	17_{II}, %	Univalents , %		Tetravalents, %		Hexavalents, %	Other aberrations, %
						$16_{II} + 2_{I}$	$15_{II} + 4_{I}$	$15_{II} + 1_{IV}$	$13_{II} + 2_{IV}$	$14_{II} + 1_{VI}$	
H. annuus H-151 × X908-B	233	23.80 ±0.14	1.40 ±0.01	6.59 ±0.12	91.0±1.9	0.4±0.4	0	2.6±1.0	0.9±0.6	0.9±0.6	4.2±1.3
H. annuus ANN 1064 × X908-B	188	22.62 ±0.14	1.33 ±0.01	5.55 ±0.13	99.0±0.7	1.1±0.8	0	0	0	0	0
H. annuus ANN 1064 × X1006-B	287	22.02 ±0.12	1.30 ±0.01	4.99 ±0.12	87.1±2.0	1.0±0.6	0	0.3±0.3	0	0	11.5±1.9
H. annuus ANN 1366 × X1012-B	260	21.92 ±0.11	1.29 ±0.02	4.83 ±0.10	94.6±1.4	3.1±1.1	0.4±0.4	0	0	0	2.3±0.9
H. praecox × X908-B	206	19.94 ±0.13	1.17 ±0.01	2.88 ±0.13	76.2±3.0	1.9±1.0	1.5±0.8	5.3±1.6	0	1.9±0.5	15.0±2.5
H. praecox × X1006-B	192	19.72 ±0.11	1.16 ±0.01	2.67 ±0.10	79.2±2.9	9.4±2.1	2.1±1.0	4.7±1.5	0.5±0.5	1.0±0.9	3.1±1.3
H. argophyllus × X908-B	154	20.59 ±0.13	1.21 ±0.01	3.53 ±0.13	83.8±3.0	2.6±1.3	0	9.1±2.3	1.3±0.9	0	3.2±1.4

The analysis of chromosome pairing in the interspecific hybrids showed univalents (maximum of their number occurred in *H. praecox* × X1006-B: $16_{II} + 2_{I}$ - 9.4±2.1% and $15_{II} + 4_{I}$ - 2.1±1.0%), tetravalents (they predominated in *H. argophyllus* × X908-B: $15_{II} + 1_{IV}$ - 9.1±2.3 and $13_{II} + 2_{IV}$ - 1.3±0.9%), hexavalents (their maximum number occurred in *H. praecox* × X908-B: $14_{II} + 1_{VI}$ - 1.9±0.5%) as well as other anomalies including chromosome elimination (from 1 to 7 pairs per meiocyte), their increase to 34 pairs per meiocyte, polyvalents in combination with univalents whose frequencies were 11.5±1.9% and 15.0±2.5% in the crosses *H. annuus* ANN1064 × X1006-B and *H. praecox* × X908-B, respectively (Table 1).

The subsequent cytological analysis of meiosis in the interspecific hybrids showed that anomalies often occurred at the stage of metaphase I. Those were the univalents and the chromosomes not included into the metaphase plate (Table 2, Figure 1).

