HELIA, **36**, Nr. 58, p.p. 1-18, (2013) UDC 633.584.78:631.527.53:631.526.325
DOI: 10.2298/HEL1358001A

CONTRIBUTION OF INTERSPECIFIC AND INTERGENERIC HYBRIDIZATION TO SUNFLOWER BREEDING

Christov, M.*

Dobroudja Agricultural Institute, 9520 General Toshevo, Bulgaria

Received: November 02, 2012
Accepted: June 10, 2013

SUMMARY

This investigation was directed to sunflower improvement using hybrid forms resulted from wide hybridization. The aim was to create new B/A and R lines from interspecific and intergeneric hybrids resistant to diseases, parasite broomrape, herbicides, other stress factors and with high combining ability in highly productive oil-type sunflower hybrids with varied fatty acid contents. The confectionary hybrids should have a high kernel protein content and amino acid content.

The investigation was carried out during the period 1983-2010. The programme included 16 cultivars and 18 B lines with their analogues. Interspecific, intraspecific, and intergeneric hybridization produced hybrid materials originating from 38 *Helianthus* species with different ploidy levels, 9 annuals and 29 perennials, and 28 species from other genera of family *Compositae*. New sunflower forms and lines created possessed resistance to downy mildew, Phomopsis, Phoma and Alternaria, tolerance to Sclerotinia and total resistance to the different races of parasitic broomrape. The new forms had distinctive plant architecture, different vegetation periods, and seeds of different sizes and coloration. New B/A and R lines, characterized with high combining ability, seed oil, and fatty acid content and varying protein amino acid contents were obtained. Fifteen sources of cytoplasmic male sterility (*cms*) were obtained from interspecific hybrids and 271 sources of fertility restoration (*Rf*) genes from interspecific and intergeneric hybrids. Five new oil type hybrids and one confectionery type were developed and registered.

The results from this investigations showed that by wide hybridization new genetic material can be transferred to the cultivated sunflower. These results supplemented the contribution of interspecific and intergeneric hybridization for sunflower breeding.

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

INTRODUCTION

The practical implementation of interspecific hybridization began at the beginning of the 20th century by Satziperov (1916). These and other later investigations showed that as a result of crossing of different *Helianthus* species with cultivated sunflower (*Helianthus annuus* L.), new sunflower forms resistant to different dis-

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

eases and the broomrape parasite could be obtained (Panchenko, 1954; Pustovoit V., 1960; Pustovoit G., 1963, 1975; Putt and Sackston, 1957, 1963; Leclercq *et al*., 1970; Hoes *et al*., 1973; Fick *et al*., 1974; Zimmer and Fick, 1974; Cvetkova, 1976; Škorić, 1985; Jan and Chandler, 1985; Christov, 1990a; Christov *et al*., 1996; Fernandez-Martinez and Ruso, 1997; Gulya *et al*., 1997; Bachvarova, 2004; Hristova-Cherbadzhi, 2007, 2012; Christov, 2008). Sunflower forms with higher seed oil content were obtained from crosses between different species and cultivated sunflower (Hristova-Cherbadzhi, 2007, 2012; Christov, 2008). Morized *et al*. (1984) and Iurras and Voinescu (1984) obtained drought tolerant forms from wild species *H. argophyllus* Torr. & Gray. Leclercq (1969, 1971) found the first *cms* source originating from the cross between *H. petiolaris* Nutt. and cultivated sunflower. Other *cms* sources were found by Anashchenko (1974), Whelan (1980, 1981), Heiser (1982), Vranceanu *et al*. (1986), Serieys and Vincourt (1987), Christov (1990b, 1999). Soon after discovery of the first *cms* source, sources of *Rf* genes were found by Kinman (1970) and Enns *et al*. (1970). Vranceanu and Stoenescu (1971, 1976,1978), Fick *et al*. (1974), Škorić *et al*. (1978), Serieys (1986), Škorić *et al*. (1987), Christov and Petrov (1988), and *etc*. followed in this direction.

Compared to interspecific hybridization, intergeneric hybridization has been rarely attempted. Crossing of *Helianthus* species with species from *Tithonia* and *Viguera* were some of the first attempts. Heiser *et al*. (1969) successfully crossed four *Helianthus* species with *Viguiera porteri*. Intergeneric hybridization between cultivated sunflower and the species *Carthamus tinctoris* L. and *Onopordon acanthium* L. was carried out by Morozov (1947). This indicated that the borders of sunflower crossability were vastly increased. Georgieva-Todorova (1971, 1976) carried out intergeneric hybridization between sunflower and species *Onopordon acanthium* L. indicating that some experiment plants were intergeneric hybrids. Unfortunately, did not explain exactly how they were obtained the intergeneric hybrids with sunflower. Our investigation in this field began in 1985. The first intergeneric hybrid was obtained in 1987 from cross between cultivated sunflower *Helianthus annuus*, variety Peredovik and *Tithonia rotundifolia* L., accession No. 567 (Christov and Panayotov, 1991). After obtaining positive results from the initial hybridization, additional genera from the *Compositae* family were used (Christov *et al*., 1994; Christov and Vassilevska-Ivanova, 1999; and Christov *et al*., 2009).

This report presents the results of interspecific and intergeneric sunflower hybrids and their use in obtaining hybrid material for developing lines with economically important characters suitable as parental lines for developing new sunflower hybrids.

MATERIAL AND METHODS

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

Plant material

The investigation included 16 cultivars of cultivated sunflower *Helianthus annuus* (2n=34): Peredovik, Progress, Voronejskii 272, Skorospelii, Nadejdnii, Pervenets, Harkovski 101, Start, VNIIMK 8931, 6540, 1646, 3497, 8883, Vihren, Bal-

kan, Stadion and 18 lines: No. 130, 1234, 1607, 1721, 2418, 2607, 2942, 3004, 6046, 6054, 6065, 6075, 6068, 6633, HA 89, HA 300, HA 341, HA 821 and their sterile analogues and hybrid material originated from 38 *Helianthus* species and 28 species from other genera of *Compositae* family.

