HELIA, **35**, Nr. 57, p.p. 37-46, (2012) UDC 633.854.78:631.523:631.527.53
DOI: 10.2298/HEL1257037C

CONTRIBUTION OF INTERSPECIFIC HYBRIDIZATION TO SUNFLOWER BREEDING

Christov, M.*

Dobroudja Agricultural Institute, 9520 General Toshevo, Bulgaria

Received: November 02, 2012
Accepted: December 10, 2012

SUMMARY

This investigation is directed at improving sunflower using hybrid forms resulted from interspecific hybridization. The aim is to create new B/A and R lines from interspecific hybrid forms that are resistant to diseases, the parasite broomrape, herbicides, and other stress factors and are characterized with high combining ability and to obtain on this basis highly productive oilseed sunflower hybrids with varied fatty acid composition of oil.

The investigation was carried out during the period 1983-2010. It involved 16 cultivars and 18 B lines with their analogues as well hybrid material originated from 38 *Helianthus* species - 9 annuals and 29 perennials.

Intraspecific hybridization and purposeful selection were the methods used. 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 performed.

New sunflower forms and lines were created as a result of this investigation. A greater part of them possess resistance to downy mildew, phomopsis, phoma and alternaria, tolerance to sclerotinia and full resistance to the different races of the parasite broomrape. The new forms were distinguished by new plant architecture, different vegetation period and seeds with different size and coloration. New B/A and R lines having high combining ability and seed oil and fatty acid contents were obtained. Fifteen sources of cytoplasmic male sterility (*cms)* were obtained from interspecific hybrid forms, as were 251 sources of genes for restoration of fertility (*Rf* genes). Five new hybrid cultivars of the oilseed type that were obtained on the basis 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 have augmented the contribution of interspecific hybridization to sunflower breeding.

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

* Corresponding author: e-mail: misho_h5@abv.bg

INTRODUCTION

The implementation of interspecific hybridization for practical purposes began at the start of the 20^{th} century with Saciperov's attempts (1916). These and other later investigations showed that as a result of crossing different *Helianthus* species with cultivated sunflower new sunflower forms resistant to different diseases and the parasite broomrape could be obtained (Pustovoit V., 1960; Pustovoit G., 1975; Put and Sackston, 1957 and 1963; Leclercq *et al.*, 1970; Fick *et al.*, 1974; Škorić, 1985; Jan and Chandler, 1985; Christov, 1990; Christov, 1996; Christov *et al.*, 1996; Hristova-Cherbadji, 2007; Christov, 2008; *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 others. Soon after the discovery 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 others followed in this direction. Investigations of *Helianthus* species were carried out in some other directions.

In this report we present the results of investigation of interspecific sunflower hybrids and the use of the hybrid material obtained for developing lines with economically important characters suitable for use as parental components of new sunflower hybrid cultivars.

MATERIAL AND METHODS

The investigations were carried out at the Dobroudja Agricultural Institute, General Toshevo during the period 1983-2010.

Plant material

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

Methods used

Methods of intraspecific hybridization and selection were used. They were in the form of 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 of resistance to diseases and broomrape of the studied plant material was carried out using methods confirmed at 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 contents and fatty and amino acid contents were evaluated according to methods confirmed at the Institute (Rushkovskiy, 1957; Stoianova and Ivanov, 1968; Ivanov *et al.*, 1996). A Nuclear Magnetic Resonance apparatus and the analyzer Hitachi L-8500 were used for the evaluation of seed oil content and for amino acids, respectively.

Morphological characteristics were assessed based on phenological observations and biometric measurements during the vegetation period and on the basis of laboratory studies of whole plants and of seed material obtained from them.

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

Firstly, sources of *Rf* genes were sought in crosses **sterile sunflower lines** × **wild species**. The presence of *Rf* genes in the genome of wild species was established in F_1. Forms with *Rf* genes were also found in materials obtained from crosses cultivated sunflower (B line or cultivar) × wild species and wild species × cultivated sunflower.

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

The development of sterile analogues - A lines began with the establishment of the fact that there were not any *Rf* genes in the studied material. After the BC_3 or BC_4, the study of the general combining ability of the A lines developed began, and after that their specific combining ability was analyzed.

