Turkish Journal of Agricultural and Natural Sciences
Special Issue: 1, 2014

TÜRK
TARIM ve DOĞA BİLİMLERİ
DERGİSİ

TURKISH
JOURNAL of AGRICULTURAL
and NATURAL SCIENCES

www.turkjans.com

Application of Classical Methods at Sunflower Breeding Program in Dobroudja Agricultural Institute General-Toshevo

Julia ENCHEVA, Galin GEORGIEV, Nina NENOVA, Daniela VALKOVA, George GEORGIEV, Penka PEEVSKA and Valentina ENCHEVA

Dobroudja Agricultural Institute, General Toshevo 9520, Bulgaria
*Corresponding author: July_26@abv.bg

Abstract

Study was carried out in Dobroudja Agricultural Institute-General Toshevo, Bulgaria. Using classical approaches hundreds inbred lines were developed and collected, distinguished with their valuable agricultural characters and resistance to economic important diseases and parasite *Orobanche cumana.* Classical sunflower breeding methods applied in DAI-General Toshevo included intraspecific, interlinear, interspecific and intergeneric hybridization, and experimental mutagenesis. Evaluation of resistance to diseases and the parasite broomrape as well as seed oil content were done on confirmed and adapted for the conditions of the institute methods.

A lot of sunflower commercial cultivars, own and joint hybrids were created during last 30 years at DAI-General Toshevo. Hybrid Maritza, Mura, Veleka, Vokil, Gabi and Velko were widespread in abroad.

Keywords: *Helianthus annuus*, classical breeding, mutant line, IMI, SU, hybrid

Introduction

Sunflower is the main oleaginous crop for Bulgaria where the total cultivated sunflower areas are approximately 73000 ha. The larger part of this territory is situated in North East region of the country. Sunflower production increses each year and in 2013 Bulgaria is on the second place after Romania in Europe.

An important direction in the breeding work with sunflower occupies heterosis breeding, which shows its advantages over the breeding of the direct cultivars. It became possible after the discovery of the first CMS source in sunflower by Leclerq (1969) and the discovery of efficient fertility restorers (Enns *et al.,* 1970; Kinman, 1970; Leclerq, 1971; Vranceanu and Stoenescu, 1971). The purposeful breeding activity in sunflower is carried out on the basis of the reproductive potential and adaptation with a view of its seed production. This had lead to development of a crop with lower genetic variability and narrowing of the heritability basis of the developed cultivars.

The improvement of the plasticity and the enrichment of the genetic potential of sunflower can be realized through obtaining of recombinations and gene mutations. This is achieved through the method of interlinear, interspecific and intergeneric hybridization and experimental mutagenesis. By using distant hybridization, forms resistant to diseases, pest and the parasite broomrape have been developed, the productivity and quality of sunflower oil are increased and new sources of cytoplasmic male sterility (CMS) are obtained.

For many of the species it was found out that they can be used as carriers of Rf genes controlling cytoplasmic male sterility, the high content of protein, the resistance to stress factors and other new properties important for the breeding of cultivated sunflower.

The aim of this study was to:

а) Development and testing of new inbred lines of sunflower by using different conventional methods,

б) Development and testing of lines resistant to herbicides,

c) Testing of experimental hybrids of sunflower with enhanced productivity, resistance to stress factors or resistance to herbicides.

Materials and Methods

The plant material includes: Bulgarian and foreign direct and hybrid varieties of sunflower, local and foreign populations, old B and R lines, wild species of genus *Helianthus* and species of other genera of family *Compositae*, hybrids obtained through interaspecific, interspecific and intergeneric hybridization, new forms developed by applying experimental mutagenesis.

Breeding methods: hybridization (intraspecific, interlinear, interspecific and intergeneric), experimental mutagenesis-gamma rays ^{60}Co and ^{137}Cs, ultra sonic, Ethyle-methanesulphonate (EMS) and selection.

At DAI work is carried out with tree lines developed by BASF with genes for resistance (CLPlus) to herbicide of the group of imidazolinones. The lines are:

-BTI-(A) M1, BTI-(B) M1 и BTI-R1.

-Line HA-425 provided by Jerry Miller, USA.