Methods used

Methods of intraspecific hybridization and selection were used. They included crossing between interspecific hybrids, between intergeneric hybrids, and crossing of interspecific and intergeneric hybrids with sunflower cultivars and lines. Self-pollination, sib-pollination, backcrossing with pollen from cultivated sunflower and pollination with pollen from different interspecific and intergeneric hybrids were used.

Evaluation for resistance to diseases (Iliescu, 1955; Panchenko, 1965; Pustovoit *et al.*, 1976; Saliman *et al.*, 1982; Tourvieille *et al.*, 1988; Christov, 1990a; Christov *et al.*, 1992; Christov, 1996a, 1996b; Christov *et al.*, 1996; Encheva and Kiryakov, 2002; Christov *et al.*, 2004) and parasite broomrape (Panchenko, 1975; Alonso, 1996; Pacureanu-Joita *et al.*, 1998; Fernandez-Martinez *et al.*, 2000; Shindrova, 2006a, 2006b) was carried out using standardized methods used at the DAI.

Seed oil and protein contens and fatty and amino acid contents were also evaluated according to standardized methods (Rushkovskii, 1957; Stojanova and Ivanov, 1968; Ivanov *et al.*, 1996). Nuclear Magnetic Resonance was used for evaluation of seed oil content and a Hitachi, L-8500 analyzer for amino acids.

Morphological characteristics were based on phenotypic observations and biometric measurements during the vegetation period and on laboratory studies of whole plants and seeds.

Sources of *cms* were searched for among the materials obtained from crosses of **wild species × cultivated sunflower**. Cytoplasmic male sterility was maintained by using pollen from B lines or cultivars. After sterility maintenance and confirmation of cytoplasmic type, comparative studies with other *cms* sources obtained at DAI, and others from all over the world were begun. Evaluation of the cytoplasmic effect on some agronomic characteristics of lines and hybrids included in some *cms* sources was also studied.

***Rf* genes** were searched for in crosses of **sterile sunflower lines × wild species**. Presence of *Rf* genes in the genome of wild species was established in the F_1. Lines with *Rf* genes were also found in materials obtained from crosses of cultivated sunflower / B line or cultivar / × wild species and wild species × cultivated sunflower. Confirmation was carried out when pollen of this material was used for pollination of sterile plants of cultivated *cms* sunflower sources. In all cases *Rf* genes were tested on several *cms* sources and the genetic determination of fertility restoration was studied.

Development of B lines was carried out by selection of hybrid materials, which in most cases began after the third generation and usually continued to the 9 through 12 generations. Evaluation and selection of materials was based the basis

of morphological, biochemical and phytopathological characteristics, absence of *Rf* genes, and presence of good combining ability.

Development of sterile analogues, A lines, and B lines, began with establishment of the fact that there were no *Rf* genes in the material. After the BC_3 or BC_4, study of general combining ability of the A lines, and after that their specific combining ability for developing the best hybrid combinations began.

Creation of self-pollinated lines, restorers of fertility - R lines was carried out mainly from crosses of male sterile lines with different wild *Helianthus* species and species from other genera of the *Compositae*. Repeated selection and self-pollination of fertile plants was implemented until obtaining homozygous *Rf* genes. The R lines possessing 100% fertility restoration and other important characters were identified from both parental forms included in the hybridization (Christov, 2002; Christov *et al*., 1996, 2009). R lines obtained from crosses of cultivated sunflower / B line or cultivar/ × wild species and wild species × cultivated sunflower can be used to produce hybrids.

Hybrid combinations were created using sterile analogues, A lines, of B lines, obtained from interspecific hybridization and experimental mutagenesis, including several *cms* sources and R lines, obtained from interspecific and intergeneric hybridization. Different Bulgarian and foreign A lines were also used. The new hybrids, A × R, were tested and the best were entered in competitive trials and recommended to be included in testing trials of State Variety Commission of Bulgaria and of similar authorized agencies of other countries.

RESULTS AND DISCUSSION

Origin of interspecific hybrids

As a result of hybridization between cultivated sunflower *Helianthus annuus* and 38 species from genus *Helianthus*, some 67 000 F_1 hybrid plants were obtained from all species combinations (Table 1). Only F_1 hybrids originating from *H. simulans* did not produce 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,* and *H. annuus* (wild)**
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,* and *H. simulans*
Perennial tetraploid species (2n=68)	*H. decapetalus, H. hirsutus, H. laevigatus, H. scaberimus*,* and *H. tomentosus**
Perennial hexaploid species (2n=102)	*H. eggertii, H. pauciflorus* (*rigidus*), *H. strumosus, H. resinosus, H. tuberosus, H. ciliaris, H. x laetiflorus,* and *H. californicus*

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

Hybrid plants from the different combinations produced great phenotypic diversity. Some combined useful characters of the parental forms. The diversity provided an opportunity to select for a large number of characters. Selection directed toward valuable characters and self-pollination lead to uniformity in plants in the next generations.

Genes controlling resistance to diseases, parasites and other stress factors were discovered in many of the hybrids. Plants with a new architecture, different vegetation period, and different coloration of seeds were obtained. Many of the new forms had high combining ability and high seed oil content; higher than that of cultivated sunflower. A large number of new *cms* sources and genes for fertility restoration (*Rf*) were obtained (Table 2).

Table 2: Sources of new characters transferred into cultivated sunflower

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

Origin of intergeneric hybrids

A total of 805 F_1 hybrid plants were obtained from direct crosses and 19 F_1 hybrid plants from reciprocal crosses originating from sunflower and 28 species from other genera of the *Compositae* (Table 3). Second generation hybrid plants were obtained from the combination *H. annuus* × *Simsia foetida*, and from all 19 F_1 hybrid plants from the reciprocal crosses. Only those from the combination *Tithonia speciosa* × *H. annuus* reached the sixth generation.

