CYTOLOGICAL STUDIES ON HYBRIDS AND BACKCROSS GENERATIONS OF *HELIANTHUS ANNUUS* /2n=34/ and SOME TETRAPLOID HELIANTHUS SPECIES /2n=68/

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SUMMARY

The hybridization between the cultivated sunflower Helianthus annuus /2n=34/ and perennial tetraploid species /2n=4x=68/ - H.decapetalus L., H.hirsutus Raf. and H.scaberimus A.Gray was realized. It was found that hybridization was possible only if the wild species H.decapetalus, H.hirsutus were used as pollinators and H.scaberimus - as a mother, i.e. the existing compatibility is unilateral. The barriers of sterility between the species differning in ploidy can be overcome by the in vitro methods.
The results from the cytogenetical analysis of hybrids PMC in F_I, BC_I-BC_6 showed the connections between the type of chromosome associations in diakinesis, the degree of disturbances /univalents, polyvalents, fragments/ in later meiotic phases and the degree of pollen and seed sterility.
Continued backcrossing with the cultivated sunflower resulted in rapid elimination of meiotic aberrations and restored the chromosome number of H.annuus /n=17/. On a later backcross, populations possessing valuable biological and economical characters have been obtained. Conclusion, concerning the possibilities of utilization of H.decapetalus, H.hirsutus, and H.scaberimus as a source of male sterility, high protein content of seeds and tolerance of some diseases have been drawn.

INTRODUCTION

As it is known, the genus Helianthus is a polyploidy complex consisting of diploids, tetraploids and hexaploids, all with basic chromosome number of x=17. The tetraploid group /2n=2x=68/ includes the species: H.ciliaris DC, H.laevigatus T.and Gray, H.hirsutus Raf., H.decapetalus L. and H.strumosus. Some of them were included in hybridization with cultivated sunflower / Heiser and Smith 1964, Georgieva 1976, Whelan 1977, Georgieva et al.1979, Bohorova 1988/. We have in our collection a form, under the name of H.scaberimus, which also proved tetraploid.

The interest towards the tetraploid species is due to the genes controling resistance to various diseases, the high satulained oil acid, high protein content and the CMS genes /Thompson et al.1981, Vannozzi et al. 1987/.

Results from utilization of tetraploid species H.decapetalus, /Georgieva, in press/, H.hirsutus /Bohorova and Georgieva 1987/ and H.scaberimus /Bohorova 1988/ in hybridization with cultivated sunflower have been presented, in our previous papers.

MATERIALS AND METHODS

Different numbers of crosses were performed in each combination to

establish the degree of crossability between cultivated sunflower H.annuus L. cv. Peredovik and the tetraploid species H.decapetalus H.hirsutus and H.scaberimus. In F_I-BC_6 / with H.annuus, the morphological characteristics growth period, pollen stainability and plant fertility were investigated.

The detailed analysis of diakinesis included determination of the type, the number of bivalents and the type of chromosome association, as well as the meiotic disturbances of pollen mother cells /PMC/. The use of embryo culture method made it possible to obtain hybrid seeds in case of pronounced embryonal incompatibility / Bohorova 1982/.

The results from the comparison of cytogenetical studies on the hybrid and backcross generations H.annuus x H.decapetalus H.annuus x H.hirsutus and H.scaberimus x H.annuus are reported here.

RESULTS AND DISCUSSION

The results presented in our previous articles /Georgieva et al. 1979/ reveal that crossability between the cultivated sunflower H.annuus /2n=34/ and the tetraploid species /2n=4x=68/ H.decapetalus, H.hirsutus and H.scaberimus is extremely low, and production of hybrid seeds is exceptionally difficult. Hybridization of the cultivated sunflower with H.scaberimus was successful only if the female parent had higher ploidy level. This fact proves that the genetic relationship of H.annuus with these species is distant and for that reason the obtained F_I seeds are to a great extent non-viable.

