NEW SOURCE OF CYTOPLASMIC MALE STERILITY (CMS) IN SUNFLOWER FROM INTERSPECIFIC HYBRIDIZATION *HELIANTHUS ANNUUS X H.HIRSUTUS* AND *IN VITRO* METHODS

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SUMMARY

New source of Cytoplasmic Male Sterility (CMS) obtained by interspecific crosses between the cultivated sunflower *H.annuus L.* and wild tetraploid perennial species *H.hirsutus Raf.* (2n=4x=68), of the reason to use *in vitro* methods. *In vitro* germination of seeds in BC_1P_1 and callus formation and plant regeneration from stem parenchima gave male sterile plants. Test with 3 lines fully maintain the male sterility in F_1 and show its cytoplasmic nature. The analysing crosses with 4 lines restoring the male fertility show that its restoration is the same as from *H.petiolaris.* On the basis of restriction endonuclease patterns of mitochondria DNA , a type of CMS tested is the same of *H.petiolaris.* These results confirm that CMS named B AN-2 (Bulg. H.annuus-2) is a very complicated phenomenon originated from interspecific hybridization and some genetical modifications by callus cultures.

INTRODUCTION

Cytoplasmic male sterility (CMS) is an extremely important factor for the heterosis breeding of sunflower, because the hermaphrodite structure of its flowers makes impossible the production of hybrid seeds.

The wild species are the main sources of sterizing cytoplasm and that why the basic method at the moment for obtaining of CMS for practical application is the interspecific hybridization (Seiler 1988). Among the cited in the literature about 24 sources of CMS, most are wild species - *H.petiolaris Nutt.* (Leclerq 1969, 1983; Whelan et al. 1980), *H.lenticularis Dougl.,H.annuus ssp.lenticularis* (Anaschenko 1974, Heiser 1982), *H.maximiliani L.* (Whelan 1978a), *H.giganteus L.* (Whelan et at.1980), *H.annuus ssp.texanus* (Vranceanu et al. 1986), *H.bolanderi Gray, H.petiolaris ssp.Falax, H.niveus ssp. canescens* (Serieys 1987) and others. However, there are reports for sources of CMS from the cultivated sunflower *H.annuus L.* (Gundaev 1966, Wolf 1966, Georgieva-Todorova 1975, Spirova 1988). This comes to show, that sterile cytoplasm is not a previlege of the wild specie .

Subject of this investugation is the cytoplasmic male sterility obtained by the interspecific cross between the cultivated sunflower *H.annuus L.* variety "Peredovik" and wild tetraploid perennial species *H.hirsutus Raf.* (2n=4x=68) of the reason to use *in vitro* methods..

MATERIAL AND METHODS

Experiments have been carried out on the field of Institute of Genetics (1975-1990) . Five to twenty crosses *H.annuus L.* (variety "Peredovik"without stigma) x *H.hirsutus Raf.* and 300 crosses-*H.hirsutus* x *H.annuus* var."Peredovik" were carried out.The hybrids were analysed by their most important morphological features. The cytological characterization and the viability of the pollen were investigated by the acceped methodology (Georgieva and Bohorova 1979). Murashige and Skoog media (1962) was used for inoculation and subcultivation, and for plantlets rooting - modified White (1963) medium. Explants of pith parenchima and stem meristema of the obtained hybrid *H.annuus x H.hirsutus* (male and female sterile) were used for induction of somatic calli and plant regeneration. Sterilization 0f the seeds and the materials from the field plants and the method of *in vitro* development was described previously (Bohorova 1982, Bohorova et al 1985). The plants in four progenies, wich were obtained though four backrosses with cultivated sunflower (Peredovik) were the subject of the experiments. These plants expressed the features of the cultivated sunflower and had high level of male sterility.

