

***Helianthus* species in breeding research on sunflower**

Michail Christov

Dobroudja Agricultural Institute - General Toshevo 9520, Bulgaria,

E-mail: michailhristov@yahoo.com

ABSTRACT

This investigation presents the results from the study on new sunflower forms obtained through hybridization between cultivated sunflower (*Helianthus annuus*) and 38 wild *Helianthus* species. Valuable genetic diversity in sunflower was developed by transfer of genetic material. The genetic system CMS - restorers of fertility was enriched with 15 new CMS sources and a lot of new Rf-gene sources. New sunflower forms, lines and hybrids with high seed oil content and high productivity, with resistance to economically important diseases and new plant architecture were produced, together with new sunflower forms and lines with high protein content in seeds suitable for birds and human consumption.

Key words: cytoplasmic male sterility – disease resistance – intergeneric hybridization – interspecific hybridization – oil – Rf gene.

INTRODUCTION

The increase in sunflower production and seed quality has been largely connected to the inclusion of wild *Helianthus* species into the improvement work on sunflower. Using this approach, the diversity in sunflower was enriched and the genetic system CMS - restorers of fertility was established. A real possibility for a complete practical application of heterosis breeding was created. The transfer of useful characters from *Helianthus* species to the cultivated sunflower started at the beginning of the 20th century and continues nowadays purposefully and at accelerated rates. The results from the investigations made by Satzaiperov (1916), Pustovoit (1960), Putt and Sackston (1963), Pustovoit (1975), Georgieva-Todorova (1976), Leclercq (1969), Leclercq et al. (1970), Fick et al. (1974), Jan and Chandler (1985), Vrânceanu et al. (1986), Christov (1990, 1999), Christov et al. (1993), and many other authors showed that the wild *Helianthus* species are rich sources for genes determining resistance to different diseases, parasites, pests, drought and other important traits. Some of these genes were already transferred to the cultivated sunflower and, on this basis, the new sunflower forms, resistant to some diseases (downy mildew, rust, phomopsis, etc., pests, broomrape and drought were developed, which also possess high production potential, high oil content in seed, presence of Rf genes, etc. (e.g. Morizet et al., 1984; Laferriere, 1986; Serieys and Vincourt, 1987; Seiler, 1992; Christov, 1991; Christov, 1996; Christov, 1996a; Christov et al., 1996; Perez-Vich et al., 2002; Christov et al., 2004; Hristova-Cherbadzi, 2004 and 2007, Hristova-Cherbadzi et al., 2006). This investigation aims to demonstrate that by transfer of genes controlling important economic characters from wild *Helianthus* species to cultivated sunflower, new sunflower forms, lines and hybrids were developed, which possess: resistance to diseases and parasites, good combining ability, high production potential and high oil content in the seeds, and which are suitable for growing in Bulgaria and other sunflower production regions worldwide.

MATERIALS AND METHODS

Plant material: New sunflower hybrid forms and completed B and R lines obtained from hybridization between sunflower cultivars and lines and 37 wild species from genus *Helianthus*.

Methods and directions of the investigation: The methods and directions of the investigation were: 1) Investigation on F₁ obtained from hybridization between sunflower and wild *Helianthus* species and the hybrid material from the next generations; 2) Individual selection and self-pollination or backcrossing; 3) Developing B and R lines and sterile analogues (A lines) of B lines; 4) Developing hybrid varieties by production and testing of hybrid combinations (A x R); 5) Developing other interesting sunflower forms.

*Evaluation of hybrid progenies and comparison to the parental forms - accessions from wild *Helianthus* species and cultivars and lines of cultivated sunflower:* The methods used in this investigation were practised, adapted to and confirmed for the conditions of DAI (Christov, 1990a; Christov, 1996a,b; Christov et al., 2004).

Biomorphological characterization: It was made on the basis of phenological and biometrical observations during the vegetation period, as well as on the basis of laboratory measurements of whole plants and seeds.