For detailed analysis of the F_I H.annuus x H.decapetalus diakinesis in PMCs - types of chromosome configurations and of bivalents were determined. The number of univalents varied within the range of 9-17, while the number of bivalents - within the 17-22 range. In F_I H.annuus x H.hirsutus various number of univalents 7-34 were observed in all the cells studied at metaphase I, because analysis of diakinesis was difficult to perform. Analysis of diakinesis in F_I H.scaberimus x H.annuus showed 22 II - 32 II, I III - 4 III, I IV - 2 IV and 2 I - 4 I. Since some of the bivalents from the hybrids of the combinations investigated were heteromorphic and open, it was suggested that, in this case conjugation was not between homologous chromosomes. Probably, these are bivalents of homoelogous chromosomes from the A genome of H.annuus and the genomes of the tetraploid species, while the closed bivalents resulted from autisyntesis chromosomes of the tetraploid species. This might also explain the more strongly expressed phenotypic and biological characteristics of the wild-growing parent in the F_I Hybrids.

The detailed analysis of the karyotype of the interspecific hybrid H.annuus x H.hirsutus shows its stability, in whose structure only individual chromosomes may have been obtained. There is exchange of chromatin material between individual chromosomes from the different genomes of the parent species /Georgieva and Bohorova 1980/.

In order to rescue the hybrids, seeds from F_I were grown in vitro and hybrid plants from H.annuus x H.hirsutus and H.scaberimus x H.annuus were yielded /Bohorova 1982/. Under ordinary conditions the seeds either do not germinate or do with great difficulty, due to the genetic incompatibility of the

species included in the hybridization.

Data from investigations of chromosome configurations at the diakinesis and PMC meiosis of BC_1-BC_6 plants are presented in Table I and Table 2.Variations in chromosome number of the different phenotypical groups were observed in BC_1 to BC_4 H.annuus x H.decapetalus. They indicate the existance of a cytological instability even after three and four backcrosses with the cultivated species. /Georgieva, in press/. The new form obtained though phenotypically uniform with the cultivated parent, differs from it, by some new features- higher field resistance to Sklerotinia and Plasmopara, higher oil content. These results show that the genome constitution of this phenotypically stabilized form is not the same, as that of the cultivated species. The additional chromosomes registered in the examined BC_6 plants may be a result of a translocation between non-homologous chromosome.

Backcrossing H.annuus x H.hirsutus with cultivated sunflower restored the chromosome number of H.annuus (after the fifth backcross/, but some structural chromosomal differences probably still existed, therefore considerable phenotypical diversity was observed. Male sterile plants with high seed fertility were obtained as a results of this hybridization and backcrossing./Bohorova, Georgieva 1987/. They are being tested for determining the type of sterility.

Bivalents, polyvalents and univalents were observed in PMC of F_1-BC_2 hybrids /Bohorova 1988/. The presence of configurations of more than two chromosomes susbstantiates the assumption that the genomes of the two species are partially homologous and interchromosome pairing occurs. A part of the chromosomes of the wild-growing specie H.scaberimus do not pair and are represented as univalents which, up to BC_2, are separated in the form of typical disturbance of the PMC phases. Populations /H.scaberimus x H.annuus/ providing opportunities for the selection of plants possessing valuable characters are obtained as a result of the hybridization. Male sterile plants have been produced and are presented for testing as well as plants with interesting economic characters such as with short stems of up to I m., with diameter of the inflorescence 25-28 cm., early blooming/up to. Yuly 15 / and early ripening with high protein content of the seeds.

CONCLUSIONS

Despite their valuable features, the use of tetraploid species H.hirsutus, H.decapetalus and H.scaberimus actually proved to be very difficult in interspecific hybridization with H.annuus. Low crossability and almost complete sterility of the hybrids resulting from considerable differences in their genomes, appear as a serious barrier for the transfer of valuable characters from the wild species to H.annuus.However, in the first backross with the cultivated species, single seeds of some combinations H.annuus x H.decapetalus, and the use of in vitro methods of H.annuus x H.hirsutus, H.scaberimus x H.annuus were obtained. It enable to include these species in the process of hybridization.

