A NEW MALE STERILE FORM OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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Interspecific hybridization is definitely reckoned of late as one of the promising methods for developing male sterile forms. Many data concerning the experimental transference of the genome of one species into the cytoplasm of another species are known in the scientific literature. Such data were reported in flax (Gaeski, 1973; Rogash, 1938, etc.), in tobacco (Kostoff, 1941; Smith, 1962; Clayton, 1950; Burg, 1960; Ternovski, 1964, 1966; Tzikov and Tzikova, 1966, etc.), in wheat (Kihara, 1951, 1968; Fukasawa, 1959; Wilson, 1962; Kihara, Tsunewaki, 1961; etc.), in potato (Koopmans, 1954, 1955; etc.), in cotton (Mayer, 1969) and in a number of other genera. By interspecific hybridization within *Helianthus* genus, transferring the genome of *H. annuus* into the cytoplasm of the wild annual species *H. petiolaris*, Leclercq (1966, 1969, 1971) obtained forms with cytoplasmic male sterility (CMS). Experiments for the development of sunflower forms with CMS through transference of the genome of one species into the cytoplasm of another species are in progress at the Experimental station in Pergamino, Argentina. The following annual species of *Helianthus* were used as initial material: *H. debilis* ssp. *cucumerifolius*, *H. argophyllus*, *H. petiolaris* and the wild species *H. annuus*. Spontaneous occurrence of CMS forms in *Helianthus* was reported by Gundaev (1966) and Volf (1966).

Vrânceanu (1970, 1973), Vrânceanu and Stoenescu (1960, 1971, 1972) studied genetically the forms with CMS obtained by them, and the use of these forms in hybrid sunflower breeding.

We have received a number of hybrids with many valuable characters which could be used in sunflower breeding. One of these valuable traits is the CMS. The object of this report is the study of a male sterile form of *Helianthus annuus* x *H. grosseserratus*.

MATERIAL AND METHODS

The investigation of the male sterile form was carried out in 1970—1973 period. The morphological, genetic, breeding and cytological peculiarities were studied. In 1973, 204 progenies with the total number of plants of 2000 were studied. Selection of male sterile plants with some valuable economic characters was made in view to their inclusion in further genetic and breeding studies.

RESULTS AND DISCUSSION

In the entire investigation of F_1 from different interspecific crosses including single hybrid plants obtained by crossing *H. annuus* to *H. grosseserratus,* we paid special attention to the behaviour of chromosomes at meiosis in pollen mother cells (PMC) in order to explain the causes of the low fertility which, as a rule, is a frequent phenomenon in crossing of remote forms. In one of the progenies of the above combination, four plants (two from progeny 988 and two from 922) with completely normal meiosis and absolutely sterile pollen occurred for the first time in 1967. The plants were extraordinary vigorous and above 3 m in height. Two of them were strongly branched. They flowered late (in the first decade of August) while the initial variety flowered normally in the first decade of July and matured in the beginning of October. At maturity it was found that the plants had normal fertility. This showed that these were cases of typically expressed male sterility but not male sterility with the ordinary unregular formation of the male gametophyte which was frequently observed in a large number of interspecific hybrids in other combinations, including different species of *Helianthus,* studied by us. The male sterile plants were pollinated with pollen from the cultivated parent.

The progenies of these plants preserved their character in BC_1. The plants were also of the same height and vigour. Some of them were strongly branched, others unbranched, but on the whole they were all with long growing period. A part of them proved again to have undisturbed meiosis, but they had completely sterile pollen and normal fertility..

Along with this type of plants, however, single plants occurred with more cultural phenotype and they approached the cultivated parent in seed type and length of growing period. Five plants of this type were again pollinated by the variety Peredovik. In the progenies of these plants (BC_2) sunflower cultivated forms appeared, but they were more vigorous than those typical for Peredovik, with large, densely arranged leaves, with big discs (20—25 cm) and full pollen sterility but with normal fertility. Since this form presented interest for sunflower breeding it was subjected to more detailed investigations.