Despite *Rf* genes, some other characters with economic importance were transferred. Resistance to downy mildew, Phomopsis, Sclerotinia, Alternaria, and broomrape were obtained. Other forms were identified with smaller stems and shorter vegetation periods. Some forms had higher seed oil content, while others had variation in amino acids and protein contents. Table 4 shows the species from which these characters were transferred from.

Creating of new sunflower forms from interspecific and intergeneric hybrids

The scheme for creating of new sunflower forms and lines from the interspecific and intergeneric hybrids was similar. The difference between them was that the hybrid material mainly originated from the intergeneric hybridization in this case where the forms and lines carry *Rf* genes.

The main reason for including wild *Helianthus* species and some other species from the *Compositae* in the research was the presence of resistance to diseases, parasites, and pests. The artificial testing of the new hybrids, obtained as a result of wide hybridization, to diseases began in different generations and depended on the quantity of seeds obtained and the phenotype of the plants. In most cases, F_1 plants were tested if there was sufficient seed. Sufficient number of plants was obtained mainly from crosses with annuals and from crosses using *cms* lines × *Helianthus* species or another genus of the *Compositae*. Studies for downy mildew resistance were a priority followed by studies for Sclerotinia, Phomopsis, Phoma, and Alternaria resistance. A high priority was also given to evaluating broomrape resistance.

Breeding to develop high oil hybrids from the interspecific and intergeneric hybrids was based on some important characters such as high ratio kernel/hull and increasing the oil content in the kernel. The next priority related to the high seed productivity and seed oil content was 1000 seeds weight, which depends on the ratio of percent kernel/percent seed and percent oil content in the kernel. Another important index was number of seeds obtained per plant which had intermediate inheritance and a positive relationship between the number of seeds per plant and its productivity.

Other characters important for sunflower breeding were: seed size and hull coloration; friability of seeds; percent seed set; plant height; head diameter; vegetation period; branching, lodging; number of leaves per plants lines; hybrid cultivars; and number of plants/unit area.

Table 3: Species from different genera of the *Compositae* used in hybridization with cultivated sunflower

Groups of species	Species from the *Compositae*
Species with the same chromosome number as sunflower (2n=34)	*Gaillardia speciosa*, *Onopordum acanthium*, *Simsia foetida*, *Tithonia rotundifolia*, *Tithonia speciosa, Verbesina alata*, *V. helianthoides*, *V. encelioides*, and *Viguera trachyphylla*
Species with a different chromosome number (2n=xx)	*Arctium lapa* (2n=32), *Aster speciosa* (2n=72), *Bidens tripartita* (2n=48), *Calendula officinalis* (2n=28), *Carduus acanthoides* (2n=22), *Carthamus lanatus** (2n=44), *Carthamus tinctorius* (2n=24), *Carlina vulgaris* (2n=20), *Chrysanthemum leucanthemum** (2n=18), *Cichorium intybus* (2n=18), *Cirsium lanceolatum** (2n=68), *Cosmos bippinatus* (2n=24), *Grindelia speciosa* (2n=24), *Ehinacea purpurea* (2n=22), *Evmolpia* sp. (2n=?), *Inula helenium* (2n=20), *Matricaria chamomila* (2n=18), *Rudbeckia hirta** (2n=36), *Silphium perfoliatum* (2n=24), *Silybium marianum*, *Telekia speciosa* (2n=20), *Tithonia tagetiflora* (2n=32), *Zinnia angustifolia* (2n=22), and *Xanthium strumarium* (2n=36)

*Species from which intergeneric hybrids were not obtained.

Table 4: Sources of new characters transferred to cultivated sunflower

Characters Resistance/tolerance to:	Species
Plasmopara helianthi	Race 731 - *Inula helenium*, *Tithonia rotundifolia*, and *Grindelia speciosa*
Phomopsis helianthi	*Arctium lapa*, and *Carduus acanthoides*
Erysiphe cichoracearum	*V. encelioides*, *Grindelia speciosa*, *Ehinacea purpurea*, *Tithonia rotundifolia*, and *Telekia speciosa*
Orobanche cumana	*Calendula officinalis*, *Carduus acanthoides*, *Grindelia speciosa*, *Inula helenium*, *Tithonia rotundifolia*, *Tithonia speciosa*, and *V. helianthoides*
Phoma helianthi	*Arctium lapa*, *Grindelia speciosa*, and *V. encelioides*
Sclerotinia sclerotiorum	*Tithonia rotundifolia*, *Arctium lapa*, *Carduus acanthoides*, *Grindelia speciosa*, *Inula helenium*. *Matricaria chamomile*, *Silphium perfoliatum*, *Telekia speciosa*, and *Zinnia angustifolia*
Earliness	*Aster speciosa*, *Verbesina alata*, and *Telekia speciosa*
Seed size	*Carduus acanthoides*, *Aster speciosa*, and *Carlina vulgaris*
High oil content	*Carduus acanthoides*, *Gaillardia speciosa*, and *Grindelia speciosa*
Various amino acid protein content	*Carduus acanthoides*, *Bidens tripartite*, *Arctium lapa*, *Grindelia speciosa*, and *V. helianthoides*
Rf genes	*Gaillardia speciosa*, *Onopordum acanthium*, *Tithonia rotundifolia*, *Tithonia speciosa Verbesina alata*, *V. helianthoides*, *V. encelioides*, *Viguera trachyphylla*, *Arctium lapa*, *Aster speciosa*, *Calendula officinalis*, *Carduus acanthoides*, *Carthamus tinctorius*, *Carlina vulgaris*, *Cichorium intybus*, *Cosmos bippinatus*, *Grindelia speciosa*, *Ehinacea purpurea*, *Evmolpia* sp., *Inula helenium*, *Matricaria chamomila*, *Silphium perfoliatum*, *Silybium marianum*, *Telekia speciosa*, *Tithonia tagetiflora*, *Zinnia angustifolia*, and *Xanthium strumarium*

Another direction of the breeding work was to increase the protein content of large seeded forms seeds suitable for human consumption, confectionery type with diverse amino acids and protein contents, and small colored seeds suitable for bird feeding.

