Cytogenetical Studies on the Hybrids and Back Cross Generations of the Cross *Helianthus annuus* ($2n=34$) $\times$ *Helianthus decapetalus* ($2n=68$)

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Genetic and cytogenetic studies on interspecific hybridization between cultivated sunflower and *Helianthus* species with different ploidy level are not numerous. Investigations are known only on cytogenetic analysis of genome constitution of hybrids between *H. tuberosus* ($2n=102$) and *H. annuus* ($2n=34$) (Kostoff 1939, Cauderon 1965).

Some results from the investigations on the possibilities to include several tetraploid *Helianthus* species in crosses with cultivated sunflower were discussed in our previous article (Georgieva-Todorova 1984). In this paper data summed up from the cytogenetic study of F_1 and BC_1 – BC_8 hybrids of *H. annuus* $\times$ *H. decapetalus* are presented.

Materials and methods

The cultivar Peredovik of *H. annuus* and the tetraploid form *H. decapetalus* were components of the hybridization. It is known that the latter species exists in nature as a diploid form also (Heiser *et al.* 1955).

The most important biomorphological characters of the hybrids produced: phenotype, earliness, type and colour of disk, pollen viability, plant fertility were studied and the type of segregation in BC_3 – BC_8 was phenotypically traced.

For meiotic studies anthers were fixed in modified Carnoy (3:1), pretreated with 4% aqueous solution of ferroammonium alum and stained in acetocarmine. 0.3% acetocarmine solution was used to determine pollen sterility. The chromosome configurations in diakinesis and the disturbances in metaphase I, anaphase I and telophase II were recorded in detail.

Results and discussion

F_1 *H. annuus* $\times$ *H. decapetalus*

Despite the great number of crosses (about 1000 inflorescences in the course of several years) only twelve well shaped seeds have been obtained. Six plants out of them reached complete development. They resembled both phenotypically and in their cycle of plant reproduction the wild growing parent, which was used as a pollinator in the cross. The inflorescences of these plants were small (5–12 cm) and with complete pollen and seed sterility. During the second year of growing the hybrids were back crossed with the cultivated species. It was obtained only 12 seeds.

Analysis of the course of meiosis in hybrid PMC revealed that complete pollen sterility is due to the pronounced disturbances typical for triploids. Univalents—from 9 to 17, and bivalents—from 17 to 22, were observed in all 20 cells of the hybrid 1/77 analysed in diakinesis. The percentage of cells with univalents was high and reached 100 in the hybrids 2/77 and 3/77. Disturbances in anaphase I (lagging chromosomes and chromosome bridges) varied from 70.16 to 82.70% in some plants.

BC₁ *H. annuus* × *H. decapetalus*

Only 4 plants developed from the 12 seeds obtained in the backcross of F₁ plants with the cultivated sunflower. They resembled phenotypically the wild parent but they occupied an intermediate position concerning leaf size and shape, inflorescence size, vegetative period, etc. Although the plants were perennial, their root system was poorly developed, without well formed rhizomes. Pollen viability was low (0% for hybrids 1/78 and 4/78). However, the hybrid plants 2/78 and 3/78 had relatively high pollen viability (57.70 and 33.34% respectively). All plants produced seeds, their number varying from 1 to 135 per plant.

Table 1. Meiosis in the BC₁ hybrids between *H. annuus* and *H. decapetalus*

Plant	Diakinesis	Metaphase I		Anaphase I		Telophase II		Viability of pollen (%)	Fertility of plants (%)
	Configurations (No. of cells)	Total cells	Disturbed cells (%)	Total cells	Disturbed cells (%)	Total cells	Disturbed cells (%)		
2/78	34II (20) 33II+2I (21) 32II+4I (4) 30II+4I+1IV (4)	357	13.77	267	8.85	208	23.08	57.70	0.53
3/78	34II (7) 33II+2I (24) 29II+4I+1IV (4)	170	23.53	336	13.39	209	25.36	33.34	4.23

Fig. 1. Viability of pollen (a) and fertility of plants (b) in BC₂ hybrid.

