CONTRIBUTION OF INTERSPECIFIC AND INTERGENERIC HYBRIDIZATION TO THE SUNFLOWER BREEDING

Prof. Dr Michail Christov

Dobroudja Agricultural Institute, General Toshevo,

Sunflower Breeder, Mihsan Ltd, 1750 Sofia, Bulgaria,

E-mail: misho_h5@abv.bg

The investigation covered 30-year period.

It was aimed at improving sunflower *Helianthus annuus* L. by conducting wide hybridization – interspecific and intergeneric.

MATERIALS AND METHODS

Plant material

36 species of *Helianthus* and

28 species of other genera of the Compositae family

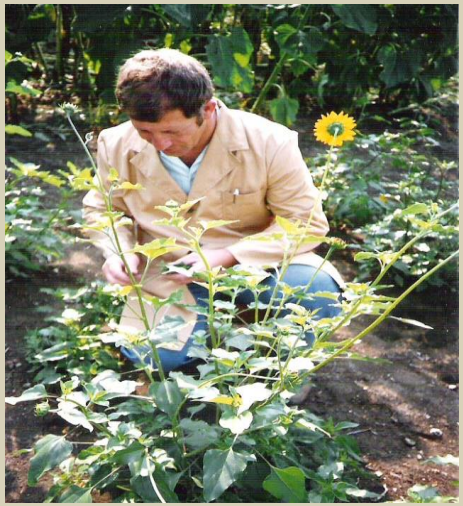
were included in the study.

From cultivated sunflower, 16 varieties and 18 lines were used with their sterile analogues.

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

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

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

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Helianthus species

H.eggertii - M-001

H.glaucophyllus - M-012

H.decapetalus - M-043

H.smithii - M-008

Table 2. Species from different genera of family *Compositae*, used in hybridization with cultivated sunflower.

Groups of species	**Species from family *Compositae***
Species with equal to sunflower chromosome number - (2n=34)	*Gaillardia speciosa*, *Onopordum acanthium*, *Simsia foetida*, *Tithonia rotundifolia*, *Tithonia speciosa, Verbesina alata*, *V. helianthoides*, *V. encelioides*, *Viguera trachyphylla*.
Species with different to sunflower chromosome number (2n= ?)	*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 hybirds were not obtained.

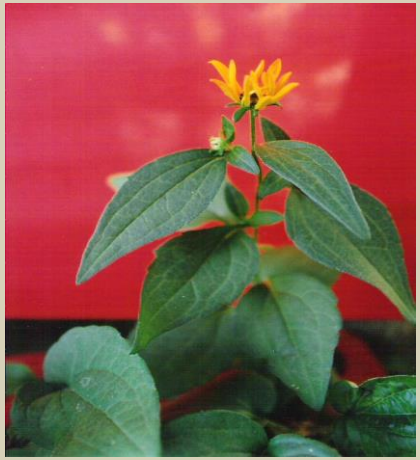
Species of other genera of the *Compositae* family







Species of other genera of the *Compositae* family



Methods and direction of the investigation

- Intraspecific hybridization
- Interspecific hybridization
- Intergeneric hybridization
- Individual selection
- Self-pollination
- Sib-pollination
- Backcross with cultivated sunflower
- Selection of new qualities

Evaluation of new forms, lines and hybrids

Morphological characteristic

It’s carried out on the base of phenological observations and biometric measurements during the vegetation period and on laboratory studies of whole plants and of obtained seed material from them.

Phytopathological characteristic

Evaluation for resistance to diseases:

downy mildew /*Plasmopara helianthi* Novot./,
phoma /*Phoma helianthi Munt*.-Cvet. et al./,
phomopsis /*Phomopsis helianthi Munt.*-Cvet. et al./,
powdery mildew /*Erisiphe cihoracearum* D. C./ ,
sclerotinia /*Sclerotinia sclerotiorum* Lib./,
alternaria /*Alternaria helianthi* (Hanasf.) Tub. and Nish./,
rust /*Puccinia helianthi* Schw./,
verticillium /*Verticilium helianthi* Keeb./

and to the parasite **broomrape** /*Orobanche cumana Wallroth*./

of the studied plant material was carried out on methods confirmed in the institute /Panchenko, 1975; Tourvieille et al., 1988; Christov, 1990; Christov et al., 1992; Alonso, 1996; Christov, 1996; Christov, 1996a/, Christov et al. /1996/, Pacureanu-Joita, 1998; Fernandez-Martinez et al., 2000; Encheva and Kiryakov, 2002; Christov et al., 2004; Shindrova, 2006a; Shindrova, 2006b/.

Biochemical characteristic of seeds

Seed oil and protein content and fatty and amino acid content of accessions from different *Helianthus* species, species of other genera of the *Compositae* family, interspecific and intergenerc hybrids and new sunflower lines and hybrids was evaluated according to methods confirmed in the institute /Rushkovskii, /1957/; Stoianova and Ivanov /1968/; Ivanov et al. /1996/.

Apparatus Nuclear Magnetic resonance for evaluation of seed oil content and Analyzer for amino acids Hitachi, L-8500 were used.

Sources of CMS

were searched among materials obtained from crosses **wild species** x **cultivated sunflower**.

For obtaining of sterile inflorescences the pollination was carried out with pollen from B lines or cultivars.

After sterility maintenance and proving of its cytoplasmic type, the comparative study with other CMS sources, obtained in DAI General Toshevo and all over the world began.

Evaluation of cytoplasmic effect on some agronomical characteristics of lines and hybrids included in some CMS sources was carried out.

First of all sources of *Rf* genes were searched in crosses **sterile sunflower lines** x **wild species**.

Presence of *Rf* genes in the genome of wild species was established in F1. Forms with *Rf* genes were found also in materials obtained from crosses **cultivated sunflower /B line or cultivar/ x wild species** and **wild species x cultivated sunflower**.

The establishment was carried out when pollen of these materials was used for pollination of sterile plants of cultivated sunflower on the base of CMS source.

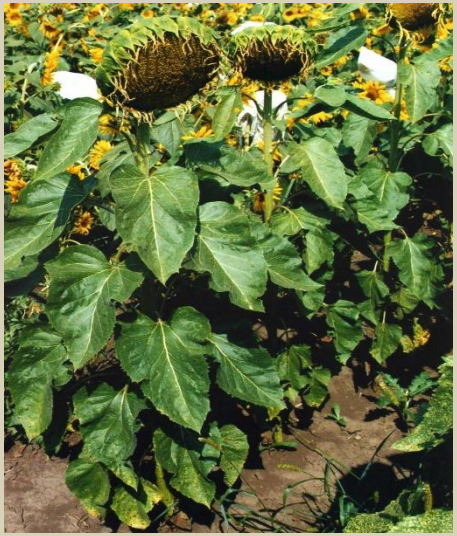
In all cases *Rf* genes were searched for several CMS sources and the genetic determination of restoration was studied.

Developing of sunflower B lines was carried out by purposeful selection in the hybrid materials, which in most cases began after third generation and continued usually to 9 - 12 generation.

Evaluation and selection of materials began on the base of their morphological, biochemical and phytopathological characteristics, absence of *Rf* genes and presence of good combining ability.

Developing of sterile analogues /A lines/ and B lines began with establishment of the fact that there are not any *Rf* genes in the studied material. After BC3 or BC4 began study of general combining ability of created A lines, and after that their specific combining ability for developing the best hybrid combinations.