Utilizing wide hybridization provides an opportunity to find new sources of *cms* and different sources of fertility restoration gens for the *cms* PET 1 and other *cms* sources, obtained at DAI and other research institutions.

New sunflower resistance/tolerance to diseases and parasites

Among the hybrids developed, resistance genes for downy mildew were the most frequent. Complete resistance to downy mildew races 300, 330 and 700 was observed in more than 2600 combinations obtained from crosses of 36 *Helianthus* species and 16 species from other genera of the *Compositae* (Tables 1 and 2). Resistance to races 731, considered as the most virulent in Bulgaria, was found in more than 400 hybrid forms, originating from the *H. divaricatus, H. hirsutus, H. pauciflorus* (*rigidus*), *H. debilis* ssp. *debilis*, *H. paradoxus, Inula helenium*, *Tithonia rotundifolia*, and *Grindelia speciosa*. More than 96% of the resistant forms possessed *Rf* genes. Many of them are finished R lines. Some of these materials possess resistance to other diseases and to the parasite broomrape (Table 5). A group of materials resistant to the most virulent race was selected. The present work was connected to transfer of this resistance into R lines with other interesting characters obtained before the appearance of the newest races.

Table 5: Characterization of sunflower lines obtained by interspecific and intergeneric hybridization resistant to downy mildew race 731 harvested in 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
PR-51/8 /c.s. × *C. acanthoides*/	100	-	52.96	18
PR-56/8 /c.s. × *Aster speciosa*/	100	100	49.56	17*
PR-57/8 /c.s. × *Inula*/ x *Tith.*	100	100	50.35	16

*unbranched form

Resistance/tolerance to *Phomopsis helianthi* was observed in more than 80 forms. They originated from species *H. annuus* (wild), *H. argophyllus*, *H. debilis*, *H. glaucophyllus, H. laevigatus*, *H. eggertii*, *H. pauciflorus*, *Arctium lapa*, and *Carduus acanthoides* (Table 6).

High resistance to *Phoma helianthi* was observed in several interspecific hybrids with *H. eggertii, H. laevigatus*, *H. argophyllus*, and *H. debilis.*

Table 6: Characterization of sunflower lines obtained by interspecific and intergeneric hybridization for resistance to Phomopsis, Phoma, Alternaria, and Sclerotinia harvested in 2009

Accession, pedigree	Resistance to (grades)			
	Phomopsis, gr. 0-4	Phoma, gr. 0-4	Alternaria, gr. 0-4	Sclerotinia, gr. 0-5
Sc-1 /c.s. × C. acanth./	3	0	0	1
Sc-2 L-6116B	1	0	0	2
Sc-3 /c.s. × *H. debilis*/	0	0	1	2
Sc-5 /c.s.× *H.pauciflor*/	2	0	3	0
Sc-8 /c.s. × *H. argophyllus*/	0	0	0	0
Sc-9 /c.s. × *H. argophyllus*/	0	0	0	1
Sc-16 /c.s. × *Silfium* sp./	3	0	0	1
Sc-18 /c.s. × *Grindelia* sp./	3	0	2	1
Sc-23 /c.s. × *Telekia* sp./	2	0	1	0
Sc-27 /c.s. × *Inula* sp./	1	0	0	0
Sc-31 /c.s. × *Gaillardia* sp./	3	0	3	1
Sc-33 /c.s. ×*Carduus* sp./	0	0	2	1
Sc-39 /c.s. × *Inula* sp./ × *Tith*.	2	0	0	1
Sc-51 /c.s. ×*Carduus* sp./	1	0	2	1
Sc-53 /c.s. × *Tith.* sp./ × *Arct*.	1	0	3	1
Sc-56 /c.s. ×*Arctium* sp./	2	1	0	1
Sc-60 /c.s × *Tith.* sp./ × *Verbes*.	2	0	2	1
Sc-62 /c.s × *Grindelia* sp./	1	0	2	1
Sc-62 /c.s. × *Zinnia* sp./	2	0	0	1

Studies on Sclerotinia resistance (*Sclerotinia sclerotiorum)* were carried out under field conditions and in greenhouses. Different methods of artificial inoculation were used with the most effective with direct mycelium setting in different uncovered parts of the plant. The evaluation was based on a 0 to 5 scale (Encheva and Kiryakov, 2002; Christov *et al*., 2004). High tolerance to *Sclerotinia sclerotiorum* was observed on some crosses originating from *Helianthus* species, *H. eggertii*, *H. pauciflorus*, *H. smithii*, *H. praecox*, *H. petiolaris*, *H. argophyllus*, *H. annuus* (wild) and species from other genera, *Tithonia rotundifolia*, *Arctium lapa*, *Carduus acanthoides*, *Grindelia speciosa*, *Inula helenium*, *Matricaria chamomile*, *Silphium perfoliatum*, *Telekia speciosa*, and *Zinnia angustifolia*. Tolerance to the three forms of this pathogen that infects the head, stem, and the basal part of the sunflower stem was observed.

Total resistance to powdery mildew (*Erysiphe cichoracearum*) was found in hybrid forms originating from *H. decapetalus*, *H. glaucophyllus*, *H. giganteus*, *H. mollis*, *H. ciliaris*, *H. laevigatus*, *H. debilis*, *H. tuberosus*, and *H. resinosus*. The resistance transferred from the *H. decapetalus* was determined to be a single dominant gene.

The investigations on Alternaria resistance (*Alternaria helianthi*) was 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 used. Some of the results are presented in Table 6.