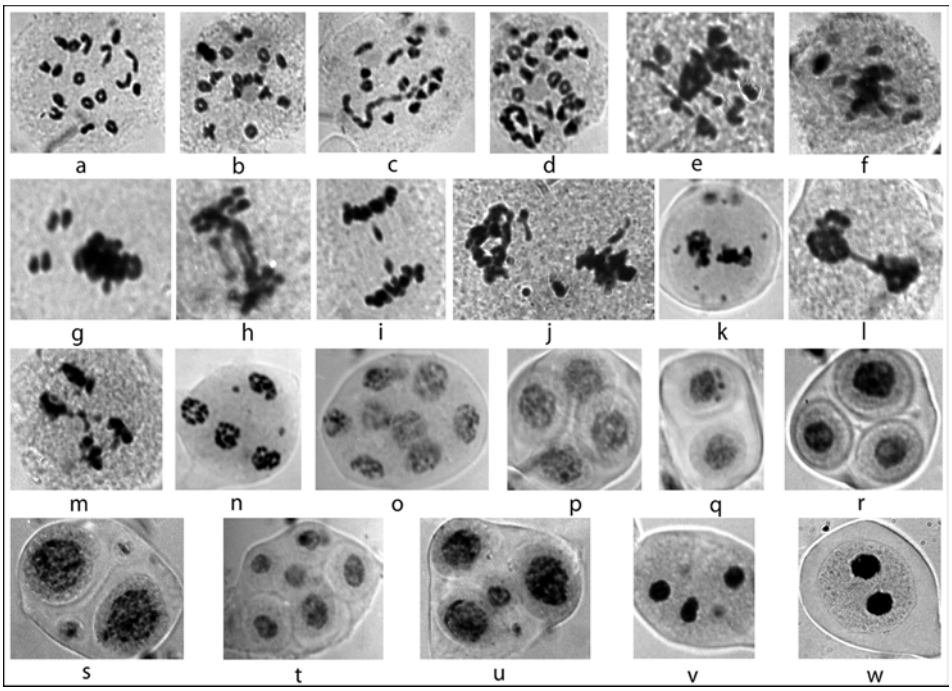

Figure 1: Photomicrographs of meiosis in the interspecific F_1 hybrids of sunflower: a - 17_{II} in diakinesis, b - 18_{II} in diakinesis, c and d - polyvalents in diakinesis, e - lagging chromosomes in metaphase I, f - chromosome asynapsis in metaphase I, g - chromosome desynapsis in metaphase I, h - bridges in anaphase I, i - lagging chromosomes in anaphase I, j - lagging chromosomes in metaphase II, k - micronuclei in metaphase II, l - bridge and lagging chromosomes in metaphase II, m - lagging chromosomes in anaphase II, n - micronuclei in telophase II, o - 8 nuclei in telophase II, p - the normal tetrad, q - the diad with 3 micronuclei, r - the triad, s - tetrad with 2 reduced cells, t - the hexad, u - the tetrad with irregular nuclei, v and w - the picnotic nuclei.

Table 2: Meiosis study in interspecific hybrids of the genus *Helianthus*

Hybrid combination	Meiocytes studied		Metaphase I		Anaphase I		Metaphase II		Anaphase II		Telophase II	
	total	aberrant meio-cytes, %	total	aberrant meio-cytes, %	total	aberrant meio-cytes, %	total	aberrant meio-cytes, %	total	aberrant meio-cytes, %	total	aberrant meio-cytes, %
H. annuus H-151 × X908-B	3760	7.3±0.4	623	17.7±1.5	278	14.0±2.1	524	2.7±0.7	288	5.9±1.4	504	0.8±0.4
H. annuus ANN-1064 × X908-B	4041	6.5±0.4	703	15.2±1.4	578	12.6±1.4	508	1.6±0.6	499	1.8±0.6	504	0.2±0.2
H. annuus ANN-1064 × X1006-B	4343	8.9±0.4	603	17.1±1.5	533	11.6±1.4	432	8.1±1.3	399	6.8±1.3	493	41.8±2.2
H. annuus ANN-1366 × X1012-B	4460	8.6±0.4	588	15.0±1.5	585	7.7±1.1	562	10.1±1.3	481	13.9±2.5	535	2.6±0.7
H. praecox × X908-B	3962	50.0±0.8	556	47.1±4.5	261	66.7±2.9	442	51.4±2.4	371	70.1±2.4	603	19.5±1.6
H. praecox × X1006-B	2811	29.6±0.9	401	25.2±2.2	256	41.1±3.1	237	64.6±3.1	327	63.0±2.7	370	6.5±1.3
H. argophyllus × X908-B	3433	6.7±0.4	469	14.7±1.6	372	7.5±1.4	456	9.0±1.3	414	10.9±1.5	442	1.8±1.6

Table 3: Aberration frequency at meiosis, during the stage of tetrad formation in interspecific hybrids of the genus *Helianthus*

