Contribution of interspecific hybridization to the sunflower breeding

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ABSTRACT

- This investigation is directed to sunflower improving, using hybrid forms resulted from interspecific hybridization. The aim is creating of new B/A and R lines from interspecific hybrid forms, resistant to diseases, parasite broomrape, herbicides, other stress factors and characterized with high combining ability and to obtain on this base the high productive oil-type sunflower hybrid cultivars with varied fatty acid content of oil.
- The investigation was carried out during the period 1983-2010. There have been included 16 cultivars and 18 B lines with their analogues as well the hybrid material, originated from 38 *Helianthus* species - 9 annuals and 29 perennials.
- Intraspecific hybridization and purposeful selection were the used methods. Crossing between interspecific hybrids and crossing of interspecific hybrids with sunflower cultivars or lines were applied. Self-pollination, sib-pollination, backcross with pollen from cultivated sunflower and pollination with pollen from different interspecific hybrids were carried out. Phytopathological and biochemical evaluation of seeds and plants, and morphological characterization of sunflower forms, lines and hybrids were carried out.
- New sunflower forms and lines were created as a result of this investigation. Greater part of them possess resistance to downy mildew, phomopsis, phoma and alternaria, tolerance to sclerotinia and full resistance to the different races of parasite broomrape. The new forms were distinguished with new plant architectonic, different vegetation period and seeds with different size and coloration. New B/A and R lines, characterized with high combining ability, seed oil and fatty acid content were obtained. Fifteen sources of cytoplasmic male sterility /CMS/ were obtained from interspecific hybrid forms as well as 251 sources of genes for restoration of fertility /*Rf* genes/. Five new hybrid cultivars oil-type, which were obtained on the base of these lines, were created and registered.
- The obtained results from the investigations showed that by interspecific hybridization new genetic material was transferred to the cultivated sunflower. These results supplemented the contribution of interspecific hybridization for sunflower breeding.

Key words: *Helianthus* - hybridization- hybrids - lines - sunflower

INTRODUCTION

The implementation of interspecific hybridization with practical purposefulness began at the beginning of XX century with Saciperov's attempts (1916). These and other later investigations showed, that as a result of crossing of different *Helianthus* species with cultivated sunflower new sunflower forms resistant to different diseases and the parasite broomrape could be obtained (Pustovoit V., 1960; Pustovoir G., 1975; Put and Sackston, 1957 and 1963; Leclercq et al., 1970; Fick et al., 1974; Skoric, 1985; Jan and Chandler, 1985; Christov, 1990; Christov, 1996; Christov et al., 1996; Hristova-Cherbadji, 2007; Christov, 2008 and etc./. Leclercq (1969) found the first CMS source originated from the cross between *H. petiolaris* Nutt. and cultivated sunflower. Other CMS sources were found by Whelan (1980), Vranceanu et al. (1986), Serieys and Vincourt (1987), Christov (1990a), Christov (1999) and etc. Soon after discovering of the first CMS source, the sources of *Rf* genes were also found by Kinman (1970) and Enns et al. (1970). The discoveries of Leclercq (1971), Fick et al. (1974), Christov and Petrov (1988) and etc. followed in this direction. Investigations of *Helianthus* species were carried out in some other directions.

In this report were presented the results of investigation of interspecific sunflower hybrids and using of obtained hybrid material for developing lines with economically important characters suitable for parental components of new sunflower hybrid cultivars.

MATERIAL AND METHODS

The investigations were carried our in Dobrudzha Agricultural Institute, General Toshevo during the period 1983-2010.

Plant material

In the investigation were included 16 cultivars of cultivated sunflower *Helianthus annuus* and 18 lines and their sterile analogues and hybrid material originated from 38 *Helianthus* species.

Methods used

Methods of intraspecific hybridization and selection were used. They were expressed by crossing between interspecific hybrids and crossing of interspecific hybrids with sunflower cultivars and lines. Self-pollination, sib-pollination, backcross with pollen from cultivated sunflower and pollination with pollen from different interspecific hybrids were implemented.

Evaluation for resistance to diseases and parasite broomrape of the studied plant material was carried out on methods confirmed in the institute (Panchenko, 1975; Tourvieille et al., 1988; Christov, 1990; Christov et al., 1992; Christov, 1996; Christov et al., 1996; Fernandez-Martinez et al., 2000; Encheva and Kiryakov, 2002; Christov et al., 2004; Shindrova, 2006).

Seed oil and protein content and fatty and amino acid content was evaluated according to methods confirmed in the institute (Rushkovskii, 1957; Stoianova and Ivanov, 1968; Ivanov et al., 1996). Apparatus Nuclear Magnetic resonance for evaluation of seed oil content and Analyzer for amino acids Hitachi, L-8500 were used.