Hybrid combinations were created using sterile analogues /A lines/ of **B** lines, obtained from interspecific hybridization and experimental mutagenesis included in 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 x R/, were tested by previous testing. The best among them were tested in competition trials and suggested 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

More than 67,000 F1 hybrid plants were obtained from the hybridization between sunflower *Helianthus annuus* and 36 species from genus *Helianthus* included in the investigation (Table 1).

(More than 2000 F1 plants have died)

Despite F1 hybrids originated from *H. simulans* all other F1 hybrids gave seeds.

The following generations were derived - F2, F3, BCF1, etc. until obtaining stable hybrid forms and creating lines from them.

Genes, controlling resistance to diseases, parasites and other stress factors were established in many of the hybrids.

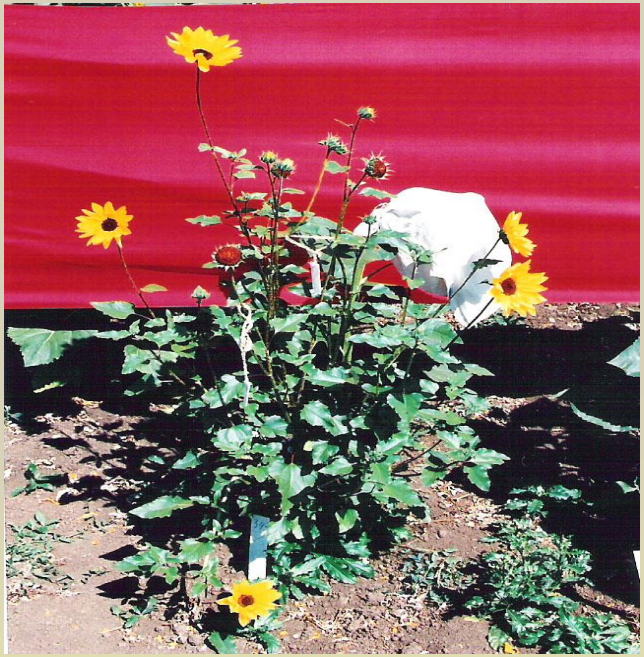
Forms with new plant architectonics, with different vegetation period and different coloration of seeds were obtained.

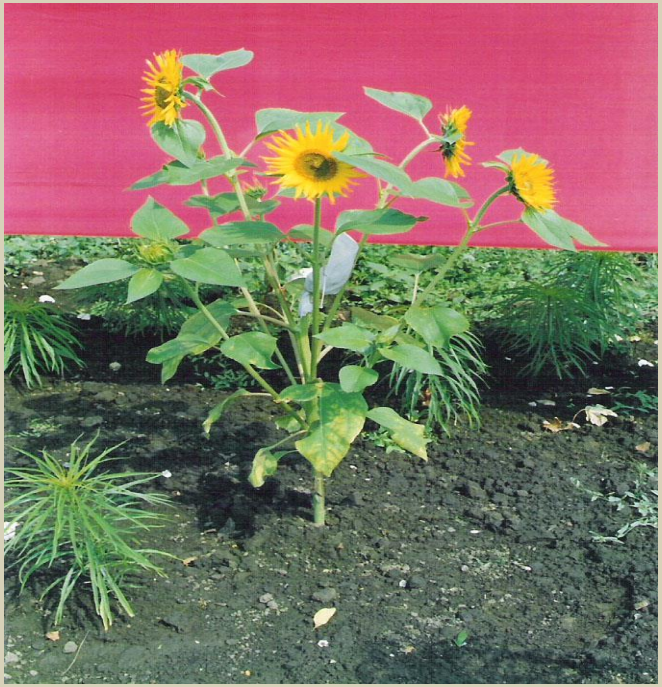
Many of the new forms were with high combining ability and high seed oil content, higher than that of cultivated sunflower, included in the hybridization.

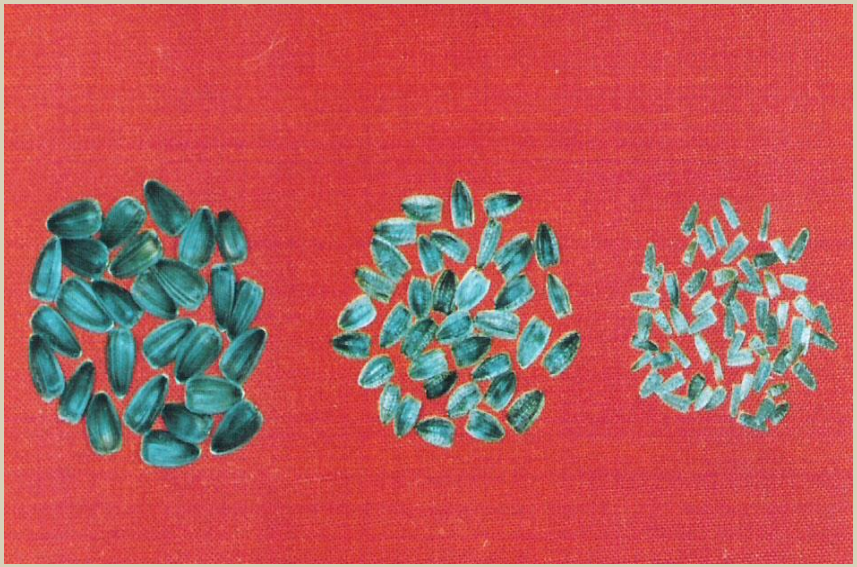
Great number of new CMS sources and genes for fertility restoration (*Rf*) were obtained (Table 3 and 4).

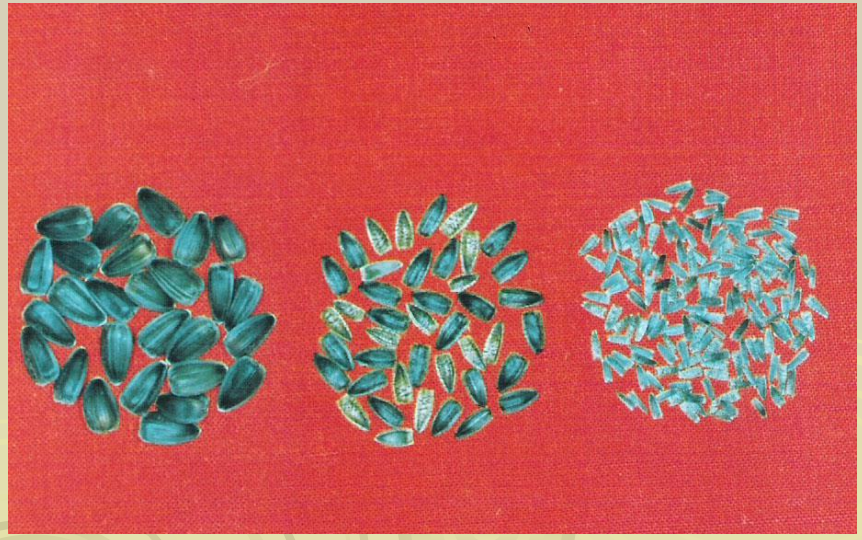
Table 3. Sources of new characters transferred into cultivated sunflower.