Data from investigations of PMC meiosis in the BC₁ plants are presented in Table 1. As evident, the percentage of disturbances at the individual phases was high. Cells with univalents reached up to 76%. The number of bivalents in diakinesis varied from 29 to 34. One quadrivalent was observed in individual cells. Generally, these data show that there are less disturbances in the course of the meiotic division of BC₁ hybrids. Therefore the pollen produced was with a relatively high percentage of viability.

BC₂ *H. annuus* × *H. decapetalus*

The larger number of plants at our disposal in this case provided an opportunity for a precise hybridological analysis. The plants observed in BC₂ were included in the following groups: of intermediate type with dominating features of the wild parent, of intermediate type with dominating features of the cultivated parent and typically intermediate plants. About 10% of the plants were perennial. It is interesting that the way of propagation is not in relation with a definite phenotype. In the progeny 4/78, for instance, out of three perennial plants one was of intermediate type, but with dominating characters of the parents, the second resembled phenotypically the cultivated parent and the third was of typically intermediate type. The pollen viability of the examined plants varied from 0% to 79.10% (Fig. 1a), and the fertility of the plants differed too (Fig. 1b). Highest percentage of plants belonged to the group with 0%–10% fertility. Comparison between the type of segregation of pollen viability with that of plant fertility showed that the latter is considerably lower.

The meiotic behaviour of the chromosomes in the BC₂ plants investigated differed from that in the BC₁ hybrids. Cells with incomplete chromosome pairing and cells with different

number of bivalents (15–35) and univalents (3–12) as well as single cells with one quadrivalent were observed in diakinesis. In metaphase I were found cells with univalents and in anaphase I cells with lagging chromosomes and chromosome bridge (Fig. 2). Cells with lagging chromosomes and micronuclei were observed in telophase II. As a result of these disturbances inviable microsporocytes were formed. Their percentage in the various plants and progenies

Fig. 2. Meiosis in BC_2 *H. annuus* $\times$ *H. decapetalus*. a, b, different chromosomal associations at diakinesis. a $\times 1980$, b $\times 2250$. c, metaphase I with preceding chromosomes. $\times 2250$. d, anaphase I with lagging chromosomes. $\times 1980$.

was different (Table 2). Plant fertility of BC_2 plants was still low although it was considerably higher than that of BC_1 . Only 8.62% of the plants belonged to the group with highest percentage of fertility (20–40%). Most of the plants belonged to the group with 1–10% fertility and 22.41% of the plants were completely sterile. These data show that despite the two backcrosses of F_1 hybrid between *H. annuus* $\times$ *H. decapetalus* with the cultivated parent chromosome disbalances were still great and caused low or complete pollen and seed sterility.

Table 2. Meiosis in the BC₂, BC₃ and BC₆ hybrids between *H. annuus* and *H. decapetalus*