In order to elucidate the type of sterility in the newly obtained form, genetic and cytological studies were performed. The variety Peredovik, used as female parent, was crossed to a hetrozygous for

male sterility (Msms) pollinator and the segregation was studied in F_2. The results showed that part of the progenies segregated to fertile and male sterile plants which enabled us to suggest that male sterility in this form is conditioned by the effect of a recessive nuclear gene because the genes for male sterility can be transferred by the male gametophyte and can exert their action independently of the S-factor of cytoplasm. Other authors have drawn a similar conclusion in their study of forms with gene male sterility (GMS) obtained spontaneously or experimentally in sunflower (Kuptzov, 1912; Putt and Heiser 1966; etc.). More than 100 progenies were analyzed in detail for pollen viability in 1973. The results showed that the ratio of male sterile to male fertile plants was quite different. This fact suggests that most probably the factors conditioning sterility are more than one and the genetic nature of this phenomenon is rather complex.

In order to find some character, such as branching of plants, anthocyanin pigmentation, etc., that is related to male sterility, a detailed analysis was made. The results indicated that there is no such relation. The plants therefore have to reach flowering and then the male fertile ones should be eliminated.

The moment of degeneration of microsporocytes was established by a more detailed cytological investigation on meiosis in pollen mother cells (PMC).

The newly obtained male sterile form was cytologically studied. The study comprised meiosis in PMC. In three of the progenies with highest percentage of male sterile plants, 3 to 5 plants were examined. Special attention was devoted to the character of sequence of the separate phases, and pollen viability. Fertility of the same plants was also scored. Some of the results from the study of maiosis are presented in table 1. The data show that the reduction division in PMC of these plants proceeded normally. Complete pairing was observed at diakinesis. The chromosomes paired forming 17 bivalents.

Table 1

Meiosis in the male sterile form 117/70

No. of the investigated plant	Cells observed at different phases of meiosis												
	Diakinesis		Metaphase I		Anaphase I			Metaphase II		Anaphase II		Telophase II	
	Normal cells with 17"	Deviations	Normal	Fast	Normal	Laggards	Bridge	Normal	Fast	Normal	Laggards	Tetrads	Triads
117/70	66	—	65	—	70	2	—	44	1	32	—	42	3
117/70	17	—	43	—	41	—	—	32	—	61	—	95	2
995/72	30	—	118	—	71	4	—	163	—	145	10	147	—
1130/73	17	—	23	—	27	—	—	12	—	20	—	52	—
1130/73	94	—	91	1	103	—	—	45	—	44	—	123	1
1164/73	64	—	53	—	62	1	—	70	1	129	—	64	—
1164/73	—	—	38	—	57	2	—	182	4	96	2	222	5
1164/73	70	—	91	9	128	8	7	53	—	56	—	115	2

At metaphase I all chromosomes were included in the metaphase plates. Anaphase I and II proceeded normally. Deviations from the type of bridge associations and laggards were observed in only small percentage (2—5) of the cells but such disturbances and at that rate are also found in the cultivated sunflower variety Peredovik. At telophase II, as a rule, normal tetrads with four microspores are formed. Degeneration of these microspores, however, takes place after the tetrad stages in the later phases of microsporogenesis and as a result of this completely sterile pollen is obtained. In some of the plants studied cytologically, the disturbances at microsporogenesis occurred before meiosis and due to this pollen grains did not form at all. These data thow that the moment of pollen degeneration cannot be used as a method for preliminary distinction of the two types of male sterility (GMS and CMS), as assumed by some authors (Hadjinov, 1968; Riabota, 1969; etc.).

The most interesting progenies of the male sterile form were evaluated in the laboratory for 1000 seed weight, percentage of husk, oil content in the kernel and seed. Part of the data are set out in table 2. They show that some of the plants are not of special interest due to their low 1000 seed weight and high percentage of husk (a character inherited from the wild parent *H. grosseserratus).* Another part of the