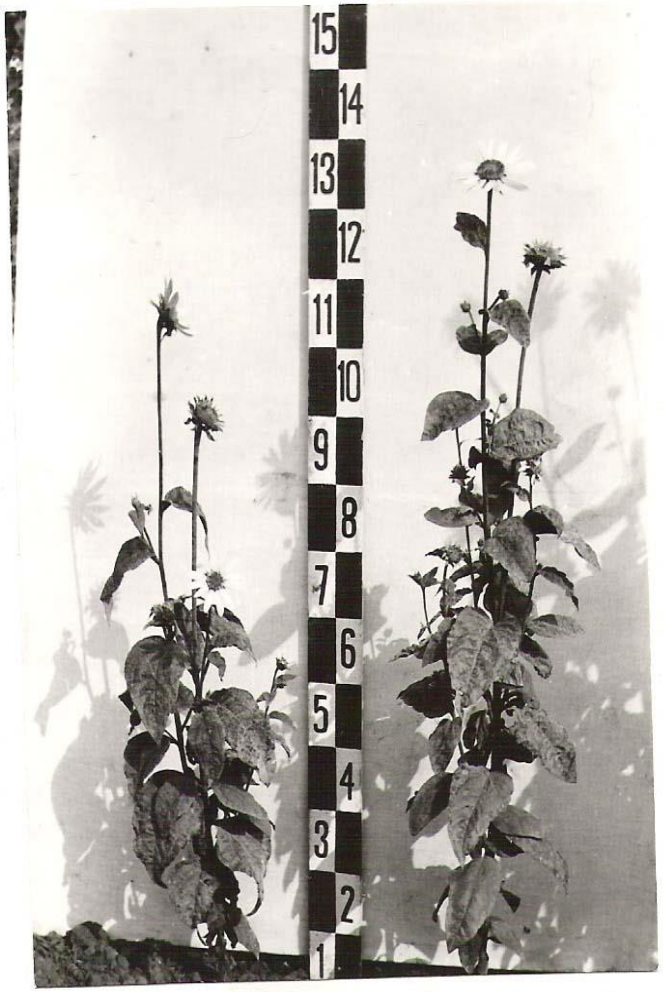
Characters	Species
Resistance/tolerance to:	*H.annuus* (w.f.), *H.argophyllus, H. bolanderi*, *H.debilis*, *H. exilis, H. neglectus*., *H. paradoxus*, *H.petiolaris*, *H.praecox*, *H.divaricatus*, *H.doronicoides, H.giganteus*, *H.glaucophyllus*, *H.grosseserratus*,
Plasmopara helianthi	*H.mollis*, *H.maximiliani*,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



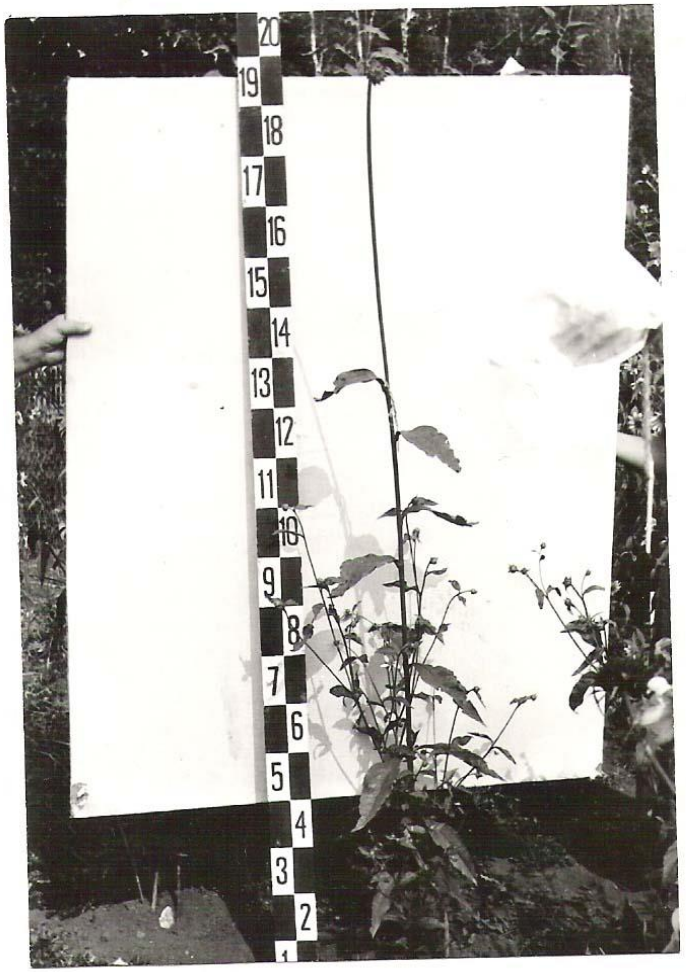








F_1 - H.annuus x H.eggertii



F_1 - H.laevigatus x H.annuus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BC_1 – (H.eggertii x H.annuus) x H.annuus

Origin of intergeneric hybrids

From all 1927 F1 hybrid plants in this investigation 805 F1 hybrid plants from direct crosses and 19 F1 hybrid plants from reciprocal crosses were included and they originated from sunflower and 28 species from other genera of family *Compositae* /Table 4/.

Hybrid plants second generation were obtained from the combination *H.annuus* x *Simsia foetida*, and from all 19 F1 hybrid plants from the reciprocal crosses, only those from combination *Tithonia speciosa* x *H. annuus* reached the sixth hybrid generation.

Despite *Rf* genes, some other characters with economical importance were also transferred.

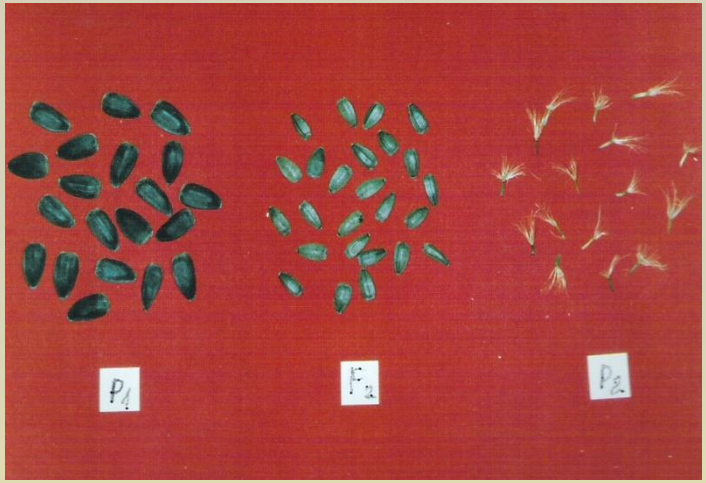
Forms, resistant to downy mildew, phomopsis, sclerotinia, alternaria and broomrape were obtained.

Other forms were distinguished with smaller stem and shorter vegetation period.

Some forms with higher seed oil content, or others with various amino acid protein content were established. In table 4 were presented the species, which these characters were transferred from.

Table 4. Sources of new characters transferred to cultivated sunflower.