Races E, F and G of parasitic broomrape (*Orobanche cumana*) have spread in Bulgaria. The last two races appeared one after another and have complicated the sunflower breeding process. During the last 20 years a sufficient number of sunflower lines had broomrape resistance to race E, the common race. However, since 2004 the aim of the breeding program was directed towards developing lines resistant to race F and since 2008 to race G.

Seventeen 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* (wild), *H. argophyllus*, *H. debilis*, *H. petiolaris* and *H. praecox*) and 5 species from genera *Calendula*, *Carduus*, *Grindelia*, *Inula* and *Tithonia* of family *Compositae* to obtain resistance to broomrape. Total resistance to this parasite have been developed in some lines such as 7019 R, 7203 R, C 23/1, C 41, C 46, C 48, C 55, and C 56. Table 7 shows the new developed lines.

Table 7: Characterization of sunflower lines obtained by interspecific and intergeneric hybridization and resistant to broomrape harvest in 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
PR-56/8 /c.s × *Aster speciosa*/	100	100	49.56	17
PR-57/8 /c.s. × *Inula*/ × Tith.	100	100	50.35	16
PR-61/8 /c.s. × *Aster speciosa*/	100	100	51.41	17
PR-63/8 /c.s. × *H. pauciflorus*/	100	100	48.80	25
PR-68/8 /c.s × *Tithonia*/ × Verb.	100	100	48.36	16

Resistance to imidazolinone herbicide

Three sources for resistance to the herbicide Pulsar were used. About 80% of the obtained materials were R lines. The most advanced generations were lines obtained from the source for herbicide resistance from the USA (J. Miller) transferred into our materials using HA 425, HAR 426, and HAR 427. The best line was C 41. The lines based of the source BASF - BTI-M1 and BTI-R1 and our source (An 17) were obtained.

Table 8: Amino acid content of seed protein of species' accession from the family *Compositae*, g / 100 g protein

Species / Origin	Protein	Lysine	Threonine	Cysteine	Valine	Methionine
	%	%	%	%	%	%
Arctium lapa	45.32	5.14	3.61	1.10	4.54	0.91
Bidens tripartita	47.50	5.82	3.67	1.03	4.80	1.56
Cirsium lanceolatum	44.06	4.92	3.46	1.31	4.50	1.03
Onopordum acanthium	-	4.42	3.70	1.04	4.71	1.54
Tithonia rotundifolia	64.11	3.60	3.73	1.45	4.31	1.52
Tithonia speciosa	66.82	3.73	3.82	1.36	4.35	1.78
Viguera sp.	60.68	3.50	3.10	1.69	4.05	1.77
Verbesina alata	78.74	3.73	2.96	1.60	4.10	1.27
V. helianthoides	79.82	3.51	2.95	1.29	4.11	1.51
V. encelioides	60.86	4.12	3.42	1.56	4.46	1.66
Gaillardia hibrida	-	3.50	3.10	1.69	4.05	1.77
H. annuus - h. Albena	-	3.41	4.63	0.63	4.73	2.1

Table 9: Amino acid content of seed protein of 11 lines (F_5), originating from the cross *H. annuus* x *Carduus acanthoides*, g / 100 g protein

No.	Form / Sample	Lysine	Threonine	Cysteine	Valine	Methionine
		%	%	%	%	%
1	1493/1-p	3.58	4.47	-	5.37	0.29
2	1494/1	3.35	4.29	0.25	5.60	0.27
3	1495/1	3.16	4.16	0.22	5.49	0.47
4	1496/1-p	3.88	4.69	0.21	5.40	0.37
5	1500/1	3.25	4.24	0.23	5.52	0.39
6	1500/2	3.19	4.25	0.21	5.36	0.43
7	1503/1-p	4.11	4.86	0.31	5.73	0.63
8	1504/1-p	4.07	4.84	0.46	5.86	1.01
9	1508/1-p	3.38	4.38	0.23	5.46	0.54
10	1511/1	3.01	4.11	0.20	5.42	0.51
11	1511/2	3.16	4.30	0.30	5.38	0.63
12	*C. acanthoides*	5.06	4.81	-	-	-
13	*H. annuus* - h. Albena	3.41	4.63	0.63	4.73	2.1

New sunflower forms with high seed oil content

Some accessions of wild *Helianthus* species could be used as sources for high seed oil content. This conclusion is based on results obtained by interspecific hybridization. Sunflower forms and lines with high seed oil content were obtained from hybrids with *H. eggertii*, *H. pauciflorus* (*rigidus*), *H. smithii*, *H. hirsutus, H. annuus* (wild), *H. nuttallii* ssp. *rydbergii*, and *H pumilus*. From intergeneric hybridization we obtained forms with high seed oil content originating from species *Carduus acanthoides*, *Gaillardia speciosa*, *Grindelia speciosa*, and *Telekia speciosa.* Results for seed oil content are presented in Tables 5, 7 and 11.

New forms with high protein content in the seeds and rich diversity of amino acids

Some *Helianthus* species and some other species from the *Compositae* were sources of high seed protein content and higher amino acid contents for humans and animals. Amino acid content of seed protein of some species from the family *Compositae* was higher of that in cultivated sunflower (Table 8). From valuation of the new hybrid forms was established that some of them were with higher amino acid contents, too (Tables 9 and 10).

New sources of *cms*

The total number of the new *cms* sources was 15 (Table 11). Some of the sources are different from *cms* PET 1. Fertility restoration genes were found for all *cms* sources.

Sources of new Restorer fertility (*Rf*) genes

The investigation was directed towards discovery of *Rf* genes for the common *cms* PET1 from *H. petiolaris* and for new *cms* sources discovered at DAI. In total, 271 crosses with 37 *Helianthus* species and some other species from the *Compositae* were found to carry *Rf* genes for *cms* PET 1. *Rf* genes were also found in *Helianthus argophyllus*, *H. debilis* and *Helianthus rigidus* (*pauciflorus*) for *cms* RIG-1, and *Rf* genes in *Carduus acanthoides* for *cms* ARG-3-M-1.