The development of self-pollinated lines, the 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 until homozygous *Rf* genes were obtained. The obtained R lines, which possessed 100% fertility restoration and other important characters, were collected from both parental forms included in the hybridization. For creating R lines hybrids obtained from crosses **cultivated sunflower** × **wild species** and **wild species** × **cultivated sunflower** could be used.

RESULTS AND DISCUSSION

Origin of interspecific hybrids

As a result of hybridization between sunflower *Helianthus annuus* and 38 species from the genus *Helianthus* a total of 67,000 F_1 hybrid plants were obtained from all the species included in the investigation (Table 1). Except for F_1 hybrids originated from *H. simulans* all the 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. xlaetiflorus, H. californicus*

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

Creation 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 mildew resistance were the priority, and they were followed by studies on resistance to sclerotinia, phomopsis, phoma, alternaria, *etc*. Great priority was given to studies on broomrape resistance.

New sunflower forms resistant / tolerant to diseases and parasites

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

Full resistance to *Plasmopora helianthi* race № 700 was observed in more than 2,600 accessions, obtained with participation of 36 *Helianthus* species (Table 1). Resistance to race № 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*, *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 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. × *H. pauciflorus*)	100	100	48.48	23
PR-9/8 (c.s.× *H. tuberosus*)	100	100	47.27	25
PR-13/8 (c.s.× *H. pumilus*)	100	-	58.28	16
PR-25/8 (c.s. × *H. pauciflorus*)	100	100	46.89	25
PR-35/8 (c.s. × *H. hirsutus*)	100	100	48.80	16
PR-41/8 (c.s. × *H. divaricatus*)	100	100	47.03	18

Resistance / tolerance to the pathogen *Phomopsis helianthi* was exhibited by more than 80 forms. They originated from the 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. argophyllus* 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 the most effective was that with direct mycelium setting in different uncovered parts of the plant (Christov *et al.*, 2004). High tolerance to *Sclerotinia sclerotiorum* was observed in some forms originated from *Helianthus eggertii*, *H. pauciflorus*, *H. smithii*, *H. praecox*, *H. petiolaris*, *H. argophyllus*, and *H. annuus* (w.f.). This tolerance referred to those pathogens forms which infect 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 the species *H. decapetalus*, *H. glaucophyllus*, *H. giganteus*, *H. mollis*, *H. ciliaris*, *H. laevigatus*, *H. debilis*, *H. tuberosus*, and *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 *A. zinniae* Pape) began later. A 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 hybridization for resistance to phomopsis, phoma, alternaria and sclerotinia

Accession, 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. × *H. debilis*)	0	0	1	2
Sc-5 (c.s. × *H. pauciflorus)*	2	0	3	0
Sc-8 (c.s. × *H. argophyllus*)	0	0	0	0
Sc-9 (c.s. × *H. argophyllus*)	0	0	0	1

Races E, F and G of the parasite broomrape (*Orobanche cumana* Wallr.) were spread in Bulgaria. The last two races appeared in quick succession and this aggravated sunflower breeding. During the last 20 years a sufficient number of sunflower lines resistant to race E of broomrape were developed. From 2008 on the aim of the breeding work has been to develop lines resistant to race G of the parasite.

In creating forms resistant to broomrape 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. *rydbergii*, *H. salicifolius*, *H. smithii*, *H. annuus* (w.f.), *H. argophyllus*, *H. debilis*, *H. petiolaris* and *H. praecox*). Full resistance to the parasite was exhibited by some new lines such as 7019 R, 7203 R, C 23/1, C 41, C 46, C 48, C 55, C 56, *etc.* (Table 4).

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

Accession, pedigree	Resistance to		Seed oil content, %	Generation
	broomrape, %	downy mildew, %		
PR-1/8 (c.s. × *H. pauciflorus*)	100	100	48.48	23
PR-9/8 (c.s. × *H. tuberosus*)	100	100	47.27	25
PR-19/8 (c.s. × *H. divaricatus*)	100	100	45.25	19
PR-25/8 (c.s × *H. pauciflorus*)	100	100	46.89	25
PR-35/8 (c.s × *H. hirsutus*)	100	100	48.80	16
PR-41/8 (c.s. × *H. divaricatus*)	100	100	47.03	18
PR-47/8 (c.s. × *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 based on the results established for different hybrid forms obtained by applying 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*, *etc*. Some of the results for seed oil content are 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 differed from *cms* PET 1 significantly. Genes restoring fertility were found for all the sources.

