Transfer of New Gene Material from Wild *Helianthus* Species to Sunflower

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Abstract

Through utilization of interspecific hybridization between sunflower and wild *Helianthus* species a new potential of forms with useful for the sunflower culture qualities was produced. The investigations established that genetic material from 94 accessions of 6 annual and 20 perennial species was transferred into the hybrid material. Transfer of qualities, concerning resistance to pests, early maturity and fertility-restorer genes (Rf-genes) was registered still in F_1, and other qualities - in the next few generations. The applying of single and double backcross, sib-pollination and self-pollination made it possible not only to overcome the barrier of sterility of the interspecific hybrids, but to create sunflower forms with interesting for the breeding qualities. New material with resistance to *Plasmopara helianthi*, *Phomopsis helianthi*, *Phoma helianthi*, *Erysiphe cichoraceanum* and *Orobanche cumana*, with high seed oil content, early maturity and high seed size was produced with the participation of great number of annual and perennial species. Twelve new CMS-sources were obtained from 5 annual and 1 perennial species. Significant number of forms, carriers of fertility-restorer genes was obtained from 6 annual and 20 perennial species. R-lines were created from these forms with high combining ability and resistance to pests.

Key Words: - Sunflower, *Helianthus*, wild species, resistance, R-lines, CMS.

Introduction

The heterosis breeding is the main direction in the sunflower breeding during the last few years. The wild *Helianthus* species are one of the best opportunities for valuable utilization of the heterosis selection. According to Thompson et al.(1978) wild *Helianthus* germplasm besides contributing the basic stock from which cultivated sunflower originated, continues to contribute specific characteristics for sunflower improvement. A number of investigations show that *Helianthus* species present themselves as a rich genetic source for disease and pest resistance, cytoplasmic male sterility, fertility restoration, increased seed protein content, high oil quality and other features.

Saziperov (1916) first reported that a genetic material has been transferred from wild *Heliantus* species into cultivated sunflower. Marchenko (1975), Pustovoit (1960), Leclercq et al.(1970), Pustovoit (1975), Georgieva-Todorova (1976), Tsvetkova (1976), Skoric (1985), Jan and Chadler (1985), Christov (1990), etc., also report a transfer of genetic material and development of new sunflower forms with the participation of

wild *Helianthus* species. The discovery of the cytoplasmic male sterility (CMS) in sunflower (Leclercq, 1969) was very important for the heterosis breeding. The same was the significance of the fertility restoration gene (Leclercq, 1971; Fick et al., 1974; Skoric et al., 1978; etc.).

This paper examined the results from the study of the transfer of genetic material from wild *Helianthus* species into sunflower and the development of new cultivated sunflower forms.

Materials and Methods

The new cultivated sunflower forms have been created and investigated for a period of 12 years. Interspecific hybrids were used. They were obtained with the participation of 26 wild *Helianthus* species and cultivated sunflower, using reciprocal hybridization. From the annual species *H.annuus* (w.f.), *H.argophyllus*, *H.debilis*, *H.neglectus*, *H.petiolaris* and *H.praecox* (diploid - 2n=34) were included and from the perennial - *H.divaricatus*, *H.giganteus*, *H.glaucophyllus*, *H.grosseserratus*, *H.maximiliani*, *H.mollis*, *H.nuttallii*, *H.salicifolius*, *H.smithii* (diploid - 2n=34); *H.decapetalus*, *H.hirsutus*, *H.laevigatus*, *H.scaberimus*, *H.tomentosus* (tetraploid - 2n=68); *H.eggertii*, *H.ciliaris*, *H.pauciflorus* (*H.rigidus*), *H.resinosus*, *H.strumosus* and *H.tuberosus* (hexaploid - 2n=102). Material obtained with the participation of *H.x laetiflorus* (2n=102) was also studied. Cultivated sunflower was represented by fertile and male sterile forms. The material was investigated under field conditions. Phenological and biometric observations were made. The female fertility of the plants was determined by the quantity of seeds obtained under open pollination. The 1000 seed weight was calculated when 2 samples of 250 seed each were weighed. The seed oil content and the fatty-acid composition of the oil were determined according to standard methods. The resistance to pests and parasites was established by methods, approved and used in IWS (Panchenko, 1965, 1975; Pustovoit et al., 1976; Saliman et al., 1982; Tourvieille et al., 1988 and etc.).