Generation	Plant	Diakinesis Configurations (No. of cells)	Metaphase I		Anaphase I		Telophase II		Viability of pollen (%)	Fertility of plant (%)	Cycle of plant- repro- duction
			Total cells	Disturbed cells (%)	Total cells	Disturbed cells (%)	Total cells	Disturbed cells			
Second backcross (BC ₂)	3/79	25''+4', 17''+1', 32''+4', 34'', 19''+8'	119	100	18	88.88	39	71.79	50.48	14.46	annual
	4/79	19''+6', 20''+8', 27''+10', 30''+10'	194	100	27	100	74	100	0	11.61	annual
	29/79	30''+4', 31''+2', 33''+1', 30''+2'	143	77.62	37	24.32	17	28.00	50.25	26.08	annual
	33/79	33''+1', 32''+2', 31''+4', 30''+1'	76	78.94	24	37.50	33	30.20	1.80	0	perennial
	3a/80-25 9/80-5	34''+2'+1 fr (5) 20''+10', 34''+1 fr, 33''+2', 24''+4'	424 267	6.15 100	181 49	5.42 99.91	181 25	5.23 95.96	0 0	6.50 0.71	annual annual
Third backcross (BC ₃)	15/80-2 18/80-1	(15-19)''+(1-2)' 17''+7', 20''+2', 18''+3', 19''+5'	393 199	90.34 100	117 38	77.77 100	302 102	82.45 85.71	6.99 0	2.40 0	preenial perennial
	72/83-1	17''(26) 16''+2'(12) 16''(10) 18''(5)	654	99.06	240	1.04	408	91.20	69.0	94.5	annual
Sixth backcross (BC ₆)	71/83-2	17''+2'(6) 17''(15) 16''+2'(8) 15''+1'(10)	400	30	56	0.8	380	0	96.4	95.6	annual

Notice: The plants included in this table have been taken at random out of a total of 35 tested plants in BC₂, 15 plants in BC₃ and 12 plants in BC₆

BC₃-BC₇ *H. annuus* × *H. decapetalus*

The phenotypical diversity of the hybrids *H. annuus* × *H. decapetalus* was highest in the third backcross (Fig. 3). In this case along with the two intermediate groups of plants having dominating characteristic features of the male and of the female parent, a third group of typically mother's phenotype was differentiated. The last group presented a reversion to the cultivated species used as a mother parent. It is interesting to notice that new plants similar to *H. debilis*, were obtained too. Pollen viability in the different phenotypical groups observed in the hybrids varied between 0.3 and 89.15%. However the group of plants with highest plant viability was considerably higher than that of the previous generations. The percentage of plants with complete pollen and seed sterility was still high, 16.67%. Total sterility of plants was evident in 34.78% of the plants, indicating that the processes of female gametophyte formation and of embryo differentiation were still greatly disturbed. This is a fact often observed in crosses between genetically distant species.

Data from the analysis of PMC meiosis in 15 examined plants belonging to various phenotypical groups, parts of which are presented in Table 2, demonstrated the great diversity of chromosome number in the individual plants and proved their heterogeneity. Plant 3a/80, for example, which was of intermediate type of inheritance, had in diakinesis the following chromosome configurations—34II+2I+1 fragment, while in plant 18/80-1, also of intermediate type of inheritance but with dominating characters of the cultivated parent the number of bivalents in the various cells varies from 17 to 20 and of univalents from 2 to 7 respectively. In plant 26/80-1, besides cells with different number of bivalents in diakinesis and in the later phases disturbances of the kind described for the earlier generations, restitutional nuclei in separate cells were observed, too. As it is known the presence of different number of bivalents in one and the same hybrid plant in F₁ and in next generations obtained of genetically remote species is very often phenomenon (Georgieva-Todorova 1976, Fedak 1982, Georgieva-Todorova *et al.* 1984).

Elimination of chromosomes from aneuploid cells as a result of plants in later generations becomes cytologically stable. They are equal or similar in chromosome number to the low-ploidy level parent, *H. annuus*, with which the backcross was carried out. Pollen viability in BC₃ (Table 2) was still considerably reduced, but this was not related, as could be expected, with the plants phenotype. These plants of the cultivated sunflower type and with chromosome number relatively closer to that of *H. annuus* (17II+2I or 18II+5I) had 6.02% and 5.9% viable pollen respectively. Out of 456 BC₃ plants 30.70% were completely sterile (Fig. 3).