Morphological characteristic was made on the base of phenological observations and biometric measurements during the vegetation period and on laboratory studies of whole plants and of obtained seed material from them.

Sources of CMS were searched among materials obtained from crosses **wild species** x **cultivated sunflower**. For obtaining of sterile inflorescences the pollination was carried out with pollen from B lines or cultivars.

First of all sources of *Rf* genes were searched in crosses **sterile sunflower lines** x **wild species**. Presence of *Rf* genes in the genome of wild species was established in F_1. Forms with *Rf* genes were found also in materials obtained from crosses cultivated sunflower /B line or cultivar/ x wild species and wild species x cultivated sunflower.

Developing of sunflower B lines was carried out by purposeful selection in the hybrid materials, which in most cases began after third generation. Evaluation and selection of materials began on the base of their morphological, biochemical and phytopathological characteristics, absence of *Rf* genes and presence of good combining ability.

Developing of sterile analogues - A lines began with establishment of the fact that there are not any *Rf* genes in the studied material. After BC_3 or BC_4 began study of general combining ability of created A lines, and after that their specific combining ability.

Creating of self-pollinated lines, restorers of fertility - R lines was carried out mainly from crosses of **male sterile lines with different wild *Helianthus* species**. Purposeful and repeated selection and self-pollination of fertile plants was implemented till obtaining of homozygous *Rf* genes. The obtained R lines, which possess 100 % fertility restoration and other important characters were collected from

both parental forms included in the hybridization. For creating of R lines could be used hybrids, obtained from crosses **cultivated sunflower x wild species** and **wild species x cultivated sunflower**.

RESULTS AND DISCUSSION

Origin of interspecific hybrids

As a result of hybridization between sunflower *Helianthus annuus* and 38 species from genus *Helianthus* totally 67 000 F1 hybrid plants were obtained from all species included in the investigation (Table 1). Despite F_1 hybrids originated from *H. simulans* all other F_1 hybrids gave seeds.

Table 1. Species of genus *Helianthus*, used in hybridization.

Groups of species	Species
Annual species (2n=34)	*H.argophyllus, H. bolanderi, H.debilis,H. exilis, H.neglectus, H. paradoxus, H.petiolaris, H.praecox, H.annuus (w.f.)* **
Perennial diploid species (2n=34)	*H.divaricatus, H.doronicoides*, H.giganteus, H.smithii, H.glaucophyllus, H.grosseserratus, H.maximiliani, H.microcephallus, H.mollis, H.nuttallii, H.occidentalis, H.orgialis*, H.pumilus, H.salicifolius, H.silphioides, H. simulans*
Perennial tetraploid species (2n=68)	*H.decapetalus, H.hirsutus, H.laevigatus, H.scaberimus *, H.tomentosus **
Perennial hexaploid species (2n=102)	*H.eggertii, H.pauciflorus /rigidus/, H.strumosus, H. resinosus, H.tuberosus, H.ciliaris, H.x laetiflorus, H.californicus*

*Not included in classification of Shilling and Heiser (1981); **Wild form.

Creating of new sunflower forms from interspecific hybrids

The main reason for including wild *Helianthus* species in the research work on biological improvement of sunflower crop was the presence of resistance to diseases, parasites and pests. Studies on downy midew resistance were the priority and they were followed by studies on sclerotinia, phomopsis, phoma, aternaria resistance and etc. Great priority was given to studies on broomrape resistance.

New sunflower forms resistance / tolerance to diseases and parasites

Among the hybrid forms with resistance to diseases and parasite broomrape, the highest percentage were those with resistance to the pathogen *Plasmopora heliathi*.

Full resistance to *Plasmopora heliathi*, races № 700 showed more than 2600 accessions, obtained with participation of 36 *Helianthus* species /tables 1/. Resistance to races № 731 considered as the most virulent in Bulgaria was established in more than 400 hybrid forms, originated from the species *H. divaricatus, H. hirsutus, H. pauciflorus* (*rigidus*), *H. debilis, H. paradoxus* and etc. Some of these forms possessed resistance to some other diseases and to the parasite broomrape (Table 2).

Table 2. Characterization of sunflower lines, obtained by interspecific and intergeneric hybridization, resistant to downy mildew - race 731, harvest 2009.