For great part of the material the selection started in F_1. Self-pollination, sib-pollination and backcross were used to obtain next generations. The male sterile plants obtained from interspecific hybrid (wild species x cultivated sunflower) were pollinated with pollen from different sunflower inbreds and cultivars with the aim to establish the type of sterility and to maintain it. When fertile plants were produced from crosses between sunflower A lines (sterile analogues) and different accessions of wild *Helianthus* species, self-pollination was made. At the same time new male sterile plants were pollinated with pollen of F_1, F_2 or F_3 plants. All was done with the purpose to confirm the presence of fertility restorer genes. After repeated self-pollination the degree of restoring ability was determined for the newly obtained forms. Great part of these materials were included as participants in the heterosis breeding for the production of new hybrid combinations.

Results and Discussion

1. Some characteristics in the work with hybrid progenies.

The progenies of interspecific hybrids could be characterized by extremely wide formative process, which makes it possible that new sunflower forms are produced. The first hybrid generation is usually uniform in morphological features. The plants are intermediary in phenotype, with dominating features of the wild parent. The F_1 hybrids, obtained with the participation of the annual species, give sufficient seed set, when they are backcrossed or sib-pollinated once and sometimes even self-pollinated. It is possible that F_1 plants are pollinated with pollen of just one line or variety and sufficient quantity of seeds and F_2 plants are produced. These abilities of reproduction and production of next generation help to obtain forms, which could combine useful characters of both parents.

There are some difficulties, which go along with the producing of second hybrid generation of hybrids, obtained with the participation of perennial species, such as partial or total sterility of the F_1 plants. It is useless to self-pollinate them, because it is almost impossible to obtain seed set in this way. Great part of the F_1 plants do not give seed set even when they are open-pollinated. This is especially true for the hybrids, produced from *H.decapetalus*, *H.hirsutus* and *H.scaberimus*. We found out in our study that backcrossing (pollination with cultivated sunflower pollen) is the most successful procedure for overcoming this barrier. The pollen is collected from a group of lines and varieties. Part of it is mixed with pollen of F_1 plants (producing pollen) of the same combination. Those F_1 plants are pollinated with the new pollen mixture. This is the way, in which sufficient quantity of seeds and plants of the next hybrid generation are produced. In this way also the partial sterility of the F_2 hybrids is overcome.

Evaluation of the disease and parasite resistance of the F_2 hybrids is made mainly with the purpose to determine the character of genes, which control particular resistance. At the same time plant selection is made, especially if a resistance is combined with some other useful features. The heterogeneous character of the hybrids, regarding some features, favors selection even in first hybrid generation. When the generation number increases, selection becomes more purposeful. Sterile analogues of cultivated sunflower lines are used once or twice as maternal parents in order to produce materials, carriers of fertility-restoring (Rf) genes. It is preferable in the second case to use sterile analogues of two sunflower lines.

The specificity of obtaining F_2 (BC_1) and F_3 (BC_2) complicate in some cases the maintenance and evaluation of the useful characters, transferred from the wild *Helianthus* species. Nevertheless, it gives the opportunity to eliminate the undesired ones. The applying of successive repeated self-pollination for the production of next generations makes it possible to reduce great part of the features to homozygous state and to lead more effective selection of desired forms. Thus forms are developed with better self-fertility, higher seed oil content, etc.

2. Development of new sunflower forms

Sunflower forms were developed from the crosses cultivated sunflower x wild *Helianthus* species, in which the cytoplasm and half or greater part of the nuclear material belonged to the cultivated sunflower. Some of these forms had in their nuclear material Rf-genes, transferred from the wild parent. Thus, lines with normal cytoplasm without Rf nuclear genes and lines with normal cytoplasm with Rf nuclear genes, called R-lines, have been developed from this type of crosses.

Lines were produced from the crosses CMS sunflower lines x wild *Helianthus* species, in which the cytoplasm belonged to the wild species (*H.petiolaris* or *H.annuus* (w.f.), *H.argophyllus*, *H.debilis*, *H.praecox*, *H.rigidus*). Half or greater part of their nuclear material belonged to the cultivated sunflower and the Rf-genes - to the wild parent. The cytoplasm in this case is called sterile cytoplasm, because the plant material is male sterile. R-lines with sterile cytoplasm and Rf-genes have been produced from wild species.

New sunflower forms have been developed from the reciprocal crosses wild *Helianthus* species x cultivated sunflower, called alloplasmic lines. Their cell cytoplasm came from the wild species and greater part of the nuclear material - from the cultivated sunflower. By using these crosses genetic material was transferred into cultivated sunflower both from the nuclear and the cytoplasm of the wild parent. Some alloplasmic forms had Rf-genes in their nuclear material. When male sterile plants were obtained in the progenies and the male sterility was maintained, forms were developed from this type of crosses, which had the cell cytoplasm of the wild parent and the nuclear material only of cultivated sunflower. New CMS-sources were produced.

Great diversity of sunflower forms has been produced using the above-mentioned methods and successive purposeful selection, which would be useful mainly for the heterosis breeding. Most valuable are those forms, which combine genes for resistance to some diseases or Orobanche cumana and high seed oil content, good combining ability and etc.