For testing the type of male sterility, some original and some outside inzucht lines (J) (which have proved their maintaining ability when the source is *H.petiolaris*), were used for the testing of the otaining male sterility (Spirova 1988, Spirova unpublished). The lines which have been used are the following 49/75, 233/3, 99/31, 113/7, 163/10 (personal ones) and HA-232 (USA). Therefore crosses of MS form in BC_4 (having number 1968) with the above mentioned test-lines were made and they were analized by the phenotype of the first progeny (F_1) Flowers were normal, protandric or male sterile. Viability of the pollen of the same progeny was examined by the acetocarmin method.

Test with 4 restoring lines (RF or R) RF 1201, RF 1216, RF 5566 and V 1581 were made for genetic identification of the sterilized cytoplasm of the studied male sterility. RF 1201 ,RF 1216, RF 5566 belong to the ICIC Fundulea (Rumenia) and posses three restorer genes Rf_1 - named as american (Kinman 1970), Rf_2- named as rumenian (Vranceanu and Stoenescu 1971) and the third-Rf_3-

rumenian Line V 1581 originated from Argentina (station Pergamino).

RESULTS AND DISSCUTION

Results from the hybridization of *H.annuus* with *H.hirsutus,* as well as cytogenetic characterization of the F_1 - BC_5P_1 were decribed in details in our former papers (Georgieva and Bohorova 1979, Bohorova and Georgieva 1987, Bohorova and Atanassov 1990).

We would like toreveal in brief the maintenance of the male sterile plants which are of great interest for the breeding of sunflower.

Hybrid plants combining the feature of both parents were obtained from the crosses between cultivated sunflower and the tetraploid *H.hirsutus Raf.* Plants were branchy with well developed main stem covered with trichoms with orange coloured raceme showing the presence of the wild *H.hirsutus* (10-18 cm diameter) with perennial cycle of development. The viability of the pollen was up to 18%. The backcrosses with the cultivar gave separate seeds. By *in vitro* germination of seeds (Bohorova 1982, Bohorova et al. 1985) callus formation and plant regenerations were obtained from the leave meristema and parenchima (Bohorova et al. 1985) in the medium with auxins and cytokinins added. We got many regenerated plants, only five of which reached their full growth. They were phenotypically uniform, male sterile, but the backrosses with cultivar gave 15% female fertility. Up to BC4 the number of the plants having the features of the cultivated sunflower rises up (57% of the new population are male sterile) (Fig.1). The considerable number of increased our attention on the selection of progenies by these feature

Cytological characteristics of the hybrid plants in BC4 showed the presence of 17 bivalents or 16 bivalents and 2 univalents, and the meiosis in PMS' s was normal with percentage of disturbances - Metaphase I - up to 22%, Anaphase I - up to 16% and Telophase II - up to 10%.

Results from the tests of the received in BC_4 male sterility are revealed in Table I . It can be seen that three of the father's lines used (49/75,113/7 and 163/10) fully maintain (100%) the male sterility in F_1 and one line (233/3) maintains it partly in F1 (from all the 9 plants - 7 are male sterility or this is 77.7%). With two lines - 99/31 and HA 232 there are a disintegration in F1 and the first one has a dominant number of male fertile (MF) plants (22 MF and 6 MS) and with the second , the number is equal.

Attention should be drown to the crosses with father's lines 99/31 and HA 232 with prevailing or equal quantity of MF and MS plants .The CMS lines fully maintain CMS with *H.petiolaris* as a source and the first line - also with *H.annuus* as a source (Spirova 1988). Line HA 232 is the fertyle analogue of the wellknown CMS line from USA with *H.petiolaris.* source. This means that full maintaining of CMS from *H.petiolaris* source comes out to be half-maintaining of CMS from *H.annuus x H.hirsutus* source. This makes us to think that this is a case of a source with sterilizing cytoplasm. which differs from this of *H.petiolaris.*

Table 1

Maintenance of Male sterility in F1 with *H.annuus x H.hirsutus* source of CMS

Cross	Maitenance %	Number of plants with type of flowers		
		Total	MS	MF
1 1268 MC x 49/75	100	48	48	0
2 1268 MC x 233/3	78.6	9	7	2
3. 1268 MC x 99/31	21.4	28	6	22
4. 1268 MC x 113/7	100	38	38	0
5. 1268 MC x 163/10	100	31	31	0
6. 1268 MC x HA 232	51.5	33	17	16