Accession, pedigree	Resistance to		Seed oil content, %	Generation
	downy mildew, %	broomrape, %		
PR-1/8 /c.s. x *H.pauciflorus*/	100	100	48.48	23
PR-9/8 /c.s. x *H.tuberosus*/	100	100	47.27	25
PR-13/8 /c.s. x *H.pumilus*/	100	-	58.28	16
PR-25/8 /c.s. x *H.pauciflorus*/	100	100	46.89	25
PR-35/8 /c.s. x *H.hirsutus*/	100	100	48.80	16
PR-41/8 /c.s. x *H.divaricatus*/	100	100	47.03	18

*unbranched form

Resistance/tolerance to the pathogen *Phomopsis helianthi* showed more than 80 forms. They originated from species *H.annuus* (w. f.), *H. argophyllus, H. debilis, H. glaucophyllus, H. laevigatus, H. eggertii* and *H. pauciflorus.*

High resistance to *Phoma helianthi* was established in several forms, obtained with participation of the species *H. eggertii, H. laevigatus, H. argophillus* and *H. debilis.*

Studies on sclerotinia resistance /*Sclerotinia sclerotiorum/* were carried out in field conditions and in greenhouses. Different ways of artificial inoculation were applied and as the most effective was that with direct micellium setting in different uncovered parts of the plant. (Christov et al., 2004). High tolerance to *Sclerotinia sclerotiorum* showed some forms originated from *Helianthus eggertii, H. pauciflorus, H. smithii, H. praecox, H. petiolaris, H. argophyllus, H. annuus* (w f.). This tolerance referred to those pathogens forms, which infected the head, stem, and the basal part of sunflower stem.

Full resistance to powdery mildew (*Erysiphe cichoracearum* D. C.) was established in hybrid forms originated from species *H. decapetalus, H. glaucophyllus, H. giganteus, H. mollis, H. ciliaris, H.*

laevigatus, H. debilis, H. tuberosus, H. resinosus. The resistance transferred from the species *H. decapetalus* was determined by a single dominant gene.

The investigations on alternaria resistance (*Alternaria helianthi* (Hansf.) Tubaki and Nishihara and *Al. zinniae* Pape) began later. The more detail study of wild species was done during the period 1985-1989. At that time the first crosses for creating hybrid forms with resistance to alternaria were carried out. After that only hybrid forms were tested. During the last years the method of Encheva and Kiryakov (2002) was applied. Some of the obtained results were presented in table 3.

Table 3. Characterization of sunflower lines, obtained by interspecific and intergeneric hybridization for resistance to Phomopsis, Phoma, Alternaria and Sclerotinia.

Acession, pedigree	**Resistance to, grades**			
	Phomopsis, gr. 0-4	**Phoma, gr. 0-4**	**Alternaria, gr. 0-4**	**Sclerotinia, gr. 0-5**
Sc-2 L-6116B	1	0	0	2
Sc-3 /c.s. x *H. debilis*/	0	0	1	2
Sc-5 /c.s.x *H.pauciflor.*/	2	0	3	0
Sc-8 /c.s. x *H. argophyllus*/	0	0	0	0
Sc-9 /c.s. x *H. argophyllus*/	0	0	0	1

Races E, F and G of the parasite broomrape (*Orobanche cumana* Wallroth.) were spread in Bulgaria. The last two races appeared shortly one by another and this aggravated the sunflower breeding. During the last 20 years a sufficient number of sunflower lines resistant to race E of the broomrape were developed. From 2008 the aim of the breeding work was directed to developing lines resistant to race G of the parasite.

In creating of resistant to broomrape forms 16 wild *Helianthus* species were used (*H. tuberosus, H. pauciflorus, H. eggertii, H.* x. *laetiflorus, H. decapetalus, H. hirsutus, H. divaricatus, H. giganteus, H. maximiliani, H. nuttallii* ssp. *rydbergi, H. salicifolius, H. smithii, H. annuus* /w. f./, *H. argophillus, H. debilis, H. petiolaris* and *H. praecox*). Full resistance to the parasite showed some new lines such as 7019 R, 7203 R, C 23/1, C 41, C 46, C 48, C 55, C 56 and etc (Table 4).

Table 4. Characterization of sunflower lines, obtained by interspecific and intergeneric hybridization and resistant to broomrape, harvest 2009.

Accession, pedigree	**Resistance to**		**Seed oil content, %**	**Generation**
	broomrape, %	**downy mildew, %**		
PR-1/8 /c.s. x *H.pauciflorus*/	100	100	48.48	23
PR-9/8 /c.s. x H.*tuberosus*/	100	100	47.27	25
PR-19/8 /c.s. x *H.divaricatus*/	100	100	45.25	19
PR-25/8 /c.s x *H.pauciflorus*/	100	100	46.89	25
PR-35/8 /c.s x *H.hirsutus*/	100	100	48.80	16
PR-41/8 /c.s. x *H.divaricatus*/	100	100	47.03	18
PR-47/8 /c.s. x *H.bolanderi*/	100	100	50.44	19

New sunflower forms with high seed oil content

Some accessions of wild *Helianthus* species could be used as sources for high seed oil content in sunflower. This conclusion is base on results established for different hybrid forms, obtained by applying of interspecific hybridization. Sunflower forms and lines with high seed oil content were obtained from hybrids with participation of the species *H. eggertii, H. pauciflorus /rigidus*/, *H. smithii, H. hirsutus, H. annuus* (w. f.), *H. nuttallii* ssp. *rydbergii, H pumilus* and etc. Some results for seed oil content were presented in tables 2, 4 and 6.