It became evident that after three backcrosses only single plants are with 40% seed fertility. 34.7% plants from the analysed were completely sterile. This shows that the set of chromosomes is similar to that of the cultivated species. Probably structural chromosome disturbances were of such nature that they had a pronounced influence on the normal formation of microsporocytes and the female gametophyte. While plants phenotypically are more quickly stabilized towards the cultivated species, the reproductive system of the plants reaches the normal state more slowly. For example, hybrid plants of several BC₃ progenies included for

Fig. 3. Viability of pollen (a) and fertility of plants (b) in BC₃ hybrid.

their phenotype in the group with intermediate mode of inheritance, but with dominating characters of the cultivated species and with relatively large inflorescences (12–15 cm compared to 3–5 cm for the wild growing parent) were with complete pollen and seed sterility.

Analysis of BC₃ progenies of plants which were male sterile in BC₂ showed that for this feature they segregated into plants fully sterile and plants with a considerably high pollen viability. In the progeny 49/80, out of the 20 analysed plants, 8 were completely pollen sterile and 12 had 2–72% viable pollen. However, in the progeny 2/80 out of the 9 plants analysed 8 were completely sterile and only one had 2.05% viable pollen. In the progeny 11/80 only 3 of the 24 plants analyzed were fully sterile and the remaining 21 had 1–98% viable pollen. Almost all plants produced seeds, but the range of variation of their fertility was very wide (3.67–81.25%). Data from the analysis for male sterility indicated that this was not a case of CMS, but a gene (nuclear) controlled sterility, which is a typical phenomenon in hybridization between genetically distant species. It was the same for the seed sterility in the late backcrosses.

Results of studying the progenies of further backcrosses—fourth, fifth, sixth backcrosses of F₁ *H. annuus* × *H. decapetalus* showed that the phenotypical diversity in their case was considerable limited. Out of the 96 progenies with a total of 1246 plants in BC₄, only 4 had the characters of the wild parent. The remaining progenies were phenotypically uniform with the cultivated species type and differed from it only by their smaller seeds. Some of these progenies are interest for sunflower breeding programs because of their higher resistance to *Plasmopara* and *Sklerotinia*.

Cytological analysis of 10 plants of BC₄ showed that most of the cells in diakinesis had 17II, i.e. a complete restoration of the female parents chromosome number had been achieved. In 15 of the examined plants, however, in diakinesis were observed cells (57.63%) with 18II or 17II+2I, which indicates that elimination of chromosomes from the *H. decapetalus* genome was not completed even after several backcrosses.

Data from the 800 analysed plants of BC₅ show that they are almost the same as these of BC₄, but only single plants had the type of the wild growing parent. BC₅ had been grown as a population obtained from selected BC₄ plants with valuable economic and biological characters. Out of 2126 plants only 14 deviated phenotypically from the cultivated sunflower. The remaining ones were relatively uniform, with heads with 18–20 cm in diameter. A hundred plants were selected at random for analysis of oil content in the seeds and resistance to *Plasmopara* and *Sklerotinia*.

Data obtained from the analysis show that oil content varied with in a relatively wide range (35.9–49.8%). Only plants with high oil content and with disease resistance are of interest for sunflower breeding. Selection for these characters goes on.

Cytological analysis of six plants (data about two of them are presented in Table 2) showed that of the cells described in diakinesis had 17 bivalents, therefore the trend toward cytological stabilization was evident. The different meiotic phases in the plants investigated had almost normal course.

Conclusions

The fact that single F₁ plants from the cross of *H. annuus* with the tetraploid form *H. decapetalus* were obtained indicates low gamete compatibility between these species. Heiser *et al.* (1969) showed that *H. decapetalus* is also not closely related with some diploid species of the section *Divaricati*, to which *H. decapetalus* belongs. Single hybrid plants have been produced from crosses with *H. laevigatus* (2n=68), *H. smithii* (2n=68) and *H. strumosus* (2n=102). The same author reports also for an obtained hybrid between *H. annuus* and the tetraploid form *H. decapetalus*, but since it was a sterile triploid its vegetative propagation was possible

only. Morphologically this hybrid was quite similar to *H. decapetalus*.