The cytological and fenotypical instability of BC_1 plants is very great. Plants with a completely normal course of meiosis are encountered along the plants in witch the disturbances during PMC reach 80 % /H.scaberimus x H.annuus/ The cytogenetical investigation of later backcross generations show gradual phenotypical and cytological stability. In some combinations, the restoration

of the basic haploid chromosome number takes place in BC_4. However, in all plants investigated, aneuploid cells have been observed. The presence of aneuploid cells /having one chromosome more or less/ registered in the examined BC_4 plants / H.scaberimus x H.annuus/ and BC_6 /H.annuus x H.decapetalus/ indicates that these plants do not repeat genotypically the initial female cultivar, despite of their phenotypical uniformity with the cultivated type. It is not easy to make conclusions about the degree of recombination between genetic material of the two species in the different combinations. However, the fact that the new form, although phenotypically uniform with the cultivated parent, differs from it in some new features - MS in the combination H.scaberimus x H.annuus and H.annuus x H.hirsutus in high seed productions, higher resistance to Sklerotinia and Plasmopara in H.annuus x H.decapetalus with high oil content, shows that the genome constitution of this phenotypically stabilized forms are not the same as that of the cultivated species. Probably some H.decapetalus, H.hirsutus and H.scaberimus chromosomes or part of chromosomes have been introgressed.

Our investigations show that the introduction of genetic material from wild-growing Helianthus species into the cultivated sunflower is possible, though this is a long and complicated process. The complications ensue from the fact that the genetic system of the cultivated sunflower is very different from, and thus incompatible with that of the representatives of the other taxonomic sections. Isolation mechanisms cause abortion of hybrid embryos at an early age or total sterility of plants. But using the suitable technique of embryo culture - in vitro developing of seeds, or some backcross, it is possible to overcome such incompatibility.

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Table I

CONFIGURATIONS OBSERVED IN DIAKINESIS OF BC_I-BC_5 HYBRIDS BETWEEN H.annuus AND SOME TETRAPLOID SPECIES

Generation	H.annuus x H.decapetalus / range/			H.annuus x H.hirsutus / range/			H.scaberimus x H.annuus /range/		
	bivalents	polyval.	unival.	bivalents	polyval.	unival.	bivalents	polyval.	unival.
BC_I	29 - 34	I	2 - 4	33 - 34	-	4 - 6	I7 - 34	I - 4	2 - I0
BC_2	I9 - 34	-	I - I0	22 - 28	-	4 - I0	I7 - 35	I - 4	I - 4
BC_3	I7 - 34	-	2 - I0	I6 - 32	-	2 - 4	I4 - 34	I - 5	2 -I4
BC_4	-	-	-	I6 - I7	-	2	I6 - 34	I	I - 4
BC_5	I5 - I8	-	I - 2	I6 - I8	-	I - 2	I6 - 28	-	I - 5

Table 2

DISTURBANCES OBSERVED IN PMC OF BC_I - BC_6 HYBRIDS BETWEEN

H. annuus AND SOME TETRAPLOID HELIANTHUS SPECIES

Generation	H. annuus x H. decapetalus			Poollen strain.	H. annuus x H. hirsutus			Poollen strain	H. scaberimus x H. ann.			Poollen strain
	M I	A II /range/	T II /range/		M I	A II /range/	T II /range/		M I	A II /range/	T II /range/	
BC_I	13- 23	8- 13	23- 25	0- 4	70	70	52	0	80	80	80	0-80
BC_2	77-100	24-100	28-100	0-50	90	55	60	-22	95-97	67-90	77-80	0-80
BC_3	6-100	5-100	0- 6	0-7	30-64	47-75	11-67	0-65	9-97	10-83	0-77	0-90
BC_4	-	-	-	-	4-22	4-16	3-10	0-90	1-52	1-65	4-53	0-90
BC_5	-	-	-	-	0-25	0-24	10	0-98	1-55	1-30	1-20	-90
BC_6	0- 99	0- 1	69-96	69-96	-	-	-	-	-	-	-	-