New sources of CMS

The total number of the new CMS sources was 15 (table 5). Some of the sources distinguished from CMS PET 1 significantly. For all sources were found genes restorers of fertility.

New sunflower forms with Rf genes /R lines/

Till now were selected and obtained more than 3900 new R forms including 1306 R lines which are fixed and named. All of them are resistant to downy mildew. Some of them were resistant to phomopsis and broomrape. There were lines, which showed resistance to phoma and others - even tolerance to sclerotinia. Part of these lines is presented in table 6.

New sunflower forms with normal cytoplasm /B lines/

New B lines were created only from forms, obtained by interspecific hybridization. The total number of developed /fixed/ "B" lines till 2010 is 289. The stem height varies from 45 to 180 cm, and the vegetation period - from 86 to 125 days. Thousand seed weight varies from 30 to 125 g, and the

seed oil content - from 40 to 54%. Some B lines show resistance to phomopsis and others - to downy mildew and broomrape. Such lines are 6066B, 6101B, 6134B, 6149B, 6488B, 6748B and etc. Sterile analogues were developed for all B lines in CMS PET 1. Sterile analogues for the rest CMS sources were created for four lines from the new lines with aim to supply their use in some experiments as evaluation of the cytoplasmic effect on some agricultural features of the new hybrids.

Table 5. Sources of CMS produced by interspecific hybridization.

Origin	Obtain in generatin	Year of obser-vation	Year report	DAI code	F.A.O. code
H. annuus E - 067	F_1	1985	1992	AN-67	ANN-10
H. annuus E - 058	F_6	1988	1994	AN-58	ANN-11
H. annuus E - 002	F_5	1991	1991	AN-2-1	ANN-12
H. annuus E - 002	F_6	1992	1992	AN-2-2	ANN-13
H. argophylus E - 006	F_1	1984	1990	ARG-1	ARG-1
H. argophylus E - 006	BC_1	1987	1990	ARG-3	ARG-3
H. argophylus E - 007	F_1	1985	1992	ARG-2	ARG-2
H. debilis E - 010	F_2	1990	1994	DV-10	DEB-1
H. petiolaris E - 034	BC_1F_6	1991	1991	Pet-34	PET-4
H. praecox E - 027	F_2	1990	1990	PHIR-27	PRH-1
H. praecox E - 029	F_4	1989	1989	PRUN-29	PRR-1
H. rigidus M - 028	BC_1F_2	1991	1991	Rig-28	RIG-2
H. strumosus M - 056	BC_1F_5	1991	1996	Strum-56	STR-1
H. argophyllus E-007	BC_1F_7	1995	1998	ARG-4	ARG-4
H. argophyllus E-006	new BC_1	1997	2000	ARG-3-M-1	ARG3M1

Table 6. Characterization of R lines, produced from interspecific and intergeneric hybridization, harvest 2010.

No	Origin	Plant height, cm	Head diameter, cm	Vegetation period, days	Seed oil content, %	Generatio
PR-1/8	c.s. x H.pauciflorus M-028	110	13	100	48.48	19*
PR-13/8	c.s. x H. pumilus M-172	105	14	98	58.28	17*
PR-41/8	c.s. x H.divaricatus M-044	130	16	102	47.03	18*
PR-47/8	c.s. x H. bolanderi E-009	140	15	103	50.44	15*
C 23/1	c.s. x H.debilis E-011	105	17	103	49.16	17*
C 55	c.s. x H.debilis E-011	120	16	105	52.71	15*
C 56	c.s. x H.hirsutus M-029	115	17	105	52.38	15*

*branched forms

New sunflower hybrid combinations

There were performed two groups of combinations. The first combination included crosses between old, confirmed Bulgarian A /B/ lines with R lines, obtained from interspecific hybrids and the second group included crosses between new A /B/ lines, obtained by using of mutagenesis and R lines obtained by the interspecific hybridization. There are small number of hybrid combinations, created from B lines obtained from the wide hybridization and R lines, obtained on the same method.

New sunflower hybrid varieties in registration

There are created new sunflower hybrids which increased the standard in sunflower seed yield and seed oil content per unit area. Five of these hybrids - Musala, Mura, Maritsa, Mesta and Magura were registered at the State Variety Commission at the end of 2004. The paternal forms of hybrids Musala, Mura, Maritsa, Mesta and Magura were created from materials, obtained by interspecific hybridization.

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