Phytopathological characterization: It included evaluation of the resistance to mildew (*Plasmopara helianthi* Novot.), phoma (*Phoma helianthi* Munt.-Cvet. et al.), phomopsis (*Phomopsis helianthi* Munt.-Cvet. et al.), downy mildew (*Erisiphe cihoracearum* D. C.), sclerotinia (*Sclerotinia sclerotiorum* Lib.), alternaria (*Alternaria helianthi* (Hanasf.) Tub. and Nish.), rust (*Puccinia helianthi* Schw.), verticillium (*Verticillium helianthi* Keeb.) and to the parasite broomrape (*Orobanche cumana* Wallroth).

Biochemical characterization: Study on the percentage and quality of oil and protein content in the seeds of interspecific hybrids and new sunflower lines and hybrids. The methods used for evaluation were confirmed and adapted by the biochemistry laboratory in DAI.

Cytological characterization: The investigations were mainly carried out with the aim of studying the meiosis of pollen female cells of F₁ interspecific hybrids special and pollen viability of the hybrid materials.

Cytoplasmic male sterility and restorer genes: CMS sources could be found among the hybrids obtained from crossing wild species x cultivated sunflower. For obtaining sterile inflorescence, the pollination was carried out with pollen from B lines or cultivars. After maintaining the sterility and the determination of its cytoplasmic type, the comparative study of other CMS sources began. These sources had already been established in DAI - General Toshevo as well as elsewhere in the world. Evaluation of the cytoplasmic effect on some agricultural characteristics of lines and hybrids included in several CMS sources was made. *Rf* genes could be found primarily in crosses cultivated sterile sunflower x wild species. The presence of *Rf* genes in the genome of a wild species was established in F₁. Forms with *Rf* genes were found in the materials obtained from crosses cultivated sunflower (B line or cultivar) x wild species and wild species x cultivated sunflower. The determination was done when the pollen of these materials was used for pollination of sterile plants from cultivated sunflower on the basis of CMS source. In all these cases *Rf* genes were identified in several CMS sources and the genetic basis of restoration was studied.

Developing of sunflower B and A lines. Developing of sunflower B lines was carried out by purposeful selection in the hybrid materials, which began after the third hybrid combination and lasted usually up to the 9-12 generation. The evaluation and the selection were done on the basis of their biomorphological, biochemical, phytopathological and entomological characteristics, lack of *Rf* genes and their good combining ability. For determining the combining ability of B lines, two variants were used: a) well-known (confirmed) sterilized R lines in CMS Rig 1 (Vulpe, Romania, Vulpe, 1972) and CMS Falax 1 (Serieys, France) were crossed with new B lines; b) Sterile analogue of new B line in BC₃ or BC₄ was crossed with familiar R lines. The second variant was predominantly used. Developing of sterile analogues (A lines) of B lines began with determining the fact that in the studied hybrid material *Rf* genes were absent. After BC₃ or BC₄, the study on the common combining ability of the developed A lines began, and after that on the specific combining ability for determination of the best hybrid combinations.

Developing of hybrid combinations and candidate cultivars for the State Variety Commission. Hybrid combinations were obtained when the sterile analogues (A lines) of B lines were used. They were obtained as a result of interspecific hybridization and experimental mutagenesis and were included in some CMS sources and R lines. Other Bulgarian and foreign A lines were also used. The new hybrids (A x R) were checked in preliminary tests. The best of them were tested and submitted to the Executive Agency for Variety Testing, Field Inspection and Seed Control and to other organizations all over the world. Obtaining of seeds from the hybrid combinations was carried out by growing of plants in nurseries covered with netting (net-house) or isolation of plants with paper isolators. The pollination was done manually. Bees could be used for plant pollination.

RESULTS

On the basis of the obtained interspecific hybrids, useful characters were transferred into cultivated sunflower from 183 accessions of 38 *Helianthus* species (Table 1). In many of the hybrids genes controlling different useful characters such as resistance to diseases, parasites and other stress factors

were identified. Forms with new plant architecture, with different vegetative periods and different seed colors were produced. Many of the forms were with high combining ability and high seed oil content, higher than that of the cultivated sunflower included in the hybridization. A lot of CMS sources were found in the cultivated sunflower, as well as fertility restorer genes (*Rf* genes).

Table 1. Sources of new characters transferred into cultivated sunflower.