In parallel with the development of imidazolinone-resistant sunflower, a new direction for development of lines and hybrids resistant to herbicides from the group of sulfonylurea (tribenuron-methyl) began. Work is carried out with a source of resistance in the wild species *H. annuus* L. discovered on the territory of Kansas (Miller and Al-Khatib, 2000; Miller and Al-Khatib, 2004).

Results

Dobroudja Agricultural Institute is the only breeding center in Bulgaria where purposeful research work is carried out on sunflower-the main oil seed crop for the country. The genetics of cultivated sunflower *H. annuus* L. and the wild annual and perennial species of genus *Helianthus* being investigate; breeding of population, varieties, lines and hybrids is being carried out.

The scientific work At DAI-General Toshevo began in 1941. Breeding was directed toward development of cultivars resistant to broomrape, which was a serious threat for sunflower production. In 1948 the first Bulgarian cultivar Tolbouhin 75 resistant to broomrape was released (Table 1). The main direction of work of sunflower from 1941 to 1985 was breeding of cultivars with the method of reserves (multiple individual family selections) of the Russian breeder Academician V. S. Pustovoit. Using the methods, the cultivars Tolbouhin 75, Hemus, Balkan, etc. were developed (Table 1). Cultivar Viola is with 80 % content of oleic acid in oil, and cultivar Obitel is with high content of protein in seed.

Table 1. Sunflower cultivars developed during 1948-1995

Crops	Registered	Breeder
Tolbuhin 75	1948	Assoc. Prof. Dr. P. Petrov
Stadion	1977	Assoc. Prof. Dr. P. Petrov
Balkan	1977	Assoc. Prof. Dr. P. Petrov
Hemus	1977	Assoc. Prof. Dr. V. Velkov
Vichren	1985	Assoc. Prof. Dr. V. Velkov
Viola	1988	Prof. Dr. P. Ivanov
Obitel	1988	Prof. Dr. P. Ivanov
Favorit	1994	Assoc. Prof. Dr. F. Tzvetkova
Vega	1995	Assoc. Prof. Dr. V. Velkov

After 1970 intensive work is being carried out on the use of the cytoplasmic male sterility discovered ii France by Leclerq in 1969 in crossing the species *H. petiolaris* L. to cultivated sunflower. Since 1985, the breeding work at DAI-General Toshevo has been focused on the use of the heterosis effect in first hybrid generation through development, investigating and stabilizing of inbred lines, testing of experimental hybrids, production of seeds from parental lines and registration of own and joint hybrids (Petrov *et al*., 1994).

The hybrids posses some advantages in comparison to the varietals populations. They

emerge and develop uniformly. The uniformity of the crop and the simultaneous maturation make harvesting easier and decrease the losses during it.

The hybrids possessed resistance to economically important diseases and the parasite broomrape. Due to the advantages the hybrid varieties have over the direct ones, in the last 30 years they justifiably occupy the grater part of the areas of sunflower in Bulgaria.

The first Bulgarian hybrids on the basis on CMS were tested in competitive varietals trials in 1973 (Stoyanova *et al*., 1977; Ivanov *et al*., 1988). In 1979 the first Bulgarian hybrid Start was released and distributed all over the country (Gotsov *et al*., 1981).

After a period of 7-8 years, another two Bulgarian hybrids were released – Dobrich and Albena. In 1988 hybrid Albena was registered and distributed in France, reaching 40 % of the area of sunflower in this country. Due to its good adaptability and productivity, it became a world standard for the early maturity hybrids. It has been successfully sown in Germany and Austria. For the period 1978-2014 at DAI-General Toshevo 14 joint hybrids were registered (Table 2) as well as 28 own hybrids (Table 3).

Among the joint hybrids, mostly applied in practice were hybrid Santafe and hybrid San Luka currently occupying significant areas of the territory of Bulgaria.

Several schemes for hybrid seed production have been developed at DAI (Velkov and Stoyanova, 1974), but only one is being applied in practice in the recent years-Simple hybrid with completely restorer fertility.

The main group of hybrids developed at DAI is early maturing: Albena, San Luka, Perfect, Musala, Mesta, Maritza, ets. (Table 3).