Hybrid combination	Total number	Average frequency of tetrads, %			Monads, %	Diads, %	Triads, %	Polyads, %
		normal	with micronuclei	with other aberration				
H. annuus H-151 × X908-B	919	94.7±0.7	0.1±0.1	0.2±0.1	0	3.5±0.6	1.4±0.4	0.1±0.1
H. annuus ANN-1064 × X908-B	560	89.3±1.3	1.8±0.6	5.3±0.9	0.2±0.2	1.8±0.6	0.9±0.4	0.7±0.4
H. annuus ANN-1064 × X1006-B	1137	91.5±0.8	0.4±0.2	0.7±0.2	0.3±0.2	0.1±0.1	1.0±0.3	6.0±0.7
H. annuus ANN-1366 × X1012-B	938	92.8±0.8	0.9±0.3	2.1±0.5	0.4±0.2	0.3±0.2	2.2±0.5	1.3±0.1
H. praecox × X908-B	1134	44.8±1.5	9.0±0.8	8.5±0.8	0.5±0.2	15.5±1.1	18.3±1.3	3.3±0.5
H. praecox × X1006-B	671	89.4±1.2	6.8±1.0	0.6±0.3	0	0.9±0.4	1.0±0.4	1.2±0.4
H. argophyllus × X908-B	862	99.0±0.3	0.3±0.2	0.2±0.2	0.1±0.1	0	0.3±0.2	0

It was observed that the univalents in metaphase I occurred both as a result of absence of chromosome pairing (asynapsis), disorderly placement of chromosomes over the entire spindle and premature chromosome disjunction as a result of chiasmata absence (desynapsis) relatively to the basic groups of bivalents (Figure 1).

Bridges and chromosome disjunction were the basic aberrations observed at the stages of anaphase I and anaphase II. Lagging chromosomes and fragments of chromosomes as well as the bridges between metaphase plates which might have been preserved from the stage of anaphase I were found in metaphase II (Figure 1). There were lagging chromosomes, micronuclei, 2-3 and 5-8 poles of chromosome disjunction, irregularities of nuclei in the number of chromosomes, almost the bridges in telophase II.

Table 2 shows that the percentage of the aberrant meiocytes is more significant from metaphase I to telophase II in the interspecific hybrids derived from *H. praecox* as well as in diakinesis than these criteria in the other studied hybrids at all stages of meiosis (19.5 - 70.1% in *H. praecox* × X908-B and 6.5 - 64.6% in *H. praecox* × X1006-B). Minimal numbers of aberrant meiocytes (0.2 - 15.2%) were also found in the interspesific hybrid *H. annuus* ANN1064 × X908-B in the mentioned period.

The second meotic division finishes simultaneously with tetrad formation and their location on sunflower microspores. The frequent formation of normal tetrads was reduced to 44.8% in *H. praecox* × X908-B, while this criterion was at the level of 89.3 - 99.0% in the other hybrids in this study (Table 3).

A wide range of aberrations such as tetrads with micronuclei resulting in chromosome elimination, tetrads with irregular number of chromosomes, pycnotical nuclei as well as monads, diads, triads and polyads was observed in the course of meiotic phases in the interspecific sunflower hybrids (Figure 1). Maximum numbers of these aberrations were found in the hybrid *H. praecox* × X908-B (9.0% tetrads with micronuclei, 15.5% diads, 18.3% triads, 3.3% poliads, 8.5% other aberrations).

The frequency of aberrations in telophase II was lower in the hybrid *H. praecox* × X1006-B than in *H. praecox* × X908-B. The decrease in the number of aberrant meiocytes observed in telophase II in these interspesific hybrids seems to be the result of restoration of some aberrations. The tetrads with micronuclei were predominant aberrations at the stage of the formation of tetrads in *H. praecox* × X1006-B (6.8±1.0%).