Character	Species
Resistance/tolerance to: <i>Plasmopara helianthi</i>	<i>H. annuus</i> (w.f.), <i>H. argophyllus</i> , <i>H. bolanderi</i> , <i>H. debilis</i> , <i>H. exilis</i> , <i>H. neglectus</i> , <i>H. paradoxus</i> , <i>H. petiolaris</i> , <i>H. praecox</i> , <i>H. divaricatus</i> , <i>H. doronicoides</i> , <i>H. giganteus</i> , <i>H. glaucophyllus</i> , <i>H. grosseserratus</i> , <i>H. mollis</i> , <i>H. maximiliani</i> , <i>H. microcephallus</i> , <i>H. multiflorus</i> , <i>H. nuttallii</i> , <i>H. occidentalis</i> , <i>H. orgialis</i> , <i>H. pumilus</i> , <i>H. salicifolius</i> , <i>H. smithii</i> , <i>H. decapetalus</i> , <i>H. hirsutus</i> , <i>H. laevigatus</i> , <i>H. scaberimus</i> , <i>H. tomentosus</i> , <i>H. eggertii</i> , <i>H. californicus</i> , <i>H. ciliaris</i> , <i>H. pauciflorus</i> , <i>H. resinosus</i> , <i>H. strumosus</i> , <i>H. tuberosus</i> , <i>H. x laetiflorus</i> .
<i>Phomopsis helianthi</i>	<i>H. annuus</i> (w.f.), <i>H. argophyllus</i> , <i>H. debilis</i> , <i>H. eggertii</i> , <i>H. pauciflorus</i> , <i>H. glaucophyllus</i> , <i>H. laevigatus</i> .
<i>Erysiphe cichoracearum</i>	<i>H. decapetalus</i> , <i>H. laevigatus</i> , <i>H. glaucophyllus</i> , <i>H. ciliaris</i> .
<i>Orobanche cumana</i>	<i>H. tuberosus</i> , <i>H. eggertii</i> , <i>H. smithii</i> , <i>H. argophyllus</i> , <i>H. pauciflorus</i> , <i>H. strumosus</i>
<i>Phoma helianthi</i>	<i>H. argophyllus</i> , <i>H. laevigatus</i> , <i>H. eggertii</i> , <i>H. debilis</i>
<i>Sclerotinia sclerotiorum</i>	<i>H. praecox</i> , <i>H. argophyllus</i> , <i>H. annuus</i> (w.f.), <i>H. petiolaris</i> , <i>H. eggertii</i> , <i>H. pauciflorus</i> , <i>H. smithii</i> .
Earliness	<i>H. praecox</i> , <i>H. scaberimus</i> , <i>H. glaucophyllus</i> , <i>H. giganteus</i> , <i>H. rigidus</i> , <i>H. nuttallii</i> , <i>H. ciliaris</i> and <i>H. annuus</i> (w.f.)
Seed size	<i>H. annuus</i> (w.f.), <i>H. argophyllus</i> , <i>H. tuberosus</i> , <i>H. strumosus</i>
High oil content	<i>H. annuus</i> (w.f.), <i>H. debilis</i> , <i>H. petiolaris</i> , <i>H. praecox</i> , <i>H. pauciflorus</i> , <i>H. x laetiflorus</i>
Genes controlling CMS	<i>H. annuus</i> (w.f.), <i>H. argophyllus</i> , <i>H. debilis</i> , <i>H. petiolaris</i> , <i>H. praecox</i> , <i>H. pauciflorus</i> and <i>H. strumosus</i>
<i>Rf</i> genes	<i>H. annuus</i> (w.f.), <i>H. argophyllus</i> , <i>H. bolanderi</i> , <i>H. debilis</i> , <i>H. exilis</i> , <i>H. neglectus</i> , <i>H. paradoxus</i> , <i>H. petiolaris</i> , <i>H. praecox</i> , <i>H. divaricatus</i> , <i>H. doronicoides</i> , <i>H. glaucophyllus</i> , <i>H. giganteus</i> , <i>H. grosseserratus</i> , <i>H. maximiliani</i> , <i>H. microcephallus</i> , <i>H. mollis</i> , <i>H. multiflorus</i> , <i>H. nuttallii</i> , <i>H. occidentalis</i> , <i>H. orgialis</i> , <i>H. pumilus</i> , <i>H. salicifolius</i> , <i>H. smithii</i> , <i>H. decapetalus</i> , <i>H. hirsutus</i> , <i>H. laevigatus</i> , <i>H. scaberimus</i> , <i>H. tomentosus</i> , <i>H. eggertii</i> , <i>H. ciliaris</i> , <i>H. resinosus</i> , <i>H. pauciflorus</i> , <i>H. strumosus</i> , <i>H. tuberosus</i> , <i>H. californicus</i> and <i>H. x laetiflorus</i> .