Table 2. Joint hybrids of sunflower registered during 1991-1998

Hybrid	Partners	Registered	Country
Santafe	DAI-France	1991	France
Alison	DAI-France	1993	France
Alise	DAI-France	1993	France
LG 5410	DAI-France	1993	France
DK 3795	DAI-USA- France	1994	France
San Luka	DAI-France	1994	France
Alinka	DAI-France	1995	France
Arte	DAI-France	1996	France
Alianz	DAI-France	1997	France
Florelo	DAI-France	1997	Italy
Gala	DAI-France	1997	France
Altes	DAI-France	1997	France
Aria	DAI-France	1998	France
Olstar	DAI-France	1998	France; Italy

The requirements to sunflower production impose the development of hybrids with higher content of oil in seed and obligatory resistance to downy mildew and broomrape, and if possible resistant/tolerant to phomopsis, phoma, alternaria and sclerotinia.

The increasing the oil content in seed, own and foreign hybrids, hybrids developed through distant hybridization and new mutant forms are used as sources of high oil content (Christov *et al.,* 1996; Christov and Nikolova 1996; Nikolova and Christov, 1996). The evaluation done according to improved methodologies of Rushkovsky, 1975**;** Stoyanova and Ivanov, 1968; Ivanov *et al.,*1996. Sunflower lines with high content of oil were derived from crosses with the wild species *H. eggertii, H. pauciflorus, H. smithii, H. hirsutus, H. annuus, H. nuttallii ssp.rydbergii, H. pumilus*, etc. (Christov, M., 2012).

At the present moment, hybrids San Luka, Maritza, Veleka and cultivar Favorit developed through conventional breeding are applied in practice (Table 2 and 3).

Table 3. Sunflower hybrids developed during 1978-2014

Hybrid	Registered	Breeder	Hybrid	Registered	Breeder
Start	1978	Prof. Dr. J. Stoyanova	Magura	2004	Prof. Dr. M. Christov
Albena	1986	Prof. Dr. J. Stoyanova	Mercurii	2004	Assoc. Prof. Dr. N. Nenov
Dobrich	1987	Prof. Dr. J. Stoyanova	Selena	2005	Assoc. Prof. Dr. D. Petakov
Super Start	1988	Prof. Dr. J. Stoyanova	Biozvet	2005	Prof. Dr. P. Ivanov
Zora	1994	Assoc. Prof. Dr. V. Velkov	Dobrozvet	2005	Prof. Dr. P. Ivanov
Diamond	1996	Assoc. Prof. Dr. D. Petakov	Rada	2005	Assoc. Prof. Dr. J. Encheva
Perla	1996	Assoc. Prof. Dr. V. Velkov	Yana	2008	Assoc. Prof. Dr. J. Encheva
Penka	1999	Assoc. Prof. Dr. V. Velkov	Valin	2010	Assoc. Prof. Dr. A. Piskov
Stoger	2000	Assoc. Prof. Dr. V. Velkov	Alpin	2011	Assoc. Prof. Dr. A. Piskov
Neli	2000	Assoc. Prof. Dr. V. Velkov	Vokil	2012	Dr. G. Georgiev
Perfect	2003	Assoc. Prof. Dr. D. Petakov	Veleka	2012	Dr. G. Georgiev
Maritza	2004	Prof. Dr. M. Christov	Gabi	2013	Dr. G. Georgiev
Mura	2004	Prof. Dr. M. Christov	Velko	2013	Dr. G. Georgiev
Mesta	2004	Prof. Dr. M. Christov	Michela	2014	Assoc. Prof. Dr. A. Piskov

Figure 1. Hybrid San Luka

Figure 2. Hybrid Maritza

Figure 3. Hybrid Veleka

Figure 4. Hybrid Velko

Figure 5. Hybrid Vokil

Figure 6. Hybrid Gabi

Hybrid **San Luka** (Fig. 1) is a joint product of DAI and France and was released in 1994 (Fig. 4). A simple linear hybrid derived from the cross 2607A x RW666. The lines were developed through conventional breeding. Seed yield is 320.0 kg/da, and oil yield is 144.0 kg/da. Oil percent of seed is 45.1 %, and 1000 seed weight 48-56g. Vegetation period is 123 days. Resistant to broomrape race F, to downy mildew races 700 and 731 and to phoma. It is moderately resistant to phomopsis.