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

The current research has obtained more than 3900 new R forms, including 1306 R lines that have been fixed and named. All of them are resistant to downy mildew. Some are resistant to Phomopsis and broomrape. There were lines that showed resistance to Phoma and tolerance to Sclerotinia. All lines showed high combining ability. A partial list of these lines is presented in Table 12.

Table 10: Amino acid content of seed protein of 12 lines (F_5) originating from the cross *H.annuus* × *Bidens tripartita*, g /100 g protein

No.	Form / Sample	Lysine	Threonine	Cysteine	Valine	Methionine
		%	%	%	%	%
1	1515/1-p	4.08	4.61	-	6.23	0.37
2	1515/2-p	3.51	4.26	-	6.16	0.44
3	1516/1-p	3.81	4.31	-	6.40	0.39
4	1517/1	3.18	4.17	-	6.30	0.29
5	1518/1	3.47	4.57	0.21	5.31	0.46
6	1519/1	3.10	4.13	0.23	5.30	1.06
7	1520/1	3.58	4.66	0.37	5.24	0.75
8	1520/2	3.18	4.30	0.26	5.15	0.67
9	1526/1	3.37	4.19	0.31	5.36	0.77
10	1530/1	3.61	4.47	-	5.57	0.44
11	1574/1	3.15	4.23	0.70	4.72	0.57
12	1574/2	3.30	4.32	0.24	4.88	0.22
13	*Bidens tripartita*	5.82	3.67	1.03	4.80	1.56
14	*H. annuus* - h. Albena	3.41	4.63	0.63	4.73	2.1

Table 11: Sources of *cms* produced by interspecific hybridization

Origin	Obtained in generation	Year of observation	Year of 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. 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 12: Characterization of R lines, produced from interspecific and intergeneric hybridization harvested in 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*
PR-51/8	c.s. × *Carduus acanthoides*	120	14	100	52.96	16*
PR-57/8	c.s. × *Inula* sp./ × *Tith.*	140	15	102	50.35	17*
Sc-17	c.s. × *Grindelia speciosa*	105	16	100	49.11	15*
Sc-23	c.s. × *Telekia speciosa*	90	13	95	51.39	17*
Sc-58	c.s. × *Tith.*/ × *Verbesina* sp.	110	17	104	48.12	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 forms with normal cytoplasm (B lines)

New B lines were created from forms only obtained by interspecific hybridization. The total number of developed "B" lines up to 2010 was 289. The stem height varies from 45 to 180 cm, and the vegetation period varies from 86 to 125 days. Thousand seed weight varies from 30 to 125 g, and the seed oil content varies from 40 to 54% (Table 13). Some B lines have resistance to Phomopsis and others to downy mildew and broomrape. Such lines are 6066B, 6101B, 6134B, 6149B, 6488B, and 6748B. Sterile analogues were also developed for all B lines in *cms* PET 1. Sterile analogues for the rest of the *cms* sources were used to create four lines with the aim to test theses lines in experiments evaluating cytoplasmic effect on some agricultural characters for the development of new hybrids.

Table 13: Characteristics of B lines produced from interspecific hybridization

No.	Origin	Plant height	Head diameter	Seed oil content	Vegetation period
		cm	cm	%	days
6101	*H. decapetalus* - M-043	125	18	47.35	106
6134	*H. debilis* - E-011	100	22	48.08	107
6159	*H. pauciflorus* - M-028	155	15	48.79	105
6170	*H. strumosus* - M-056	110	12	47.82	110
6202	*H. hirsutus* - M-029	105	12	45.25	105
6215	*H. salicifolius* - M-045	180	18	51.15	107
6275	*H. argophyllus* - E-007	140	23	49.96	105
6149	*H. eggertii* - M-001	140	24	48.91	103

New sunflower hybrid combinations

During the developing and investigation of new sunflower hybrids, two groups were formed. The first group included crosses between old, confirmed Bulgarian A / B / lines with R lines obtained from interspecific and intergeneric hybrids, and the second group included crosses between new A /B/ lines, obtained by using mutagenesis and R lines obtained by wide hybridization. There were a small number of hybrid combinations created from B lines obtained from the wide hybridization and R lines obtained by the same method. Each year 350 to 370 hybrid combinations are produced for testing. Three-fourth of tested hybrids were oil seed types and the rest were developed as large-seeded hybrids, and hybrids with colored seeds for birds.

New sunflower hybrid varieties in registration

New sunflower hybrids were created which increased seed yield and seed oil content per unit area higher than the standard hybrid check. Five of these hybrids, Musala, Mura, Maritsa, Mesta and Magura were registered with the State Variety Commission at the end of 2004. At the beginning of 2008, the first large-seeded hybrid Madan was registered. The paternal source of hybrids Musala, Mura, Maritsa, Mesta and Magura and the maternal source of hybrid Madan were created from materials obtained by interspecific hybridization. The paternal source of hybrid Madan was obtained by intergeneric hybridization.

CONCLUSION

The results of this investigation showed that successful interspecific and intergeneric hybridization was achieved and the transfer of new genetic material into cultivated sunflower was possible. Hybrids with valuable characters were obtained. Lines developed from these crosses can be used in developing high quality sunflower hybrids. The new sources of *cms* and *Rf* genes increases the genetic diversity of current single *cms - Rf* system widely used in heterotic breeding. The results obtained from this investigation confirm the valuable contribution interspecific and intergeneric hybridization can make to a sunflower breeding program.

REFERENCES

Alonso, C., 1996. New highly virulent sunflower broomrape (*Orobanche cumana* Wallr.) phenotypes in Spain. Advances in parasitic plant research. Proc. 6th Int. Parasitic Weed Simposium, April 16-18, 1996, Cordoba, Spain.

Anashchenko, A.B., 1974. Citoplasmaticheskie form mujskoi sterilnosti u podsolnechnika. Dokl. VASHNIL 4: 11-12.