New sunflower forms

- Resistance/tolerance to diseases and parasites:

*Resistance to *Plasmopora helianthi*: Full resistance to *Plasmopora helianthi* (races 300, 330, 700) was shown by 784 lines (765 R and 19 B lines). More than 850 new sunflower forms obtained also by interspecific hybridization (from F₆ to F₁₃) showed full resistance to the pathogen and possessed restorer genes (*Rf* genes) for the first sunflower CMS source - CMS Pet 1 and for some new CMS sources, obtained at DAI.

*Resistance to *Sclerotinia sclerotiorum*: In this case the term tolerance could be used. Using the species *H. eggertii*, *H. pauciflorus*, *H. smithii*, *H. praecox*, *H. petiolaris*, *H. argophyllus* and *H. annuus* (w.f.), plants with tolerance to *S. sclerotiorum* forms were obtained on the head as well as on the stem and roots. Lines 7043 R, 7083 R and 7092 R were of considerable importance, besides the obtained ones.

*Resistance to *Phomopsis helianthi*: New sunflower forms resistant to the pathogen *Ph. helianthi* were obtained using *Helianthus annuus* (w.f.), *H. argophyllus*, *H. debilis*, *H. glaucophyllus*, *H. laevigatus*, *H. eggertii*, *H. pauciflorus* (*rigidus*). Lines 6066B, 6748B, 7041 R, 7043 R obtained from *H. argophyllus*, *H. decapetalus*, *H. eggertii*, *H. pauciflorus* transfer resistance/ tolerance to the F₁ hybrids included.

*Resistance to *Phoma helianthi*: New sunflower forms resistant to *Phoma helianthi* were developed using *H. eggertii*, *H. laevigatus*, *H. argophyllus* and *H. debilis*.

*Resistance to *Erysiphe cichoracearum*. There are two types of resistance to this pathogen. The first one is controlled by a dominant gene. This type of resistance was found in *H. decapetalus*. The second one is controlled by a group of genes. Some accessions of *H. glaucophyllus*, *H. ciliaris*, *H. laevigatus*, *H. debilis*, *H. tuberosus* (M 004), *H. resinosus* possess such resistance. New sunflower forms resistant to this pathogen were obtained using *H. decapetalus*, *H. laevigatus*, *H. glaucophyllus* and *H. ciliaris*.

*Resistance to *Orobanche cumana*. Resistance to the parasite *O. cumana* found in the cultivated sunflower is controlled by one dominant gene. Several perennial *Helianthus* species also showed 100%

resistance to *O. cumana*. New sunflower forms resistant to the parasite were obtained using *H. tuberosus*, *H. eggertii*, *H. smithii*, *H. decapetalus*, etc. Full resistance to the parasite was demonstrated by some new lines such as 7009 R, 7019 R, 7203 R, etc.

Many lines showed resistance to two or three pathogens (downy mildew, phomopsis, phoma and sclerotinia) and the parasite broomrape.

- Cytoplasmic Male Sterility (CMS):

Until 2005, 72 CMS sources in sunflower have been reported and described by different authors. Most of these CMS sources were derived from interspecific crosses (Serieys and Christov, 2005). On the basis of the hybridization carried out till 2005, 14 new CMS sources were found plus one new type of CMS - CMS ARG3-M1. The greater part of the new CMS sources were distinguished by the first CMS source, obtained from *H. petiolaris* - CMS Pet 1 (Leclercq, 1969), as well as between them (Table 2).

Table 2. Sources of CMS produced by interspecific hybridization.