Hybrid **Maritza** was released in 2004 (Fig. 2). A simple linear hybrid derived from the cross 197 A x 7009 R. The lines were developed through conventional breeding. Seed yield is 330.0 kg/da. Oil yield is 155.1 kg/da. Oil percent of seed is 46-47 %, and 1000 seed weight-65-70 g. Plant height is 145-160 cm. Vegetation period is 123-125 days. Resistant to broomrape race F, to downy mildew races 300 and to phoma. It is moderately resistant to phomopsis.

Hybrid **Veleka** was released in 2012 (Fig. 3). A simple linear hybrid derived from the cross 217 A x 166 R. The lines were developed through conventional breeding. Seed yield is 387.2 kg/da. Oil yield is 202.8 kg/da. Oil percent of seed is 52.4 %, and 1000 seed weight-55-65 g. Plant height is 145-160 cm. Vegetation period is 123-125 days. Resistant to broomrape race F, to downy mildew races 300 and 700 and to phoma. It is moderately resistant to phomopsis (Nenova *et al*., 2012).

Hybrid **Velko** was released in 2013 (Fig. 4). A simple linear hybrid derived from the cross 3607 A x 105 R. The lines were developed through conventional breeding. Seed yield is 410.0 kg/da. Oil yield is 184.5 kg/da. Oil percent of seed is 46.0 %, and 1000 seed weight-55-65 g. Plant height is 155-160 cm. Vegetation period is 116-118 days. Resistant to broomrape race F, to downy mildew races 700 and 731 and to phoma. It is susceptible to phomopsis.

Hybrid **Vokil** was released in 2012 (Fig. 5). A simple linear hybrid derived from the cross 217 A x 340 R. The lines were developed through conventional breeding. Seed yield is 399.3 kg/da, and oil yield is 196.3 kg/da. Oil percent of seed is 49.0 %, and 1000 seed weight-65 g. Vegetation period is 123-125 days. Resistant to broomrape race F, to downy mildew races 300 and 700 and to phoma. Susceptible to phomopsis (Nenova *et al*., 2012).

Hybrid **Gabi** was released in 2013 (Fig. 6). A simple linear hybrid derived from the cross 217 A x 127 R. The lines were developed through conventional breeding. Seed yield is 372.4 kg/da, and oil yield is 191.1 kg/da. Oil percent of seed is 48.0 %, and 1000 seed weight-63 g. The height plant is 155-170 cm. Vegetation period is 125-127 days. Resistant to broomrape race F, to downy mildew races 300 and 700 and to phoma. It is moderately resistant to phomopsis.

Hybrids Maritza and San Luka are distributed in Moldavia and Ukraine. Hybrids

Yana and Alpine are spread in the European Community, Russia, Moldova, Ukraine, Serbia and Kazakhstan. Hybrid Valine is widely used in Russia, Moldavia and Ukraine. Hybrids Veleka, Vokil, Gaby, Velko and Michaela are registered in Romania.

Methods of breeding

For developing of new sunflower lines the methods of intraspecific, interlinear, interspecific and intergeneric hybridization, experimental mutagenesis and selection are applied. Now DAI-General Toshevo has at its disposal over 15 000 lines A, B and R, a part of which were included in the development of new hybrid varieties of sunflower (Table 4).

Table 4. Number of inbred sunflower lines

Types of inbred lines	2011 (no)	2012 (no)	2013 (no)	Total
Restorer of fertility (Rf)	3100	3602	2091	8793
Maintainer of sterility (B)	1801	258	258	2317
Sterile analogues A of B lines	451	1092	555	2098
R lines resistant to herbicides	500	510	502	1512
B lines resistant to herbicides	45	40	49	134

Interlinear hybridization

This is a main breeding method of sunflower. It allows obtaining high-yielding hybrids with ecological plasticity on the basis of cytoplasm male sterility (Petrov, P., 1978).