Characters	Species
Resistance/tolerance to:	Race 731 - *Inula helenium*, *Tithonia rotundifolia* and *Grindelia speciosa*.
Plasmopara helianthi	
Phomopsis helianthi	*Arctium lapa*, *Carduus acanthoides*.
Erysiphe cichoracearum	*V. encelioides*, *Grindelia speciosa*, *Ehinacea purpurea*, *Tithonia rotundifolia*, *Telekia speciosa*.
Orobanche cumana	*Calendula officinalis*, *Carduus acanthoides*, *Grindelia speciosa*, *Inula helenium*, *Tithonia rotundifolia*, *Tithonia speciosa V. helianthoides*.
Phoma helianthi	*Arctium lapa*, *Grindelia speciosa*, *V. encelioides*.
Sclerotinia sclerotiorum	*Tithonia rotundifolia*, *Arctium lapa*, *Carduus acanthoides*, *Grindelia speciosa*, *Inula helenium*. *Matricaria chamomile*, *Silphium perfoliatum*, *Telekia speciosa*, *Zinnia angustifolia*.
Earliness	*Aster speciosa*, *Verbesina alata*, *Telekia speciosa*.
Seed size	*Carduus acanthoides*, *Aster speciosa*, *Carlina vulgaris*.
High oil content	*Carduus acanthoides*, *Gaillardia speciosa*, *Grindelia speciosa*.
Various amino acid protein content	*Carduus acanthoides*, *Bidens tripartite*, *Arctium lapa*, *Grindelia speciosa*, *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*.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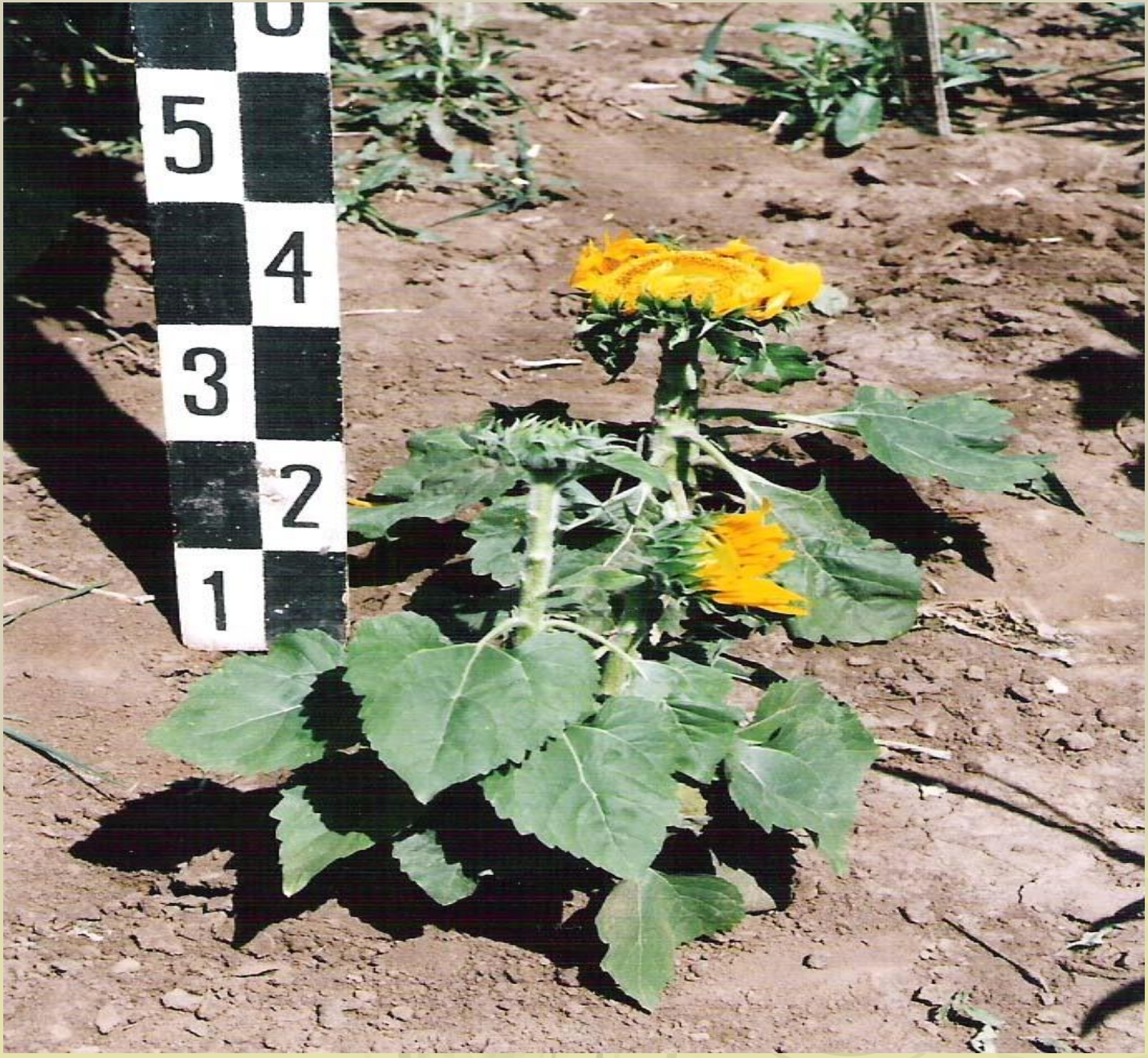
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Creating of new forms from interspecific and intergeneric hybrids sunflower

The scheme for creating of new sunflower forms and lines from the obtained interspecific and intergeneric hybrids was similar.

The difference between them was that from the hybrid material originated from the intergeneric hybridization in this case mainly, forms and lines, carriers of *Rf* genes were obtained.

The main reason for including wild *Helianthus* species and some other species from family *Compositae* in the research work on biological improvement of sunflower crop was the presence of resistance to diseases, parasites and pests.

The artificial testing of the new hybrids form, obtained as a result of wide hybridization, to diseases began in different generations and depended on the quantity of obtained seeds and the plants. In most cases F1 plants were tested if the number of obtained seeds was enough. Sufficient number of plants was obtained mainly from crosses with annuals and from crosses sterile sunflower lines x *Helianthus* species or another genus from family *Compositae*.

Studies on downy midew resistance were the priority and they were followed by studies on sclerotinia, phomopsis, phoma, aternaria resistance and etc.

Great priority was given to studies on broomrape resistance.

Breeding on developing high oil hybrids from the interspecific and intergeneric hybrids was based on some important characters such as high ratio kernel/hull and increasing of oil content in the kernel.

The next index, connected to the high seed productivity and seed oil content was 1000 seeds weight, which depended on the ratio percentage of kernel/percentage of seed and percentage of oil in the kernel.

Important index was number of seeds obtained per plant - its inheritance was intermediate and the dependence between number of obtained seeds per plant and the productivity was positive.

Other characters important for sunflower breeding work were: seed size and hull coloration; friability of seeds; percentage of seed set; plant height; head diameter; vegetation period; branching, lodging; number of leaves per plants lines and hybrid cultivar; number of plants/dka and etc.

Another direction of the breeding work was increasing of protein content of large seeded forms, seeds suitable for direct use by the man - confectionery type and small colored seeds suitable for bird feeding with diverse amino acid content of the protein.

Applying of the methods of wide hybridization gave the possibility to be found new sources of CMS and different sources of genes restorers of fertility (*Rf* genes) for CMS PET 1 and for other CMS sources, obtained in DAI and in other research institutions.

New sunflower forms resistance / tolerance to diseases and parasites

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

Full resistance to ***Plasmopora heliathi*,** races № 300, 330 and 700 showed more than 2600 accessions, obtained with participation of 36 *Helianthus* species and 16 species from other genera of family *Compositae* /tables 1 and 2/. Resistance to races № 731 considered as the most virulent in Bulgaria was established in more than 400 hybrid forms, originated from the species *H. divaricatus, H. hirsutus, H. pauciflorus* (*rigidus*), *H. tuberosus, H. debilis* ssp. *debilis*, *H. paradoxus, Inula helenium*, *Carduus acanthoides, Tithonia rotundifolia* and *Grindelia speciosa*.

More than 96 % of the resistant forms possessed *Rf* genes. Many of them are completed R lines. Some of these materials possessed resistance to some other diseases and to the parasite broomrape (Table 5).