Origin	Obtained in generation	Year of observation	Year of report	DAI code	F.A.O. code
<i>H. annuus</i> E-067	F ₁	1985	1992	AN-67	ANN-10
<i>H. annuus</i> E-058	F ₆	1988	1994	AN-58	ANN-11
<i>H. annuus</i> E-002	F ₅	1991	1991	AN-2-1	ANN-12
<i>H. annuus</i> E-002	F ₆	1992	1992	AN-2-2	ANN-13
<i>H. argophyllus</i> E-006	F ₁	1984	1990	ARG-1	ARG-1
<i>H. argophyllus</i> E-006	BC ₁	1987	1990	ARG-3	ARG-3
<i>H. argophyllus</i> E-007	F ₁	1985	1992	ARG-2	ARG-2
<i>H. debilis</i> E-010	F ₂	1990	1994	DV-10	DEB-1
<i>H. petiolaris</i> E-034	BC ₁ F ₆	1991	1991	Pet-34	PET-4
<i>H. praecox</i> E-027	F ₂	1990	1990	PHIR-27	PRH-1
<i>H. praecox</i> E-029	F ₄	1989	1989	PRUN-29	PRR-1
<i>H. rigidus</i> M-028	BC ₁ F ₂	1991	1991	Rig-28	RIG-2
<i>H. strumosus</i> M-056	BC ₁ F ₅	1991	1996	Strum-56	STR-1
<i>H. argophyllus</i> E-007	BC ₁ F ₇	1995	1998	ARG-4	ARG-4
<i>H. argophyllus</i> E-006	new BC ₁	1997	2000	ARG-3-M-1	ARG3M1

Sources of new fertility restorer (Rf) genes:

The investigation was directed towards the discovery of *Rf* genes for CMS from *H. petiolaris* (PET-1) and for the new CMS sources obtained at DAI - General Toshevo. It was found out that 181 accession of 37 *Helianthus* species carried *Rf* genes. We also identified *Rf* genes in *H. argophyllus*, *H. debilis* and *H. rigidus* (*pauciflorus*) for CMS RIG-1. Our investigations showed that fertility restoration in CMS RIG-1 was controlled by two dominant genes. Similar results were obtained for fertility restoration in CMS ARG-3-M-1. The sources of genes for restoration of fertility of plants in CMS ARG-3-M-1 were different from those for CMS RIG-1. One of the sources was found in *Carduus acanthoides* widespread in Bulgaria.

New sunflower forms with normal cytoplasm (B lines): The total number of developed (fixed) B lines till 2007 was 268. The stem height varied from 60 to 180 cm, and the vegetation period - from 86 to 125 days. Thousand seed weight varied from 35 to 118 g, and seed oil content - from 40 to 54 % (Table 3). Some B lines showed resistance to phomopsis and others - to downy mildew and broomrape. Such lines were 6066B, 6101B, 6488B, 6748B, etc. New sunflower forms with *Rf* genes (R lines): Till now 765 R lines have been fixed and named. All of them are resistant to downy mildew. Some of them are resistant to phomopsis and broomrape. There are lines which show resistance to phoma and others - even tolerance to sclerotinia. All lines show high combining ability. A part of these lines are given in Table 4.

New sunflower hybrid varieties in registration: During the developing and investigation of new sunflower hybrid varieties, two groups of combinations were performed. 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 mutagenesis and R lines produced through wide hybridization. There was a small number of hybrid combinations developed from B lines through wide hybridization and R lines, obtained through the same method. Each year 280 to 320 hybrid combinations are produced for testing. New sunflower hybrids were developed which exceeded the standard hybrid Albena by seed yield and seed oil content per unit area. Five of these hybrids - Musala, Mura, Maritsa, Mesta and Magura, were already registered in the State Variety Commission at the end of 2006. At the beginning of 2008 the first type of hybrid for direct human consumption - hybrid Madan - will be released.

Table 3. Characteristics of B lines produced from interspecific hybridization.