The work on developing of inbred sunflower lines for the purposes of heterosis breeding in DAI is carried out in three main directions: a) enhancing of productivity; b) enhancing the resistance to diseases, the parasite broomrape, some herbicides and soil and air drought; c) increasing oil content in seeds and variability of the chemical composition of oil.

The work by this method includes the following main elements:

1. Breeding of lines with normal cytoplasm

A large part of the lines with normal cytoplasm are with origin from Russian cultivars with high oil content. Others originate from the USA, Hungary, Argentina, etc. (Petrov *et al*., 1994; Christov, M., 2002).

1.1. Developing of lines with normal cytoplasm through interlinear hybridization

Through hybridization between highly productive and resistant L-lines, new 227 lines were developed, which were stabilized and are characterized with good economic indices and resistance to the most recent races of downy mildew and broomrape. The resistant materials are with origin from cultivar Vega, line HA-300, etc.

1.2. Developing of lines with normal cytoplasm through interspecific and intergeneric hybridization

In the recent years the percent of the L lines produced by applying interspecific hybridization increased (Christov, M., 2002; Christov *et al.* 2009). The collection of stabilized lines maintainers of sterility includes 555 items. Most of the lines are resistant to downy mildew race 700 and the parasite broomrape race F. In this group of breeding materials work is carried out with 16 lines used for maintainers of 4 CMS sources with origin *H. annuus* (E-126), *H. praecox* (E-028), *H. nuttallii* (M-088) and *H. argophyllus* (E-091). The lines were tested for combining ability and gave very good results.

1.3. Developing of lines with normal cytoplasm through induced mutagenesis

In the last few years the percent of lines obtained through applying experimental mutagenesis increased (Christov, M., 2002; Christov *et al.* 2009). Seeds are irradiated with 50 and 100 Gy, and the kernels- with 5, 10 and 15 Gy.

A large number of mutant forms were developed after treatment of seeds and kernels of sunflower with gamma rays-^{137}Cs, ^{60}Co, ultra sonic and Ethyl methansulfonate. The materials are in generation from M6 to M30. Some of them, registered as B-lines, possessed very good combining ability. A sunflower mutant line 6056 B was obtained by treating of dry

seeds with 150 Gy (Christov, M., 1995). The line maintained the CMS source of ARG-2, coming from *H. argophyllus.*

An increased was observed of genetic variability by applying induced mutagenesis on mature seeds. Forms with higher content of oil in seeds, modified fatty acid composition, higher protein content and changes in some morphological traits of the plants were obtained (Christov, M., 1995; Christov, M., 1996a; Christov *et al.,* 1996b; Christov and Nikolova, 1996c; Christov, M., 2002).

Twenty-eight inbred lines were developed directly from mutant forms, with Peredovik, Progress, Voronegskii-272, Trudovik, Start (Russia), Hemus, Vihren and Stadion origin. Totally new eleven lines produced from inbred 1607 B, 2607 B, 2969 B, 3004 B and HA-89 showed great differences from their origins. There were obtained 10 new restorer lines from hybrid Start, NSH-26 or 3 synthetics. Two new CMS-sources were maintained, two after treatment with gamma rays and one with ultra sound (Christov and Nikolova, 1996c).

From cultivar VINK8931 by treatment with gamma rays ^{60}Co at dose 150 Gy, mutant line M95-674 was obtained, which is characterized with altered shape of the leaves, petioles and tubular florets (Christov, M.,1996a). The line possessed also a recessive mutation for resistance to *Orobanche cumana.* Sterile analogies were developed to 204 lines with normal cytoplasm (Christov *et al*., 2009).

2. Developing of sterile analogues (A) of B lines.

For developing of sterile analogue, 2098 sunflower accessions were used. The sterile analogues were developed on the basis of 30 sources of CMS, 75 % of all analogues being based on CMS of PET-1. Back-crossing was at stages BC1 to BC 6. Till now as completed were determined 117 B lines. Lines with elite number 2607A, 217A, 3607A, 813A, 846A, 807A, 1017A, 688A, 823A, 76120A, 7419A, 7448A, 2003A, 2004A, 2008A and 2012A demonstrated highest productivity.