The data obtained on meiotic progression in interspesific hybrids derived from wild species of *H. annuus* (Georgieva-Todorova, 1990; Georgieva-Todorova, 1993) and *H. argophyllus* (Manjula *et al.*, 1999; Quillet, 1995) are similar to the results of other authors who also observed a normal meiosis with small aberrations. However, some authors noted a small number of aberrations during meiosis in hybrids of *H. praecox* (Georgieva-Todorova, 1993; Manjula *et al.*, 1999; Quillet *et al.*, 1995; Vassilevska *et al.*, 1993). Chromosome associations $15_{II}+1_{IV}$ were observed in only

8 out of 269 meiocytes (3%) analyzed at diakinesis. It seems that such differences in results are associated with genotype peculiarities of parent forms (the structure and degree of affinity of the chromosomes involved in crosses of these genotypes). The high frequency of aberrations in *H. praecox* × *H. annuus*, unlike in hybrids such as *H. annuus* (wild) ×*H. annuus* and *H. argophyllus* × *H. annuus*, may explain phylogenetic relationships of these species. It was shown that *H. annuus* and *H. argophyllus* belong to the same cluster while *H. praecox* stays separately from them (Schilling *et al*., 1998; Schwarzbach *et al*., 2001). The noted meiotic peculiarities occurring in the hybrids between the wild species of *H. annuus*, *H. argophyllus, H. praecox* and the cultivated sunflower represent a different degree of homology of chromosomes belonging to *CH* genome (*C* is a general ancestral genome for the whole genus *Helianthus* and *H* is typical for the annual species only) (Sossey-Alaoi *et al*., 1996; Sossey-Alaoi *et al*., 1998). It is supposed that the frequency and patterns of aberrations in the interspecific sunflower hybrids result from amphyploidazation of the two genomes (*C* and *H*) in the course of evolution of the genus *Helianthus* (Sossey-Alaoi *et al*., 1996). In order to evaluate and describe the exchange of genetic material between the crossed sunflower species, it is necessary to obtain data on chromosomes associated with specific marker genes, which allow to determine the genomic origin of the chromosomes, the availability of inversions in the karyotype and translocations between chromosomes. Such information are absent for the moment.

Thus, the study of meiosis in interspecific hybrids from the genus *Helianthus* has shown that the hybrids derived from the crossing with wild *H. annuus* and *H. argophyllus* are characterized by a small frequency of aberrations. It gives evidence of better balance of the chromosome complex of these hybrids than in the hybrids derived from *H. praecox*, particularly *H. praecox* × X908-B, in which the aberrations during meiosis reach 50%. The number of normal tetrads was 89.4% in spite of a high frequency of aberrations occurring in the hybrid *H. praecox* × X1006-B during meiosis.

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INVESTIGACIONES CITOGENÉTICAS DE HÍBRIDOS INTERESPECIES F_1 DE LA SECCIÓN *Helianthus*

RESUMEN

Las propiedades citogenéticas durante la fase de meiosis, han sido estudiadas en las anteras de 7 interspecies del híbrido F_1 obtenidos por el cruzamiento de las especies anuales silvestres (*Helianthus annuus*, *H. praecox*, *H. argophyllus*) con el girasol cultivado. Los híbridos derivados de las especies *H. annuus* y *H. argophyllus* se caracterizaban por baja frecuencia de aberaciones (6.5-8.6%), lo que indica el equilibrio del complejo cromosomático de esos híbridos. Los híbridos derivados del cruzamiento entre *H. praecox* y el girasol cultivado, eran mucho menos estables y tenían alta frecuencia de aberación en la meiosis (29.6-50.0%).

ÉTUDE CYTOGÉNÉTIQUE DES HYBRIDES INTERSPÉCIFIQUES F_1 DE LA SECTION *Helianthus*

RÉSUMÉ

Les caractéristiques cytogénétiques ont été étudiées au cours de la phase méiotique dans les anthères de 7 hybrides interspécifiques F_1obtenus par le

croisement des espèes sauvages annuelles (*Helianthus annuus, H. praecox, H. argophyllus)* avec le tournesol de culture. Les hybrides dérivés de *H. annuus* et *H. argophyllus* étaient caractérisés par une faible fréquence d'aberrations (6.5-8.6%), ce qui démontre un bon équilibre du complexe chromosomique de ces hybrides. Les hybrides dérivés des croisements entre *H. praecox* et le tournesol de culture étaient beaucoup moins stables à cause de la haute fréquence d'aberrations dans la méiose (29.6-50.0%).