No	Origin	Plant height (cm)	Head diameter (cm)	Seed oil content (%)	Vegetation period (days)
6101	<i>H.decapetalus</i> M-043	125	18	47.35	106
6159	<i>H.pauciflorus</i> M-028	155	15	48.79	105
6170	<i>H.strumosus</i> M-056	110	12	47.82	110
6202	<i>H.hirsutus</i> M-029	105	12	45.25	105
6215	<i>H.salicifolius</i> M-045	180	18	51.15	107
6275	<i>H.argophyllus</i> E-007	140	23	49.96	105
6134	<i>H.debilis</i> E-011	90	23	49.58	100
6149	<i>H.eggertii</i> M-001	130	24	49.88	105

Table 4. Characterization of R lines produced from interspecific hybridization.

No	Origin	Plant height (cm)	Head diameter (cm)	Vegetation period (days)	Seed oil content (%)	Generation
7009R	<i>H.tuberosus</i> M-037	80	13	92	45.99	17*
7015R	<i>H.debilis</i> E-011	120	15	102	52.73	15*
7026R	<i>H.smithii</i> M-008	140	14	106	45.34	16*
7027R	<i>H. x laetiflorus</i> M-005	135	17	102	48.86	15*
7041R	<i>H.eggertii</i> M-001	120	15	101	47.21	16*
7043R	<i>H.pauciflorus</i> M-028	135	16	106	52.46	15*
7203R	<i>H.decapetalus</i> M-043	110	16	102	49.56	15*
7082R	<i>H.glaucophyl.</i> M 012	110	16	107	49.91	16*
7090R	<i>H.paradoxus</i> E-019	120	15	104	52.12	15*
7091R	<i>H.ciliaris</i> M-092	70	21	105	45.31	16
7092R	<i>H.divaricatus</i> M-044	120	17	105	52.38	16*
7089R	<i>H.salicifolius</i> M-078	120	15	103	46.30	15*
7203R	<i>H.decapetalus</i> M-043	110	16	102	49.56	15*

*branched forms

CONCLUSIONS

As a result of a successfully ample hybridization program, new sunflower forms, lines and hybrids were developed. Many of these materials possess high productivity, high seed oil content and resistance to some economically important sunflower diseases. Besides, a great number of CMS sources and *Rf* genes were obtained. Forms and lines with oil type and protein type of seeds (for human consumption and poultry feeding) were produced.

REFERENCES

- Christov, M. 1990. A new source of cytoplasmic male sterility in sunflower originated from *H. argophyllus*. *Helia* 13:55-61.
- Christov, M. 1991. Possibilities and problems in the hybridization of cultivated sunflower with species of the genus *Helianthus* L. *Helia* 14(15):35-40.
- Christov, M. 1996a. Characterization of wild *Helianthus* species as sources of new features for sunflower breeding. p. 547-570 (vol. 2). In P.D.S. Caligari & D.J.N. Hind (eds). *Compositae: Biology & Utilization*. Proceedings of the International Compositae Conference, Kew, 1994. Royal Botanic Gardens, Kew.
- Christov, M. 1996b. Hybridization of cultivated sunflower and wild *Helianthus* species. p. 603-615 (vol. 2). In P.D.S. Caligari & D.J.N. Hind (eds). *Compositae: Biology & Utilization*. Proceedings of the International Compositae Conference, Kew, 1994. Royal Botanic Gardens, Kew.
- Christov, M. 1999. Production of new CMS sources in sunflower. *Helia* 22(31):1-12.
- Christov, M., I. Ivanova and P. Ivanov, 1993. Some characteristics of the *Helianthus* species in the Dobroudja collection. I. Protein content and amino acid composition in proteins. *Helia* 16(18):63-70.
- Christov, M., P. Shindrova and V. Entcheva. 1996. Transfer of new characters from wild *Helianthus* species to cultivated sunflower. *Genet. a Slecht.* 32(4):275- 286.