Table 5. Characterization of sunflower lines, obtained by interspecific and intergeneric hybridization, resistant to downy mildew - race 731, harvest 2009.

Accession, pedigree	Resistance to		Seed oil content, %	Generation
	downy mildew, %	broomrape, %		
PR-1/8 /c.s. x *H.pauciflorus*/	100	100	48.48	23
PR-9/8 /c.s. x *H.tuberosus*/	100	100	47.27	25
PR-13/8 /c.s. x *H.pumilus*/	100	-	58.28	16
PR-25/8 /c.s. x *H.pauciflorus*/	100	100	46.89	25
PR-35/8 /c.s. x *H.hirsutus*/	100	100	48.80	16
PR-41/8 /c.s. x *H.divaricatus*/	100	100	47.03	18
PR-51/8 /c.s. x *C.acanthoides*/	100	-	52.96	18
PR-56/8 /c.s. x *Aster speciosa*/	100	100	49.56	17*
PR-57/8 /c.s. x *Inula*/ x *Tith.*	100	100	50.35	16

*unbranched form

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

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

(*Sclerotinia sclerotiorum* (Lib) de Bary)

Studies on sclerotinia resistance /***Sclerotinia sclerotiorum***/ were carried out in field conditions and in greenhouses. Different ways of artificial inoculation were applied and as the most effective was that with direct micellium setting in different uncovered parts of the plant.

High tolerance to *Sclerotinia sclerotiorum* showed some forms originated from *Helianthus* species - *H. eggertii*, *H. pauciflorus*, *H. smithii*, *H. praecox*, *H*. *petiolaris*, *H. argophyllus*, *H. annuus* (w f.) and species from other genera - *Tithonia rotundifolia*, *Arctium lapa*, *Carduus acanthoides*, *Grindelia speciosa*, *Inula helenium*, *Matricaria chamomile*, *Silphium perfoliatum*, *Telekia speciosa*, *Zinnia angustifolia*.

This tolerance referred to those pathogens forms, which infected the head, stem, and the basal part of sunflower stem.

Grade of infection with *Sclerotinia sclerotiorum*

Lines from intergeneric hybridization, resistance to *Sclerotinia sclerotiorum*

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

The resistance transferred from the species *H. decapetalus* was determined by a single dominant gene.

The investigations on alternaria resistance (***Alternaria helianthi*** (Hansf.) Tubaki and Nishihara and ***Al. zinniae*** Pape) began later. During the last years the method of Encheva and Kiryakov (2002) was applied.

Some of the obtained results were presented in table 6.

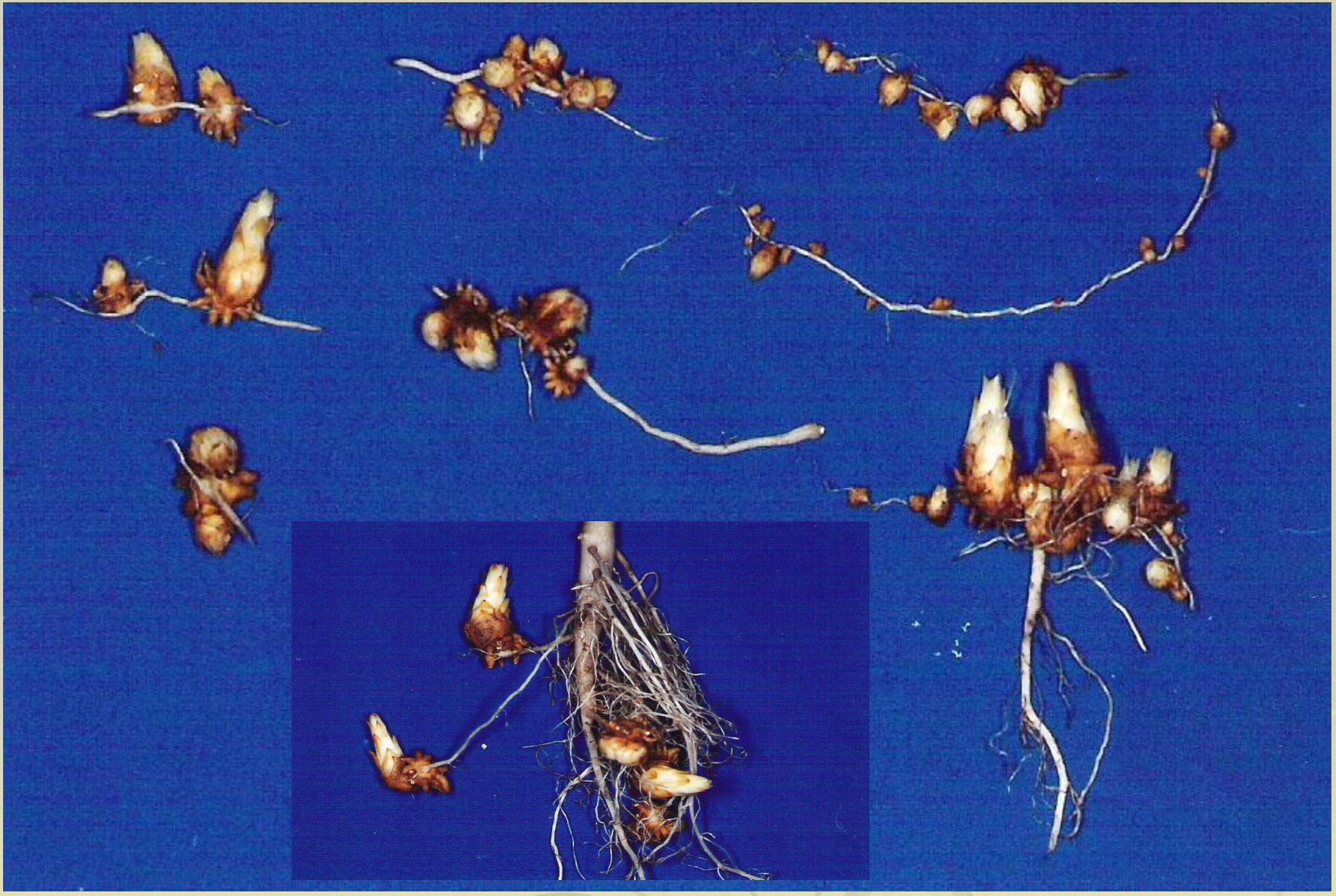
Table 6. Characterization of sunflower lines, obtained by interspecific and intergeneric hybridization for resistance to Phomopsis, Phoma, Alternaria and Sclerotinia, harvest 2009.

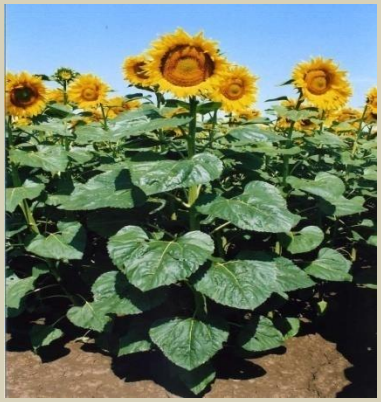
Acession, pedigree	Resistance to, grades			
	Phomopsis gr. 0-4	*Phoma* gr. 0-4	*Alternaria* gr. 0-4	*Sclerotinia* gr. 0-5
Sc-1 /c.s. x C. acanth./	3	0	0	1
Sc-2 L-6116B	1	0	0	2
Sc-3 /c.s. x *H. debilis*/	0	0	1	2
Sc-5 /c.s. x *H.pauciflor.*/	2	0	3	0
Sc-8 /c.s. x *H. argophyllus*/	0	0	0	0
Sc-9 /c.s. x *H.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

Broomrape (*Oronbanche cumana* Wallr.)