There are five trends for development of initial breeding material:

a) Development of new sunflower forms with total or high resistance to Mildew, Phoma, Phomopsis, Sclerotinia, Powdery mildew and Broomrape.

The stress is put on the development of forms, carriers of resistance. The other characters, such as high seed oil content, high 1000 seed weight, vegetation period and etc. remain in the background. The already obtained resistant forms have been put into crosses with other forms, which had other valuable features in order to combine all these characters in one form and to develop lines with normal cytoplasm or R-lines. Eighty-seven accessions of 5 annual and 20 perennial species were used in the development of resistant forms (Table 1.).

b) Development of new sunflower forms, characterized by the predominating manifestation of the features: early maturity, short stem, seed size and kernel size, high seed oil content.

The species, from which these characters have been transferred in the newly obtained sunflower forms are presented in Table 1. It is difficult to combine all these features in just one form, but nevertheless, there have been produced some new forms, which had 2 or 3 and even the four features together. At the same time forms were obtained with transferred resistance to some of the pathogens, noted in Table 1. Several of these forms represent already fixed lines, which were directly included in the heterosis breeding.

c) Development of inbred sunflower lines with normal cytoplasm ("B" lines).

The newly obtained "B" lines originated from crosses cultivated sunflower x wild *Helianthus* species and wild *Helianthus* species x cultivated sunflower. The total number of the already fixed "B" lines, developed until 1994 was 36. The stem height varied from 80 to 170 cm and the vegetation period - from 92 to 125 days. The 1000 seed weight varied from 35 to 118 g and the oil content - from 37 to 53%. Some "B" lines had a high percentage of Phomopsis resistance; others - total resistance to Downy Mildew and Broomrape. A characteristic of some "B" lines is presented in Table 2.

d) Development of inbred sunflower lines, carriers of Rf-genes (R-lines).

R-lines originated from crosses cultivated sunflower x wild *Helianthus* species , CMS cultivated sunflower x wild *Helianthus* species and wild *Helianthus* species x cultivated sunflower. Ninety-four accessions of 26 species were used for their development. Eighty-one R-lines have been fixed till now. Except for 2, all of them had total resistance to Downy Mildew. Some R-lines have been resistant to Phomopsis and Broomrape too. Those of them, which did not have resistance to Mildew, were totally resistant to Phomopsis. Line R-7006 was remarkable for her high resistance to Sclerotinia (under artificial infection with sclerotia). Two lines were notable for their high resistance to Phoma. All R-lines had a high combining ability. Data for some R-lines is given in Table 3.

e) Development and investigation of new CMS-sources.

The searching for new CMS-sources was combined with the task to develop alloplasmic sunflower lines on the basis of the cytoplasm of different wild species. It was established that sterile forms could be obtained in different generations - from F_1, BC_1 to F_5. It became necessary to use greater number of pollinators. Twelve CMS-sources have been obtained until 1994 (Table 4.). In none of the CMS-sources a negative effect of the cytoplasm was observed. The plants, produced on the basis of these CMS-sources developed normally. The seed productivity of the sterile analogues, based on the new CMS-sources was equal to that of the inbred lines ("B" lines).

Conclusion

The methods of interspecific hybridization and the hybrid material produced made it possible to develop new potential of forms with valuable for the sunflower crop qualities. New forms were produced with resistance to diseases and parasites, with economical importance for cultivated sunflower; new forms, remarkable for their short vegetation period, short or medium high stem, high oil content. On this basis 36 "B" lines and 81 R-lines have been developed, which were included as parents in the heterosis breeding. Twelve new CMS-sources have been obtained from the hybrid material. Thus, opportunities appeared to produce new hybrid combinations.

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Table 1. Characters, transferred from wild *Helianthus* species into the cultivated sunflower.