Bachvarova, R., 2004. Proutcvanija varhu sinjata kitka (*Orobanche* spp.) pri slancogled i tjutjin. Dissertation, Sofia.

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

Christov, M., 1990b. A new source of cytoplasmic male sterility in sunflower originated from *H. argophyllus*. Helia 13: 55-61.
Christov, M., 1996a. Characterization of wild *Helianthus* species as sources of new features for sunflower breeding. Proc. Int. *Compositae* Conf., Kew, 1994, Caligari, P.D.S. and Hind, D.J.N. (Eds.). *Compositae*: Biology and Utilization, Royal Botanic Gardens, Kew, 2: 547-570.
Christov, M., 1996b. Hybridization of cultivated sunflower and wild *Helianthus* species. Proc. Int. *Compositae* Conf., Kew, 1994, Caligari, P.D.S. and Hind, D.J.N. (Eds.). *Compositae*: Biology and Utilization, Royal Botanic Gardens, Kew, 2: 603-615.
Christov, M., 1999. Production of new *cms* sources in sunflower. Helia 22(31):1-12.
Christov, M., 2002. Resultati ot prilaganeto na otdalecena hybridizatija i eksperimentalen mutagenez pri podobritelnata rabota sas slancogleda. Iubileina nautcna sesija - 50 godini Dobruddzanski zemedelski institut, I: 315-335.
Christov, M., 2008. *Helianthus* species in breeding on sunflower. Proc. 17th Int. Sunfl. Conf., Cordoba, Spain, Int. Sunfl. Assoc., Paris, France, 2: 709-714.
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. and Panayotov, L., 1991. Hybrids between the genera *Helianthus* and *Tithonia* and their study. Helia 14: 27-34.
Christov, M. and Vassilevska-Ivanova, R.D., 1999. Intergeneric hybrids in *Compositae* (*Asteraceae*). I. Hybridization between cultivated sunflower *H. annuus* L. and *Compositae* Genera. Helia 22(31): 13-22.
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., Vassileva, R., Tsujimoto, H. and Panayotov, I., 1994. Intergeneric hybridization between sunflower and some species of genera from *Compositae*. Int. *Compositae* Conf., Royal Botanic Gardens, Kew, 26.07-05.08.
Christov, M., Shindrova, P. and Entcheva, V., 1996. Transfer of new characters from wild *Helianthus* species to cultivated sunflower. Genet. a Slecht. 32(4): 275-286.
Christov, M., Kiryakov, I., Shindrova, P., Encheva, V. and Christova M., 2004. Evaluation of new interspecific and intergeneric sunflower hybrids for resistance to *Sclerotinia sclerotiorum*. Proc. 16th Int. Sunfl. Conf., Fargo, North Dakota, USA, Int. Sunfl. Assoc., Paris, France, II: 693-698.
Christov, M., Hristova-Cherbadzi, M., Nikolova, V., Ivanova, I. and P. Shindrova. 2009. Medzdurodova hybridizatija pri slancogleda - rezultati i problemi. Sbornik dokladi ot medzdunarodna naucna konferencija. *In*: Dobri praktiki za ustoitcivost zemedelsko proizvodstvo. Lesotehnitceski universitet, Sofia, pp. 167-184.
Cvetkova, F., 1976. Njakoi rezultati ot izpolzvane na medzduvidovata hybridizatija pri selekcijata na slancogleda. Genetika i selekcija 9(3): 194-201.
Encheva, V. and Kiryakov, I., 2002. A method for evalution of sunflower resistance to *Diaporthe/Phomopsis helianthi* Munt.-Cvet. Bulgarian Journal of Agricultural Science 8: 219-222.
Enns, H., Dorrell, D.G., Hoes, J.A. and Chubb, W.O., 1970. Sunflower research. Proc. 4th Int. Sunfl. Conf., Memphis, Tennessee. Int. Sunfl. Assoc., Paris, France. Progress report, pp. 162-167.
Fernandez-Martinez, J. and Ruso, J., 1997. Evaluation of wild *Helianthus* species. FAO Progress Report 1995-1996, Giessen, Germany, pp. 43-46.
Fernandez-Martinez, J.M., Malero-Vara, J., Muñoz-Ruiz, 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. Proc. 4th Int. Sunfl. Conf., Bucharest, Int. Sunfl. Assoc., Paris, France, pp. 333-338.
Georgieva-Todorova, J., 1971. Further study of the form obtained after crossing *Helianthus annuus* L. with *Onopordum acanthium* L. Genetics and Breeding 4(6): 396-408.
Georgieva-Todorova, J., 1976. Megdurodovi otnoshenia v roda *Helianthus* L., Sofia, BAS.
Gulya, T., Rashid, K.Y. and Maširević, S., 1997. Sunflower Diseases. In: Schneiter, A.A. (Ed.), Sunflower Technology and Production pp. 263-379.
Heiser, C.B., 1982. Registration of Indiana - 1 *cms* sunflower germplasm. Crop Science 22(5): 1089.

Heiser, C.B.Jr., Smith, D.M., Clevinger, S.B. and Martin, W.C.Jr., 1969. The North American Sunflower (*Helianthus*). Mem. Torrey Botanical Club 22(3): 3.

Hoes, J.A., Putt, E.D. and Enns, H., 1973. Resistance to *Verticilium* wild in collections of wild *Helianthus* in North America. Phytopathology 63: 1517-1520.

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

Hristova-Cherbadzhi, M., 2012. Study of new forms of sunflower received by distent hybridization. Breeding and genetics of cultivated sunflower - methods, new lines, new crosses, new *cms* source. Lambert Academic Publishing, ISBN 978-3-659-13617-7.

Iliescu, H., 1955. Proposed methodologies for inoculation of sunflower with different pathogens and for disease assessment. Bucharest.

