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CYTOGENETIC STUDIES OF HYBRIDS HELIANTHUS BOLANDERI A. GRAY AND HELIANTHUS ANNUUS L.

Studies of the character of relationships between different species of the genus Helianthus are important for establishing their specificities. Such studies are even more important for the section "Annuii" which also includes the cultivated sunflower and for which specific delimitations based on morphological studies alone cannot be a sufficient criterion. Regardless of some cytogenetic studies in this section (Heiser, 1949, 1951; Georgieva, 1970, 1973), relationships between separate species have not been studied so far. Scholars belicoe (Heiser, 1949; Stebbins, 1950, et als) that introgression is a common phenomenon in morphologically close species and that it has played an important role in the separation of new species under natural conditions. The species H. annuus and H. bolanderi may serve as examples of such hybridization.

Generalizing our studies, we aimed to use experimental hybridization and detailed cytogenetic analysis in order to study relationships between these two systemically close species. On the other hand, introduction of H. bolanderi resistant to diseases into hybridization with cultivated sunflower may present interest to practical sunflower breeding.

H. bolanderi was used as initial source. It was grown in the field for several years. The Peredovik variety was used as the second initial source. Both species are annual diploids ($2n = 34$). Peculiar to H. bolanderi is the fact that the majority of its plants clearly show male sterility allowing their utilization as female pa-

rents. A number of biological properties were studies in hybrids.

To study meiosis in initial species and in hybrids, buds were fixed in acetalcohol in a certain stage of development and treated with acetocarmine according to the Schwach method. Meiosis was analyzed on 25-100 cells in diakinesis.

Chromosome behaviour during meiosis was studied by the following features:

a) pollen viability, judging by its ability to be coloured by acetocarmine;

b) fertility of plants under open pollination of initial species and hybrids.

Hybridization was carried out during four years under different weather conditions. Results show that in general crossability was low. Percentage of successful crosses in certain years ranged from 0.41 to 25.12%.

The first hybrid generation was characterized by diversity typical for hybrids obtained when crossing genetically distant forms. One part of hybrids inherits the wild form, another inherits the cultivated form, but the majority of plants are as a rule of intermediate type. Almost all hybrids are viable, but in telophase II retarding chromosome are observed. Pollen viability of H. annuus (Peredovik variety) is 98.0-100%, and that of H. bolanderi ranges from 0 to 38.10% for 1974 and from 0 to 23.2% for 1973.

Meiosis in FPC hybrids H. bolanderi x H. annuus in F_1 was almost normal in some plants. Bivalent associations were observed in diakinesis. In few cases associations of four chromosomes were observed in the form of closed or open ring. In metaphase I chromosomes advancing ahead are observed in some cells (2.44-5.38% of the total number). Residual chromosomes are observed in anaphase I and telophase II in 6.06-16.32% of cells. Only in few cells in anaphase I (in the hybrid 1681/74-1 in two cells out of 124) 1-2 chro-

mosome bridges were observed, and in the hybrid 1681/74-7 (in three out of 462 cells) micro nucleoli were observed. However, in spite of the normal meiosis behaviour the pollen viability in studied plants remains low, from 0 to 19.25%.

It is of interest to note that in some plants along with the formation of 17 bivalents one additional chromosome is observed in the form of univalent. Percentage of cells with an additional chromosome is variable. For example, in the hybrid 1969/74 from the total of 82 cells in diakinesis 17 bivalents and one univalent are observed or 15 bivalents, one quadrivalent and one univalent. In the hybrid 1502/75-3 in all 120 cells in diakinesis 17 vibalents and one additional chromosome were observed. As a rule, plants with additional chromosomes do not show serious deviations during meiotic phases. By their morphological properties these plants are intermediate ones, in some cases being similar to either of the parents. By the pollen viability rate and fertility these plants do not differ from the plants with normal chromosome number.

Following the detailed analysis of bivalent forms in diakinesis the average chiasma rate in bivalent has been calculated as well as the total chiasmata number for the cell. These values are variable in different plants, ranging from 1.04 to 1.23 for bivalents and from 18.0 to 20.83 for cells.

Meiosis in FPC hybrids in BC1 and BC2 is almost normal. Some deviations were observed similar to those indicated for F_1. Some plants show much larger deviations in meiosis. For example, in the plant 1502/75-13 101 cells out of 556 had unincluded chromosomes in metaphase I. In 13 cells from 22 in diakinesis additional chromosomes were observed. These plants showed some other deviations. Cells 2-3 times larger than normal ones ap-

peared with the viability of pollen being 65.12% and fertility 52.98%. BC2 progeny from the plant 1502/75 had some other deviations from meiosis as well as (chromatolysis, meiotic division delay in prophase). However, regardless of deviations, plants are able to form a certain percentage of normal pollen.

Studies of species H. bolanderi and H. annuus of section Annuii have shown that, regardless of their systemic position it is difficult to cross them under normal conditions. High pollen sterility of hybrids F_1, BC1 and BC2 and low seed fertility show their good isolation in nature, and using the Steffins classification (1950) their isolation mechanism could be defined as hybrid sterility. Considerable diversity of hybrids in certain morphological and biological properties also indicates their genetic distance. One and two backcrosses show that experimentally partial transfer of chromosomes from one species into another is possible. In this respect Heiser's supposition that these two species are introgressive in nature is rather reasonable. In our case it is interesting to determine the duration of backcrossing with an eye to obtaining a new species experimentally. Such studies were conducted by Lamprecht (1941). High sterility of hybrids after three backcrosses leads to a conclusion that hybridization between these two species under natural conditions is a lengthy process.

Absence of direct relationship between meiosis disturbances of male pollen cells and low viability of pollen indicate that sterility is genetically controlled. Normal chromosome distribution in telophase II in the majority of examined plants and formation of non-viable pollen suggest that there are qualitative differences between genomes of both species. High frequency of discovered bivalents also points to this, resulting in lower hiasma values in hybrids. The fact that quadrivalent associations are observed in some cells H. bolanderi

indicates that genomes H. bolanderi and H. annuus have different translocation, while quadrivalent associations in a small percentage of cells in F_1 hybrids and their predominant rod-like form indicate that translocation has taken place between short segments.

One additional chromosome during diakinesis in some hybrids suggests more serious chromosomal reconstructions of unknown nature.

Results of studies of BC_1 and BC_2 indicate that to obtain a hybrid of a cultivated sunflower type usable in practical breeding it is necessary to backcross hybrids to cultivated species.

The foregoing discussion suggests the conclusion that though H. bolanderi and A. annuus are sistematically very close they are genetically isolated from each other. Experimental hybridization has shown that though these species are not geographically isolated they possess isolation barriers accounting for their existence as two separate species. Though introgression is possible a long period is necessary for the genetic material of one species to find its place in the other species. In this respect introgressive hybridization is possible between these two annual Helianthus species.