New sunflower forms with *Rf* genes (R lines)

Thus far, more than 3,900 new R forms have been selected and obtained including 1,306 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 which even had 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 "B" lines developed (fixed) by the year 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 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, *etc.* Sterile analogues were developed for all the B lines in *cms* PET 1. Sterile analogues for the rest of the *cms* sources were created for four of the new lines with the aim to enable their use in some experiments such as the evaluation of the cytoplasmic effect on some agronomic characters of the new hybrids.

Table 5: Sources of *cms* produced by interspecific hybridization

Origin	Obtained in generation	Year of observation	Year reported	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. argophyllus E - 006	F_1	1984	1990	ARG-1	ARG-1
H. argophyllus E - 006	BC_1	1987	1990	ARG-3	ARG-3
H. argophyllus 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 by interspecific hybridization, harvest 2010

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

*branched forms

New sunflower hybrid combinations

Two groups of combinations were made. 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 using mutagenesis and R lines obtained by interspecific hybridization. There are small number of hybrid combinations created from B lines obtained from wide hybridization and R lines obtained by the same method.

New sunflower hybrid varieties in registration

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

REFERENCES

Christov, M., and Petrov, P., 1988. New source of *Rf* genes for *cms* on the basis of *H. petiolaris*. Genetics and Selection 21(5).

Christov, M., 1990. Study on wild species *Helianthus* with aim of using in sunflower breeding. Ph.D. Dissertation, Bulgarian Academy of Agriculture, Sofia.

Christov, M., 1990a. A new source of cytoplasmic male sterility in sunflower originated from *H. argophyllus*. Helia 13: 55-61.

Christov, M., Shindrova, P. and Encheva, V., 1992. Phytopathological characterization of wild species in the genus *Helianthus* in view of their use in breeding for resistance. Genet. Breed. 25: 45-51.

Christov, M. 1996b. Hybridization of cultivated sunflower and wild *Helianthus* species. pp. 602-615. *In:* P.D.S. Caligari and D.J.N. Hind [*eds.*], *Compositae*: Biology and Utilization, Vol. 2. Proc. Intl. Composite Conf., The Royal Botanic Gardens, 24 July-5 August 1994. Kew, London.

Christov, M., Shindrova, P. and Encheva, V., 1996. Transfer of new characters from wild *Helianthus* species to cultivated sunflower. Genet. a Slecht. 32(4): 275-286.

Christov, M., 1999. Production of new *cms* sources in sunflower. Helia 22(31): 1-12.

Christov, M., Kiryakov, I., Shindrova, P. and Encheva, V. and Christova, M., 2004. Evaluation of new interspecific and intergeneric sunflowerhybrids for resistance to *Sclerotinia sclerotiorum*. *In*: Proc. 16th Int. Sunfl. Conf., Fargo, North Dakota, USA, Int. Sunfl. Assoc., Paris, France. II: 693-698.

Christov, M., 2008. *Helianthus* species in breeding research in sunflower. pp. 709-714. *In:* L. Velasco [*ed.*], Proc 17th Intl. Sunflower Conf., Cordoba, Spain, 8-12 June, 2008. Intl. Sunflower Assoc., Paris, France.

Encheva, V. and Kiryakov, I., 2002. A method for evalution of sunflower resistance to *Diaporthe/ Phomopsis helianthi* Munt.-Cvet. *et al.* Bulgarian Journal of Agricultural Science 8: 219-222.

Enns, H., Dorrell, D.G., Hoes, J.A. and Chubb, W.O., 1970. Sunflower research. Progress report. *In*: Proc. 4th Int. Sunfl. Conf., Memphis, Tennessee. Int. Sunfl. Assoc., Paris, France. pp. 162-167.

Fernandez-Martinez, J.M., Malero-Vara, J,. Munoz-Ruz, J., Ruso, J. and Domingez, J., 2000. Selection of wild and cultivated sunflower for resistance to a new broomrape race that overcomes resistance of the Or_5 gene. Crop Science 40(2): 550-555.