It is evident from the results of our study of F_1 hybrid plants that they are similar to the F_1 hybrids between these species produced by Heiser. The aim of our investigations, however, was to study the effect of backcrossing on the phenotypical and cytological stabilization of the hybrids in view of using *H. decapetalus* properties in *Helianthus* breeding. The study of BC_3 and BC_4 diversity which proved considerable is a fact observed for other crops by other authors (Bochev *et al.* 1978—for wheat, Baeva 1981—for *Triticale*, Douglas *et al.* 1971—for cotton, Ternovski—for tobacco, etc.). BC_5 and BC_6 generations are characterized with a considerable phenotypical and cytological stabilization. They resemble the cultivated species.

It is not easy to make conclusions about the degree of recombination between genetic material of the two species. The new form, obtained by us, is phenotypically uniform as the cultivated parent, but differs from it by some new features—higher resistance to *Sklerotinia* and *Plasmopara*, higher oil content. This fact shows that the genome constitution of this phenotypically stabilized form is not the same as that of the cultivated species.

The analysis (for the first time carried out in detail by us) of F_1 hybrids between *H. annuus* and *H. decapetalus* and the study on the effect of backcrossing on cytological and phenotypical stabilization substantiate the conclusion that *H. decapetalus*, although not very closely relating to *H. annuus*, could be used in hybridization programmes.

Summary

Results presented in this paper are concerning the hybridization between the cultivated sunflower (*Helianthus annuus*, $2n=34$) and the tetraploid species *H. decapetalus* ($2n=68$).

The analysis of F_1 hybrids between *H. annuus* and *H. decapetalus* and the study on the effect of backcrossing on cytological and phenotypical stabilization substantiate the conclusion that *H. decapetalus*, although not very closely relating to *H. annuus*, could be used in sunflowers hybridization programmes.

References

- Baeva, R. 1981. Cytogenetica i celekcia na *Triticale*. BAN, Sofia (in Bulgarian).
- Bochev, B., Ganeva, G. and Tyankova, N. 1978. A study on the effects of the genetic systems in chromosomes of the 3rd and 5th homologous groups of *Triticum aestivum* on its hybrids with *Aegilops*, *Agropyron* and *Secale* species. Cereal Research Communications 6(4): 377–386.
- Cauderon, J. 1965. Analyse cytogénétique d'hybrides entre *Helianthus tuberosus* et *Helianthus annuus*. Cons. en matière de sélection. Ann. Amel. Plates 15(3): 243–261.
- Douglas, C. 1971. A study of triploid and $3x-1$ aneuploid plants in the Genus *Gossypium*. Amer. J. Bot. 58: 1.
- Fedac, C. 1982. Effect of cultivar combination on meiosis in barley–wheat hybrids. Canad. J. Genet. and Cytol. 14(5): 575–582.
- Georgieva-Todorova, J. 1976. Mezduvidovi otnoshenia v rod *Helianthus*. M. Izd. BAN (in Bulgarian).
- and Lakova, M. 1979. Hybridization of diploid sunflower *Helianthus annuus* ($2n=34$) with some tetraploid *Helianthus* species. Zeitschr. für Pflanzenzüchtung 83: 340–349.
- 1984. Interspecific hybridization in the Genus *Helianthus* L. Z. Pflanz. 93: 265–279.
- Heiser, Ch. and Smith, D. M. 1955. New chromosome numbers in *Helianthus* and related genera (Compositae). Pr. of the Indian Acad. Sci. 64: 250–253.
- Smith, D. M. and Martins, W. C. 1969. The North American Sunflowers (*Helianthus*). Indiana Univ. Press, Indiana, USA.
- Kostoff, D. 1939. Autosynthesis and structural hybridity in F_1 *Helianthus tuberosus* L. $\times$ *H. annuus* Genetica 21: 285–300.
- Ternovski, M. F. 1966. Sozdanie novih form rastenii putem mejvidovoi gibridizazii. Genetica 10: 125–133.