- Christov, M., I. Kiryakov, P. Shindrova, V. Encheva, and M. Christova. 2004. Evaluation of new interspecific and intergeneric sunflower hybrids for resistance to *Sclerotinia sclerotiorum*. p. 693-698 (vol. 2). In: Proc. 16th Int. Sunflower Conf., Fargo, ND, USA.
- Fick, G.N., D.E. Zimmer, J. Dominguez-Gimenez, and D.A. Rehder, 1974. Fertility restoration and variability for plant and seed characteristics in wild sunflower, p. 333-338. In: Proc. 4th Int. Sunflower Conf., Bucharest, Romania.
- Georgieva-Todorova, I. 1976. Medzduvidovi odnosnenija v rod *Helianthus* L. Sofia, BAN.
- Hristova-Cherbadzi, M.M. 2004. Hybridization of cultural sunflower *Helianthus annuus* L. with the annual species *Helianthus bolanderi* Gray, *Helianthus neglectus* Heiser and *Helianthus petiolaris* Nuttall. p. 699-707 (vol. 2). Proc. 16th Int. Sunflower Conf., Fargo, ND, USA.
- Hristova-Cherbadzi, M. and D. Valkova. 2006. Parvonashalni rezultati ot hybridizacija na kulturnija slanchogled *H. annuus* L. s mnogogodishnija hexaploiden vid *H. x laetiflorus* Pers. Field Crops Studies, vol. III, 4:533-537.
- Hristova-Cherbadzi, M. M. 2007. Study of new forms of sunflower by remote hybridization. Thesis.
- Jan, C.C., and J. M. Chandler. 1985. Transfer of powdery mildew resistance from *Helianthus debilis* Nutt. to cultivated sunflower. Crop Sci. 25:4.
- Laferriere, J.E. 1986. Interspecific hybridization in Sunflowers: An illustration of the importance of wild genetics resources in plant breeding. Outlook Agric. 15:104-109.
- Leclercq, P. 1969. Une sterile male cytoplasmique chez le tournesol. Ann. Amelior Plant 19:99-106.
- Leclercq, P., Y. Gauderon, and M. Dauge. 1970. Selection pour la resistance au mildiou du tournesol a partir d'hybrides topinambour x tournesol. Ann. Amelior Plant 20:363-373.
- Morizet, J., P. Cruzilat, J. Chatenoud, P. Picot and P. Leclercq 1984. Improvement of drought resistance in sunflower by interspecific crossing with a wild species *H. argophyllus*. Methodology and 1st results. Agronomie 4:577-585.
- Perez-Vich, B., B. Akhtouch, J. Munoz-Ruz, J.M. Fernandez-Martinez and C.C. Jan. 2002. Inheritance of resistance to a highly virulent race F of *Orobanche cumana* Wallr. in a sunflower line derived from interspecific amphiploids. Helia 25(36):137-144.
- Putt, E.D. and W.E. Sackston. 1963. Studies on sunflower rust. IV. Two genes R_1 and R_2 for resistance in the host. Can. J. Plant Sci. 43:490-496.
- Pustovoit, V. 1960. Medzduvidovae rdzavchinoustoichibie gibridi podsolnechnika. V: Otdilennaja gibrizatsija rastenii. M.
- Pustovoit, G.V. 1975. Segekchija podsolnechnika na gruppovoi immunitet metodom medzvidovoi gibrizatsii. V kn. Podsolnechnik, M., "Kolos", 164-209.
- Satziperov, F. 1916. Opit skreshtivaniija dvuh form podsolnechnika *Helianthus annuus* x *H. argophyllus*. Tr. prikl. bot., gen. i selek., 9.
- Seiler, G.J. 1992. Utilization of wild sunflower species for the improvement of cultivated sunflower. Field Crops Res. 30:195-230.
- Serieys, H., and P. Vincourt. 1987. Characterization of some new cytoplasmic male sterility sources from *Helianthus* genus. Helia 10:9-13.
- Serieys, H., and M. Christov. 2005. Identification, study and utilization in breeding programs of new CMS sources (1999 - 2004). X Consultation Meeting, Novi Sad, Serbia and Montenegro, July 17-20.
- Vrânceanu, A.V., M. Iuoras and F. Stoenescu. 1986. A contribution to the diversification of cytoplasmic male sterility sources in sunflower. Helia 9:21-25.
- Vulpe, V. 1972. Surce de androsterilite le floara soarelni. Analele institute de Ceretari pentru cereale si plante tehnice Fundulea. Academia Stiinte Agrisole si Silvice 38:273-277.