Fick, G.N., Zimmer, D.E., Dominguez-Gimenez, J. and Rehder, D.A., 1974. Fertility restoration and variability for plant and seed characteristics in wild sunflower. *In*: Proc. 4th Int. Sunfl. Conf., Bucharest, Int. Sunfl. Assoc., Paris, France. Pp. 333-338.

Hristova-Cherbadzi, M., 2007. Study of new sunflower forms by remote hybridization. Ph.D. Dissertation, Sofia.

Ivanov, P., Christov, M., Ivanova, I. and Nikolova, V., 1996. Study of seed oil and protrin quality of some *Bidens tripartita* accessions. Helia 19(25): 79-85.

Jan, C.C., and Chandler, J.M., 1985. Transfer of powdery mildew resistance from *Helianthus debilis* Nutt. to cultivated sunflower. Crop Science 25(4): 664-666.

Kinman, M.L., 1970. New development in the USDA and state experiment station sunflower breeding programe. *In*: Proc. 4th Int. Sunfl. Conf., Memphis, USA, Int. Sunfl. Assoc., Paris, France. Pp. 181-183.

Leclercq, P., 1969. Une sterile male cytoplasmique chez le tournesol. Ann. Amelior Plant 19: 99-106.

Leclercq, P., 1971. La sterilite male cytoplasmique du tournesol. I. Premieres etudes sur la restauration de la fertilite. Ann. Amelior. Plant 21: 45-54.

Leclercq, P., Gauderon, Y. and Dauge, M., 1970. Selection pour la resistance au mildiou du tournesol apartir d'hybrides topinambour × tournesol. Ann. Amelior Plant 20(3): 363-373.

Pancenko, A., 1975. Rannaja diagnostika zarazihoustoitcivosti pri selekcii i ulucshajutshem semenovodstve podsolnetcnika, Vestnik selskohozjaistvennoi nauki 2:107-115.

Pustovoit, V. 1960. Mezhvidovie i rzhavtcinoustoitcivie gibridi podsolnetcnika. *In:* Otdaletcennaja gibridizacija rastenii. Moskva, 376-378.

Pustovoit, G. 1975. Selyektsiya podsolnechnika na groppovoy immonityet myetodom mezhvidovoy gibridizatsii. *In:* Podsolnechnik. izd. "Kolos", Moskva, 164-209.

Putt, E.D., and W.E. Sackston. 1957. Studies on Sunflower rust. I. Some sources of rust resistance. Can. J. Plant Science 37:43-54.

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 Science 43:490-496.

Rushkovskiy, C.B. 1957. Metodi issledovaniy pri selekcii maslichnih rastenii na soderzhanie masla i ego kachestvo. M. Pischepromizdat.

Satziperov, F., 1916. Opit skreschivaniya dvuh form podsolnechnika *Helianthus annuus* × *Helianthus argophyllus*. Tr. prikl. bot., gen. i selek. 9: 207-244.

Serieys, H., and Vincourt, P., 1987. Characterization of some new cytoplasmic male sterility sources from *Helianthus* genus. Helia 10: 9-13.

Shindrova, P., 2006. Broomrape (*Orobanche cumana* Wallr.) in Bulgaria - Distribution and race composition. Helia 29(44): 111-120.

Škorić, D., 1985. Sunflower breeding for resistans to Diaporthe (*Phomopsis helianthi*). Helia 8: 21-23.

Stojanova, J., and Ivanov, P., 1968. Proutcvanija varhu podgotovkata na semenata ot slantcogleda za laboratorno opredeljane na maslenostta im. Rastenievadni nauki 5(4): 49-57.

Tourvieille, D., Vear, F. and Pelletier, C., 1988. Use of two mycelium tests in breeding sunflower resistance to Phomopsis. *In:* Proc. 12th Int. Sunfl. Conf., II: 110-114.

Vranceanu, A.V., Iuoras, M. and Stoenescu, F., 1986. A contribution to the diversification of cytoplasmic male sterility sources in sunflower. Helia 9: 21-25.

Whelan, E.D.P., 1980. A new source of cytoplasmic male sterility in sunflower. Euphytica 29: 3-46.