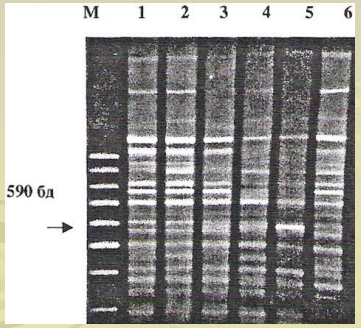
The decision of this problem is carried out on a genetic way by finding of new sources for resistance and developing of new sunflower forms resistant to the arise races of broomrape.

Another, practicable opportunity for protection of sunflower plants from the broomrape attacks is applying of suitable chemical substances /herbicides/, which can stop parasite's growth.





Our investigations on sunflower resistance to the broomrape included applying separately or in combinations certain methods (such as selection, hybridization, experimental mutagenesis and etc.) and valuations /phytopathological valuation – percentage of resistance and level of attacks, biochemical valuation and etc./



Races E, F and G of the parasite broomrape (*Orobanche cumana* Wallroth.) were spread in Bulgaria. The last two races appeared shortly one by another and this aggravated the sunflower breeding.

During the last 20 years a sufficient number of sunflower lines resistant to race E of the broomrape were developed. From 2004 the aim of the breeding work was directed to developing lines resistant to race F, and from 2008 to race G of the parasite.

In creating of resistant to broomrape forms 16 wild *Helianthus* species were used (*H. tuberosus*, *H. pauciflorus*, *H. eggertii*, *H*. x. *laetiflorus, H. decapetalus*, *H. hirsutus, H. divaricatus*, *H. giganteus, H. maximiliani*, *H. nuttallii* ssp. *rydbergi, H. salicifolius, H. smithii, H. annuus* /w. f./, *H. argophillus, H. debilis, H. petiolaris* and *H. praecox*) and 6 species from genera *Calendula*, *Carduus*, *Grindelia*, *Inula* and *Tithonia* of family *Compositae*. Full resistance to the parasite showed some new lines such as 7009R, 7019 R, 7203 R, C 23/1, C 41, C 46, C 48, C 55, C 56 and etc (Table 7).

Table 7. Characterization of sunflower lines, obtained by interspecific and intergeneric hybridization and resistant to broomrape, harvest 2009.

Accession, pedigree	Resistance to		Seed oil content, %	Generation
	broomrape, %	downy mildew, %		
PR-1/8 /c.s. x *H.pauciflorus*/	100	100	48.48	23
PR-9/8 /c.s. x H.*tuberosus*/	100	100	47.27	25
PR-19/8 /c.s. x *H.divaricatus*/	100	100	45.25	19
PR-25/8 /c.s x *H.pauciflorus*/	100	100	46.89	25
PR-35/8 /c.s x *H.hirsutus*/	100	100	48.80	16
PR-41/8 /c.s. x *H.divaricatus*/	100	100	47.03	18
PR-47/8 /c.s. x *H.bolanderi*/	100	100	50.44	19
PR-56/8 /c.s x *Aster speciosa*/	100	100	49.56	17
PR-57/8 /c.s. x *Inula*/ x Tith.	100	100	50.35	16
PR-61/8 /c.s. x *Aster speciosa*/	100	100	51.41	17
PR-63/8 /c.s. x *H.pauciflorus*/	100	100	48.80	25
PR-68/8 /c.s x *Tithonia*/ x 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 forms were R lines.

The highest generations were the lines, obtained from the source for resistance from the USA /J. Miller/, IMI type transferred to our materials using HA 425, HAR 426, HAR 427.

The best characters possessed line C 41.

Lines on the base of the source BASF - BTI-M1 and BTI-R1 and our source An 17 were obtained.

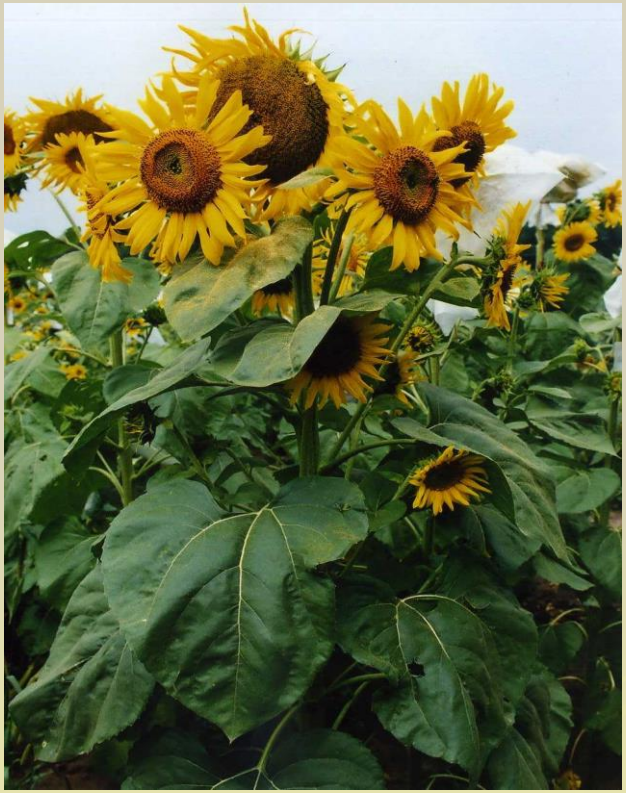
New sunflower forms with high seed oil content

Some accessions of wild *Helianthus* species could be used as sources for high seed oil content in sunflower.

This conclusion is base on results established for different hybrid forms, obtained by applying of interspecific hybridization.

Sunflower forms and lines with high seed oil content were obtained from hybrids with participation of the species *H. eggertii*, *H. pauciflorus /rigidusl*, *H. smithii*, *H. hirsutus, H. annuus* (w. f.), *H. nuttallii* ssp. *rydbergii*, *H pumilus* and etc.

From intergeneric hybridization were obtained forms with high seed oil content originated from species *Carduus acanthoides*, *Gaillardia speciosa*, *Grindelia speciosa*, *Telekia speciosa* and etc. Some results for seed oil content were presented in tables 5, 7 and 11.



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

Some *Helianthus* species and some other species from family *Compositae* are sources of high seed protein content and higher content of amino acids valuable for man and for animals Results for some of the new forms were presented in tables 8 and 9.

Table 8. Amino acid content of seed protein of 11 lines (F5), originated from the cross *H.annuus* x *Carduus acanthoides*, in malls %.

№	Form / Sample	Lisin, %	Treonin, %	Cystein, %	Valin, %	Metionin, %
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

Table 9. Amino acid content of seed protein of 12 lines (F5), originated from the cross *H.annuus* x *Bidens tripartita,* in molls %.

№	Form / Sample	Lisin, %	Treonin, %	Cystein, %	Valin, %	Metionin, %
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

New sources of CMS

The total number of the new CMS sources was 15 + 3 (table 10).

Some of the sources distinguished from CMS PET 1 significantly.

For all sources were found genes restorers of fertility.

Table 10. Sources of CMS produced by interspecific hybridization.

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

Sources of new Restorer fertility (*Rf*) genes

The investigation was directed towards discovery of *Rf* genes for CMS from *H*. *petiolaris* (Pet-1) and for the new CMS sources, obtained in DAI Gen. Toshevo.