But, for the genetic identification of the cytoplasmic type,the attention should be directed also to the analyzing crosses with the restoring lines, results from which are presented in Table 1. It can be seen that two of the R-lines RF 1201 and RF1216, corespondingly the genes Rf_1,Rf_2, fully restore the male fertility in F_1. The presence of two CMS plants in the first restorer during 1980 could be only accidental as a result of technical negligence (oversight) and comes to confirm this statement. The other two lines restore partly (50%) the fertility (corespongly 60.6% and 34.8%). The information from the respective Argentina's line shows that R line V-1581 restores the fertility about 70%. We haven't any other information about restoring ability of line 5566, exept our observations for restoring the fertility with *H.petiolaris* sources (Spirova-unpublished data).The restoring of MF with this sources is also partly, as it is in our case.

It has to be made more precise if the cytoplasm of the cultivated sunflower from the source studied with only combination with the nuclea of the wild species *H.hirsutus* has a sterilizing effect. Does

SCHEMATIC REPRESENTATION OF OBTAINING CYTOPLASMIC MALE STERILITY (CMS) FORM BY INTERSPECIFIC HYBRIDIZATION (HELIANTHUS ANNUUS L. X H.HIRSUTUS RAF.) AND IN VITRO METHODS.

H.annuus L. (2n=2x=34) x H.hirsutus Raf. (2n=4x=68)

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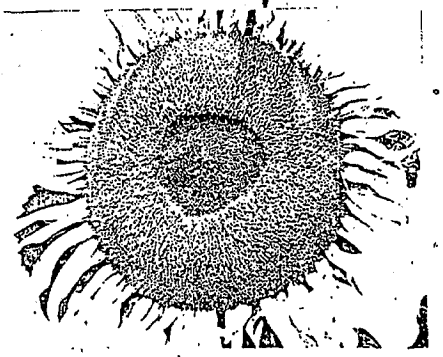
F_1 H.annuus x H.hirsutus (2n=3x=51)

In vitro germination of F_I seeds

Regeneration of plants from stem parenchyma callus

CMS plants(H.annuus x H.hirsutus)

34 chromosomes in root tip



17 II in diakinesis

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Test for CMS with different maintainer lines.

the obtaining of plants from callus by introducing of stem parenchima make possible the modification of the new form callus cells under the influence of the medium components.? As these plants exately come from such type of cells.? There is a possibility for genetic and cytoplasmic changes in them, something which can be observed in callus cells (Larkin et al. 1981, Evans 1984).

Analysis from the crosses with 4 lines restoring the male fertility show that its restoration from *H.annuus x H.hirsutus* is the same as from *H.petiolaris*. Therefore, on the basis of genetic analysis by the restoration we can make the conclution that its cytoplasm does not differ from this of *H.petiolaris*. But, the results from the maintenance of CMS show certain differences with one and the same maintenance test. These different conclutions only confirm that the CMS is a very complicated phenomenon and that only the genetic test at the organism level are not sufficient for the identification of the sterilizing cytoplasm. It is necessary a complex experimental work at cell and molecular level, which will allow to decide much more detailed differences between the cytoplasms that can't be done by macromethods.

Plant mitochondrial genomes have been intensively investigated on a molecular basis to clarify the mechanism of cytoplasmic male sterility (CMS) because several observations indicates that a major cytoplasmic factor of the CMS trait resides in mitochondria rather than in chloroplasts. On the basis of restriction endonuclease patterns of mitochondria DNA (mDNA) , a type of CMS line tested is the same of H.petiolaris (personal communication with M.Spassova carried out molecular analysis).

Our proposal for the name of the new source of cytoplasme male sterility (CMS) is Bulgarian H.annuus -2 (B AN-2) ,similar to the first Bulgarian CMS B AN-1 (Spirova 1988).

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