Table 2

Economic characteristics of some male sterile plants

No. of the investigated plant	Weight of 1000 seeds (g)	% kernel in the seed	% oil in the kernel	% oil in the seed
1246/16	62,80	77.70	67.16	52.18
1246/19	56.00	80.00	65.12	52.10
1246/10	77.60	78.86	58.79	46.36
1246/15	66.80	77.84	62.99	49.03
1246/17	64.40	77.63	63.57	49.35
1246/7	51.60	79.84	60.87	48.60
1246/4	51.60	82.55	58.33	48.15
1248/10	65.20	76.07	59.02	44.90
1248/15	62.60	77.31	59.43	45.94
1248/4	73.60	76.63	61.61	47.21
1246/5	55.60	78.05	60.87	47.51
1246/8	66.40	79.21	61.29	48.55
1246/3	70.00	80 85	57.59	46.56
1246/11	46.80	78.20	65.57	51.27
1246/18	66.40	79.21	61.29	48.55
1246/9	66.20	77.94	58.70	45.75
1246/6	70.00	80.85	57.50	46.49
1246/12	59.20	77.70	62.07	48.20
1246/1	47.60	79.41	59.76	47.45
1246/20	53.60	76.19	65.57	49.96
1157/6	37.20	77.41	56.67	43.87
1208/1	72.00	76.76	55.20	42.32
1211/1	40.80	78.43	55.33	43.39
Peredovik	86.60	78.06	62.60	48.86

plants, however, deserve interest for further investigation with a view to their using in hybrid sunflower breeding.

CONCLUSION

The preliminary investigation of the male sterile form studied by us showed that the interspecific hybridization between the cultivated sunflower and some perennial *Helianthus* species such as *H. grosseserratus* can serve as a source of such forms. The form with GMS obtained for the first time in the above cross presents interest and enriches the collection of the relatively small number of sunflower forms with genetic and cytoplasmic male sterility.

REFERENCES

Burg, L., 1960, Journal Heredity 51, 1, 27—31.
Clayton E., 1950, Journal Herdity 41, 7, 171—175.
Fukasawa, H., 1959, Japan J. Bot. 17, 55—91.
Gaevski, 1937, after Krupnov V., 1973, Gennaia cytoplazmaticeskaia mujskaia sterilnost rastenii.
Gundaev A. I., 1966, in : Sel. rast. ispolz. tzitopl. mujskoi steril., Kiev, 433—441.
Hadjinov M., 1968, in Heterozis. Teoria I praktika, 23—46.
Kihara, H., 1951, Cytologia 16, 177—193.
Kihara H., 1968, Seiken Ziho 20, 1—14.
Kihara H., K. Tsunewaki, 1961, Seiken Ziho 12, 1—10.
Koopmans A., 1954, Genetika, 27, 273—285.
Koopmans A., 1955, Genetika, 27, 384—390.
Kostoff D., 1941, Forschungsdienst, Sonderheft 14. S. 100—116.
Kuptzov A. I., 1935, Sotzialist. rastenievod, Leningrad, 14.
Leclercq P., 1966, Ann. Amél. pl. 16, 2, 135—144.
Leclercq P., 1969, Ann. Amél. pl., 19, 2, 99—106.
Leclercq P., 1971, Ann. Amél. pl., 21, 1.
Mayer V., 1969, Crop Sci., 9, 237—242.
Putt E. D., Heiser C. B., 1966, Crop Sci., 6, 2, 165—168.
Riabota A., 1969, Citologia i genetika, t. 3, 1, 22—27.
Rogash A., 1968, in Heterozis v rastenievodstve, 368—370.
Smith H., 1962, (Abstract) Genetics 47, 985—986.
Ternovski M., 1964, Tabak 2, 22—25.
Ternovski M., 1966, Genetika 10, 125—133.
Tzikov D., Tzikova El., 1966, Selskostopanska nauka 3, 3—15.
Volf V. G., 1966, in : Sel. rast. ispolz. tzitopl. mujskoi steril., Kiev, 422—423.
Vrânceanu V., 1970, Proc. Fourth. Intern. Sunfl. Conf., Memphis, U.S.A., 136—148.
Vrânceanu V., 1973, Ferma Intr. Agr. Stat. 25, 3, 6—8.
Vrânceanu V., Stoenescu Fl., 1969, Analele I.C.C.P.T.-Fundulea, *35*, C, 551—557.
Vrânceanu V., Stoenescu Fl., 1971, Analele I.C.C.P.T.-Fundulea, *37*, C, 177—182.
Vrânceanu V., Stoenescu Fl., 1972, Analele I.C.C.P.T.-Fundulea, *38*, C, 259—264.