It was found, that 271 accessions of 36 + 1 *Helianthus* species and some other species from family *Compositae* carried *Rf* genes for CMS PET 1.

We found also *Rf* genes 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/

Till now were selected and obtained more than 3900 new R forms including 1306 R lines which are fixed and named. All of them are resistant to downy mildew. Some of them were resistant to phomopsis and broomrape.

There were lines, which showed resistance to phoma and others - even tolerance to sclerotinia.

All lines showed high combining ability. Part of these lines is presented in table 11.

Table 11. Characterization of R lines, produced from interspecific and intergeneric hybridization, harvest 2010.

No	Origin	Plant height, cm	Head diameter, cm	Vegetation period, days	Seed oil content, %	Generatio
PR-1/8	c.s. x H.pauciflorus M-028	110	13	100	48.48	19*
PR-13/8	c.s. x H. pumilus M-172	105	14	98	58.28	17*
PR-41/8	c.s. x H.divaricatus M-044	130	16	102	47.03	18*
PR-47/8	c.s. x H. bolanderi E-009	140	15	103	50.44	15*
PR-51/8	c.s. x Carduus acanthoides	120	14	100	52.96	16*
PR-57/8	c.s x Inula sp./ x Tith.	140	15	102	50.35	17*
Sc-17	c.s. x Grindelia speciosa	105	16	100	49.11	15*
Sc-23	c.s x Telekia speciosa	90	13	95	51.39	17*
Sc-58	c.s. x Tith./ x Verbesina sp	110	17	104	48.12	15*
C 23/1	c.s. x H.debilis E-011	105	17	103	49.16	17*
C 55	c.s. x H.debilis E-011	120	16	105	52.71	15*
C 56	c.s. x H.hirsutus M-029	115	17	105	52.38	15*

*branched forms

New sunflower forms with normal cytoplasm /B lines/

New B lines were created only from forms, obtained by interspecific hybridization.

The total number of developed /fixed/ “B” lines till 2010 is 289.

The stem height varies from 45 to 180 cm, and the vegetation period - from 86 to 125 days.

Thousand seed weight varies from 30 to 125 g, and the seed oil content - from 40 to 54% (Table 12).

Some B lines show resistance to phomopsis and others - to downy mildew and broomrape.

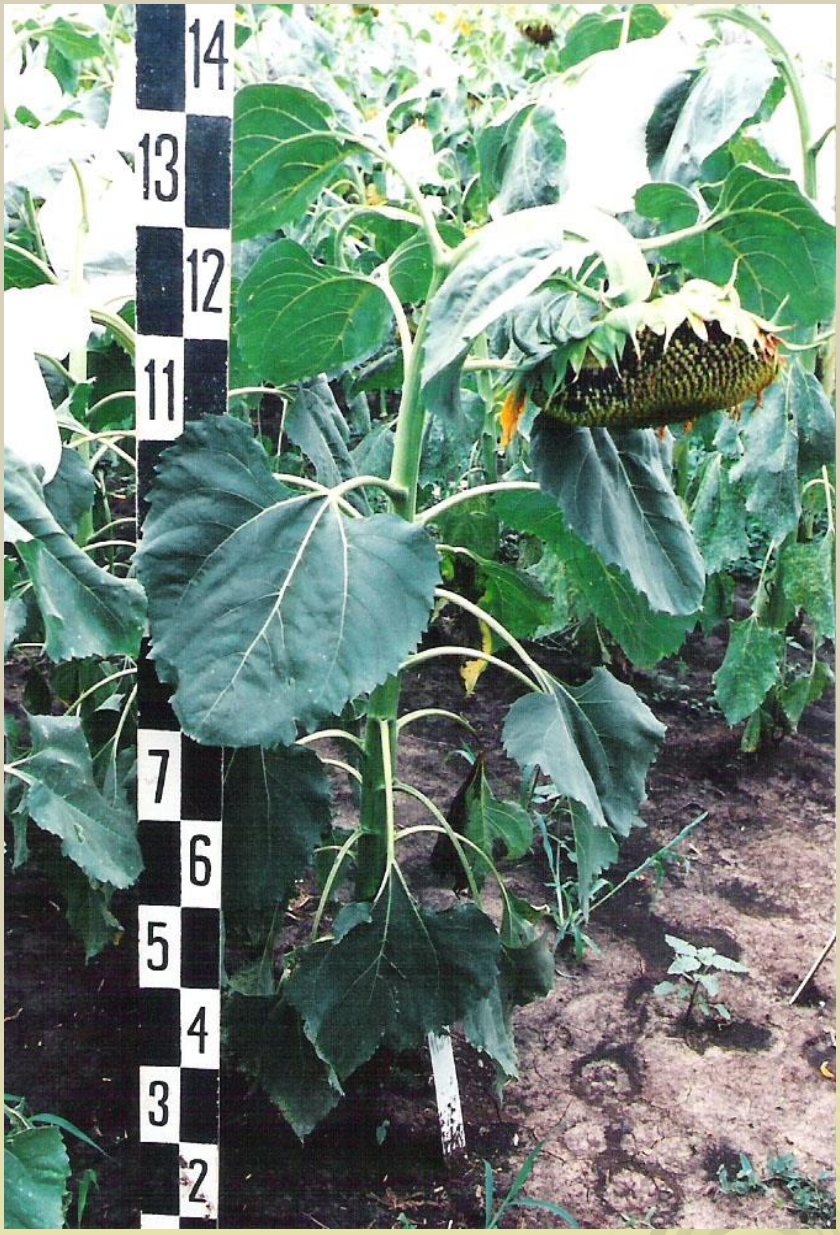
Such lines are 6066B, 6101B, 6134B, 6149B, 6488B, 6748B and etc.

Sterile analogues were developed for all B lines in CMS PET 1.

Sterile analogues for the rest CMS sources were created for four lines from the new lines with aim to supply their use in some experiments as evaluation of the cytoplasmic effect on some agricultural features of the new hybrids.

Table 12. Characteristics of B lines, produced from interspecific hybridization.

No	Origin	Plant height, cm	Head diameter, cm	Seed oil content, %	Vegetation period, 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


14
13
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2

New sunflower hybrid combinations

During the developing and investigation of new sunflower hybrid varieties were performed two groups of combinations.

The first combination 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 of mutagenesis and R lines obtained by the wide hybridization.

There are small number of hybrid combinations, created from B lines obtained from the wide hybridization and R lines, obtained on the same method.

Each year 350 - 370 hybrid combinations are produced for testing.

Three fourth of tested hybrids oleaginous and the rest are the combinations for developing large-seeded hybrids and hybrids with colored seeds for birds.

X

New sunflower hybrid varieties in registration

There are created new sunflower hybrids which increased the standard in sunflower seed yield and seed oil content per unit area.

Five of these hybrids - Musala, Mura, Maritsa, Mesta and Magura were registered at the State Variety Commission at the end of 2004.