Characters	Species
Full resistance to:	
Plasmopara helianthi	*H.annuus* (w.f.), *H.argophyllus*, *H.debilis*, *H.petiolaris*, *H.praecox*, *H.divaricatus*, *H.giganteus*, *H.glaucophyllus*, *H.grosseserratus*, *H.mollis*, *H.maximiliani*, *H.nuttallii*, *H.salicifolius*, *H.smithii*, *H.decapetalus*, *H.hirsutus*, *H.laevigatus*, *H.scaberimus*, *H.eggertii*, *H.ciliaris*, *H.pauciflorus*, *H.resinosus*, *H.strumosus*, *H.tuberosus* and *H.x laetiflorus*
Phomopsis helianthi	*H.annuus* (w.f.), *H.argophyllus*, *H.eggertii*, *H.pauciflorus* and *H.glaucophyllus*
Erysiphe cichoracearum	*H.decapetalus*
Orobanche cumana	*H.tuberosus*, *H.argophyllus*, *H.pauciflorus* and *H.strumosus*
High percent resistance to:	
Phoma helianthi	*H.argophyllus* and *H.laevigatus*
Sclerotinia sclerotiorum (infection with mycelium)	*H.praecox*, *H.argophyllus* and *H.annuus* (w.f.)
Earliness	*H.praecox*, *H.scaberimus*, *H.glaucophyllus*, *H.giganteus*, *H.rigidus*, *H.nuttallii*, *H.ciliaris* and *H.annuus* (w.f.)
Seed size	*H.annuus* (w.f.), *H.argophyllus*, *H.tuberosus*, *H.strumosus*
High oil content	*H.annuus* (w.f.), *H.debilis*, *H.petiolaris*, *H.praecox*, *H. x laetiflorus*
Genes, controlling CMS	*H.annuus* (w.f.), *H.argophyllus*, *H.debilis*, *H.petiolaris*, *H.praecox* and *H.pauciflorus*
Rf - genes	*H.annuus* (w.f.), *H.argophyllus*, *H.debilis*, *H.neglectus*, *H.petiolaris*, *H.praecox*, *H.divaricatus*, *H.glaucophyllus*, *H.giganteus*, *H.grosseserratus*, *H.maximiliani*, *H.mollis*, *H.nuttallii*, *H.salicifolius*, *H.smithii*, *H.decapetalus*, *H.hirsutus*, *H.laevigatus*, *H.scaberimus*, *H.tomentosus*, *H.eggertii*, *H.ciliaris*, *H.resinosus*, *H.pauciflorus*, *H.tuberosus* and *H. x laetiflorus*

Table 2. Characteristics of "B" lines, obtained from interspecific hybridization.

N	Origin	Plant height (cm)	Head diameter (cm)	Seed oil content (%)	Vegetation period (days)
93-1159	H.pauciflorus - M-028	155	15	48.79	105
93-1170	H.strumosus - M-056	110	12	47.82	110
93-1191	H.decapetalus - M-043	150	16	52.54	109
93-1202	H.hirsutus - M-029	105	12	45.25	105
93-1215	H.salicifolius - M-045	180	18	51.15	107
93-1217	H.x laetiflorus - M-005	120	17	49.72	110
93-1224	H.annuus - E-002	160	20	51.60	115
93-1275	H.argophyllus - E-007	140	23	49.96	105
93-1291	H.debilis - E-011	155	24	47.10	108
93-1305	H.debilis - E-014	150	21	52.68	108
93-1310	H.petiolaris - E-034	135	16	49.67	105
93-1330	H.praecox - E-029	125	16	45.34	113

Table 3. Characterization of R-lines, obtained from interspecific hybridization.

N	Origin	Plant height (cm)	Head diameter (cm)	Vegetation period (days)	Seed oil content (%)	Gene ration
7004R	H.praecox - E-028	145	16	112	52.64	10*
7006R	H.praecox - E-028	120	18	98	46.71	12
7009R	H.tuberosus - M-037	80	13	92	45.99	12*
7011R	H.annuus - E-004	145	15	103	46.85	11*
7015R	H.debilis - E-011	120	15	102	52.73	10*
7017R	H.praecox - E-028	145	16	110	52.10	12*
7024R	H.tuberosus - M-004	140	25	105	48.95	11
7026R	H.smithii - M-008	140	14	106	45.34	11*
7027R	H.x laetiflorus - M-005	135	17	102	48.86	11*
7041R	H.eggertii - M-001	120	15	101	47.21	11*
7042R	H.pauciflorus - M-028	130	15	106	49.13	11*

Table 4. New CMS-sources, obtained from interspecific hybridization until 1995 in IWS "Dobroudja", Bulgaria.

New CMS-sources	Numbering according to FAO	Origin	Obtained, Year
AN - 67	ANN - 10	H.annuus - E-067	1986
AN - 58	ANN - 11	H.annuus - E-058	1988
AN - 2 - 91	ANN - 12	H.annuus - E-002	1991
AN - 2 - 92	ANN - 13	H.annuus - E-002	1992
ARG - 1	ARD - 1	H.argophyllus - E-006	1985
ARG - 2	ARG - 2	H.argophyllus - E-007	1985
ARG - 3	ARG - 3	H.argophyllus - E-006	1987
DV - 10	DEB - 1	H.debilis - E-010	1990
PHIR - 27	PRH - 1	H.praecox - E-027	1990
PRUN - 29	PRR - 1	H.praecox - E-029	1989
Pet - 34	PET - 4	H.petiolaris - E-034	1991
Rig - 28	RIG - 2	H.rigidus - M-028	1991