Iurras, M. and Voinescu, A., 1984. Utilizarea speciei *Helianthus argophyllus* Torrey & Gray, pentru obtinerea unor forme xerofite de floarea-soarelui. In: Probleme de genetica teoretica si aplicata, Fundulea 16(2): 123-130.

Ivanov, P., Christov, M., Ivanova, I. and Nikolova, V., 1996. Study of seed oil and protein 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).

Kinman, M.L., 1970. New development in the USDA and state experiment station sunflower breeding program. 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.

Morized, J., Cruizilat, P., Chatenoud, J., Picot, P. and Leclercq, P., 1984. Improvement of drought resistance in sunflower by interspecific crossing with a wild species *Helianthus argophyllus*. Methodology and 1st results. Agronomie 4: 577-585.

Morozov, V.K., 1947. Selekcia podsolnechnika v SSSR. Pistepromizdat, Moskva, 272.

Pacureanu-Joita, M., Vranceanu, A.V., Soare, G., Marinscu, A. and Sandu, I., 1998. The evaluation of the parasite-host interaction system (*Helianthus annuus* L.) - (*Orobanche cumana* Wallr.) in Romania. Proc. 2nd Balkan Symposium on Field Crops. 16-20 June, 1998. Novi Sad, Yugoslavia, Int. Sunfl. Assoc., Paris, France, pp. 153-155.

Panchenko, A., 1954. Gibridi podsolnechnika s topinamburom. V sb.: Kratkoi otchet s nauchno-issledovatelskoi rabote VNIIMK za 1953. Krasnodar, Sovetskaia Kuban.

Panchenko, A., 1965. Rapid method of evaluation of sunflower for resistance to mildew. Selectia i semeproizvodstvo 2: 52-54.

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

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

Pustovoit, G., 1963. Selekcija podsolnetcnika na gruppovoi immunitet metodom medzvidovoi gibridizacii. Masl. i efiromaslitcnoi kulturii, Moskva, 75-92.

Pustovoit, G., 1975. Selekcija podsolnetcnika na gruppovoi immunitet metodom medzvidovoi gibridizacii. *In*: Podsolnetcnik. izd. "Kolos", Moskva, 164-209.

Pustovoit, G.V., Krasnokutskaya, O.N., Platovskii, U.P. and Slyussar, EL. 1976. Phytopathological methods of sunflower evaluation in breeding for resistance to basic diseases. Bul. Sc. Techn. Infor. Of Oil Crops. vol. 1. Krasnodar.

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

Putt, E.D. and Sackston, W.E., 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.

Rushkovskij, C.B., 1957. Metodi issledovanii pri selekcii maslitcnih pastenii na soderdzanie masla i ego katcestvo. M. Pitshepromizdat.

Satziperov, F., 1916. Opit skretshivanija dvuh form podsolnetcnika *Helianthus annuus* x *Helianthus argophyllus*. Tr. prikl. bot., gen. i selek. 9: 207-244.

Saliman, M., Yang, S.M. and Wilson, L., 1982. Reaction of *Helianthus* species to *Erysiphe cichoracearum*. Plant Dis. 66: 572-573.
Schilling, E.E. and Heiser, C.B., 1981. Infrageneric classification of *Helianthus* (*Compositae*). Taxon 30: 393-403.
Serieys, H., 1986. Characterisation of some new cytoplasmic androsterilities sources from *Helianthus* genus. F.A.O. Meeting, July 21-25, Sindos, Greece.
Serieys, H. and Vincourt, P., 1987. Characterization of some new cytoplasmic male sterility sources from *Helianthus* genus. Helia 10: 9-13.
Shindrova, P., 2006a. Downy mildew (*Plasmopara halstedii* Farl. Berlese et de Tony) distribution and race composition during 2004-2005. 70th Anniversary of Plant Protection Institute and Annual Balkan Week of Plant Health - Plant Protection Institute, May 28-31, 2006, Kostinbrod, Bulgaria, Book of Abstracts, p. 22.
Shindrova, P., 2006b. Broomrape (*Orobanche cumana* Wallr.) in Bulgaria - Distribution and race composition. Helia 29.
Škorić, D., 1985. Sunflower breeding for resistans to *Diaporthe* (*Phomopsis helianthi*). Helia 8: 21-23.
Škorić, D., Ćuk, L., Mihaljčević, M. and Marinković, R., 1978. New sources of fertility restoration (*Rf* genes) and downy mildew resistance (*Pl* genes) in Sunflowers. Proc. 8th Int. Sunfl. Conf., Minneapolis, Int. Sunfl. Assoc., Paris, France, pp. 423-426.
Škorić, D., Atlagić, J., Marinković, R., Vasiljević, L., Mihaljčević, M. and Maširević, S., 1987. *In*: Škorić, D. (Ed.), Progress report - 1984-1986, FAO Subnetwork, Szeged.
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*. Proc. 12th Int. Sunfl. Conf., II: 110-114.
Vranceanu, A.V., and Stoenescu, F., 1971. Pollen restorer gene from cultivated sunflower (*Helianthus annuus* L.). Euphytica 20(4): 536-541.
Vranceanu, A.V. and Stoenescu, F., 1976. Breeding for pollen fertility restoration in sunflower. Proc. 7th Int. Sunfl. Conf., Krasnodar. Int. Sunfl. Assoc., Paris, France.
Vranceanu, A.V. and Stoenescu. F., 1978. Genes for pollen fertility restoration in sunflower. Euphytica 27(2): 617-627.
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.
Whelan, E.D.P., 1981. Cytoplasmic male sterility in *Helianthus giganteus* L. x *H. annuus* L. interspesific hybrids. Crop Science 21(6): 855-858.
Zimmer, D.E. and Fick, G.N., 1974. Some diseases of sunflower in the United States - their occurrence, biology and control. Proc. 6th Int. Sunfl. Conf., Bucharest, Int. Sunfl. Assoc., Paris, France, pp. 673-680.