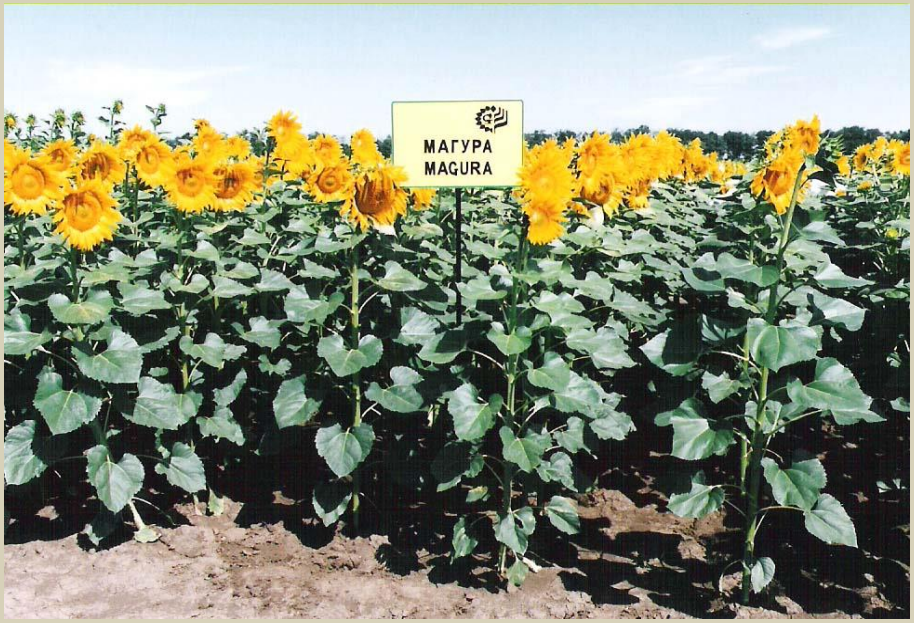
In the beginning of 2008 was registered the first large-seeded hybrid - Madan.

The paternal forms of hybrids Musala, Mura, Maritsa, Mesta and Magura and the maternal form of hybrid Madan were created from materils, obtained by interspecific hybridization.

The paternal form of hybrid Madan was obtained by intergeneric hybridization.


МУСАЛА
MUSALA


МУРА
MURA


МАГУРА
MAGURA


МЕСТА
MESTA

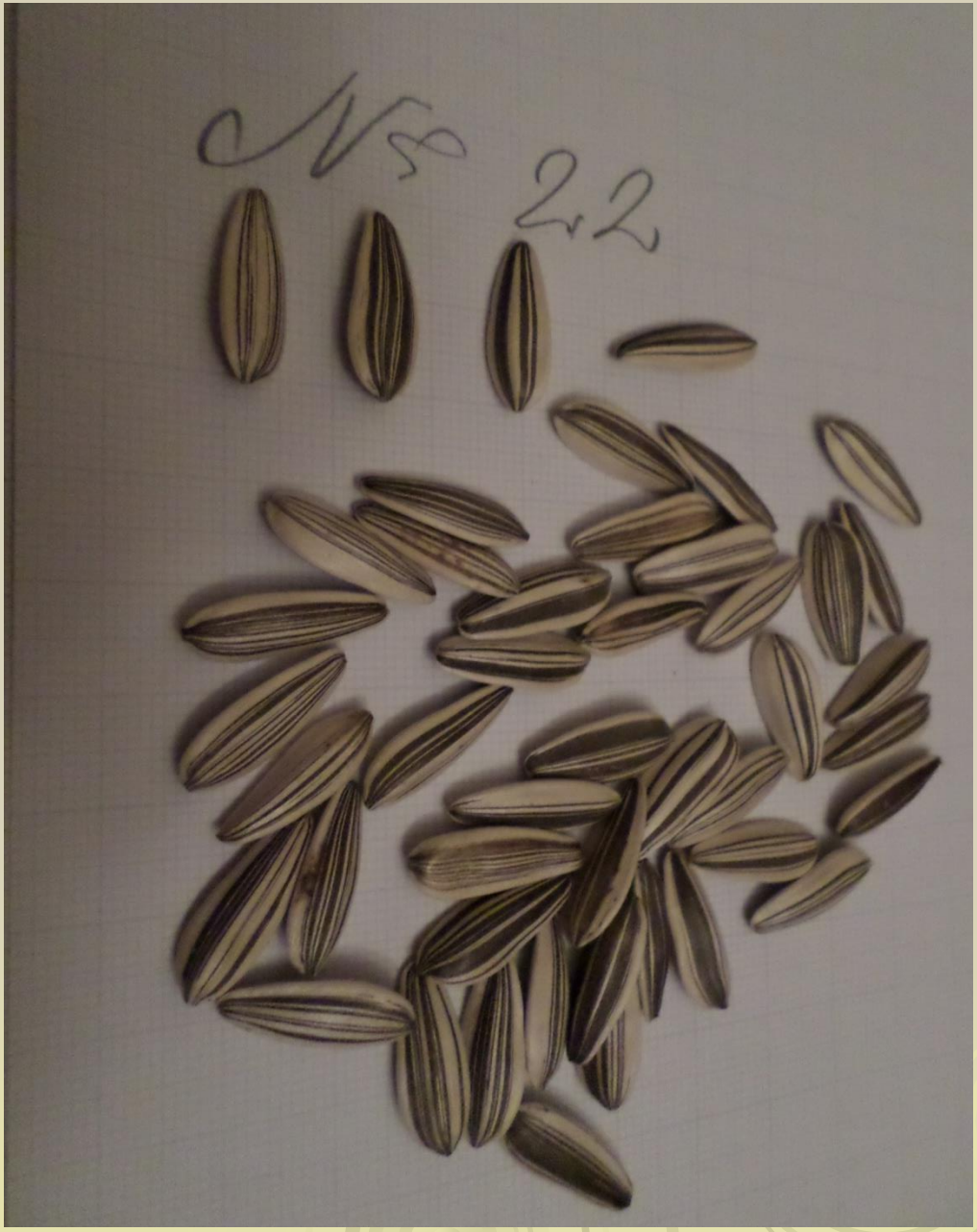

МАРИЦА


МАДАН


Ц3-70 x PR-66/9
A 124 x PR-66/9
Мадан
Aiz-70 x RK f-1
y. x.
№ 9
A 127 x PR-66/9
A 1480 x PR-66/9
A 1480 x PR-62/9
A 1480 x PR-47/5


236 B
БДС 3644-74
ЦЕНА 0.50 лв.


347 A
БДС 3644-74
ЦЕНА 0.50 лв.


№ 22

МИХСАН

МИХСАН ЕООД
София, 1750
mobile: +359 899 122 761, +359 899 792 448

MIHSAN LTD
Sofia, 1750
e-mail: mihsan2013@abv.bg

Mihsan Ltd is a Bulgarian family company, registered in 2013, with strong sides - own land and people with previous experience. Prof. Dr. Mihail Christov is a breeder who deals with plant breeding program, and Dr. Hristova-Cherbadzhi responsible for preparing the materials and documents for registration.

Main focus and activity: Selection of sunflower. The selection of the sunflower is carried out in several directions: Confectional type sunflower (Food for people); Oil sunflower seeds; Ornamental sunflower; Food for birds.

Currently, the company has completed more than 30 lines (*B/A* and *R*), resistant to the most economically important diseases for Bulgaria and Europe. Some of them are resistant to herbicides - *IMI* and *SU* type, others to *downy mildew* and *broomrape* and tolerant of diseases *phomopsis* and *sclerotinia*. We have 1000 forms with which we are working on studying the qualities and creating sterile analogues in the forms. Lines and new forms sunflower were obtained primarily through interspecific and intergeneric hybridization and experimental mutagenesis. Some of the materials are high oleic. In the moment we have 9 registered lines. In 2020 we expect to have registered hybrids, and to go on the market with variety as we will own multiply seeds.

We are ready for cooperation and discussion with everyone. We believe that our qualification, experience and ideas closely parallel your requirements.


Thank you for your attention !
Thank you for your attention !
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