3. Breeding of fertility restorer lines(R)

3.1. Development of lines through interlinear hybridization

At the current moment the department has at its disposal 11893 lines in different generations developed through various conventional breeding methods. In the recent years, hybrid varieties are used for the breeding of fertility restorer lines. Selection is carried out of plants and selfing, which continues for 6-8 generation with the aim of transforming the Rf gene into homozygous state. A series of lines were developed which possess also genes controlling the resistance to downy mildew races 700 and 731 and the parasite broomrape, races G and H. At the present moment DAI has it its disposal 1050 stabilized R lines.

3.2. Development of lines through distant hybridization

Another method for developing of fertility restorer lines is interspecific and intergeneric hybridization. A significant amount of lines were obtained by crossing male sterile lines of cultivated sunflower to different wild species of genus *Helianthus* or genera of *Compositae.* Two hundred and eight sources of Rf genes from accessions of genus *Helianthus* and 24 sources of Rf genes from accessions of other genera of family *Compositae* were found. Intergeneric hybrids between cultivated sunflower and the species *Arctium, Aster, Bidens, Calendula, Carlina, Carduus, Carthamus, Cihorium, Ehinacea, Evmolpia, Gaillardia, Grindelia, Inula, Matrikaria, Onopordum, Silphium, Silybum, Telekia, Tithonia, Verbesina, Zinnia u Xanthium* were obtained. About 8-9 generations of selfing of the hybrid fertile plants are necessary to transform the Rf gene into homozygous condition (Christov, M., 2002; Christov *et al.,* 1996d; Valkova, D., 2004, Valkova *et al.,* 2008). From the 940 lines obtained through interspecific and intergeneric hybridization, 750 were investigated. Different morphological, phonological, and biochemical traits were characterized. The total and specific combining ability has been investigated. Up to this moment 716 promising R lines are like completed.

4. Development of new sunflower lines with low oil content suitable for direct use (confectionary type) and for birds

The total number of the hybrid materials characterized with higher content of protein was 28 items. Some of them are suitable for developing of direct varieties.

5. Development of lines and hybrids resistant to herbicides

For developing lines resistant to herbicides of the group of imidazolinones, three different sources were used: one from the USA-line HA-425 provided by Jerry Miller (with gene Ahasl 1-1), one of BASF (with gene Ahasl 1-3 named ALPlus), and one Bulgarian-from the wild species *H. argophyllus*. One tousand one hundred and eighty-four fertility restorer lines and 61 lines maintaner of sterility, resistant to herbicides from the group of imidazolinones Pulsar 40+ Stomp 330 EU. During the period of investigation 302 resistant to IMI resistant hybrids were tested in coimpetitive varietal trial. Evaluation of the indices seed yield and oil yield was made. Seed yield above the mean standard was registered in 8 hybrids, in which the twq parental lines possessed gene for resistance to herbicides Pulsar 40 + Stomp 330 EC at homozygous state. Best results were found in the combinations BTI- (A) M1 x 100/1/2 R (108.70%), BTI- (A) M1 x 102/3/3 (107.90%) and BTI- (A) M1 x 100/2/3 R (107.60%).

Three hundred twenty-eight fertility restorer lines resistant to the herbicide Express from the group of sulfonylurea were developed.

Conclusions

Nine sunflower varieties, 32 own hybrids and 14 joint Bulgarian-French hybrids and one Bulgarian-American-and French hybrid were released.

Five hundred and sixty-nine mutant forms were obtained after treatment of mature seeds and kernels with gamma rays, 45 items through treatment with ultra sound and Ethyl Methansulfonate. Sterile analogs were made of 204 lines with normal cytoplasm.

DAI-General Toshevo has at its disposal 8793 R, 2317 B and 2098 A lines developed through different conventional methods.

One thousand five hundred and twelve fertility restorer lines and 134 sterility maintainer lines resistant to herbicides of the group of the imidazolinones Pulsar 40 + Stomp 330 ES were obtained.

Three hundred and twenty-eight fertility restore lines resistant to herbicide Express from the group of the sulfonylureas were obtained.

During the period of investigation at competitive trail, 302 IMI-resistant hybrids were tested.

References

Gotzov, K., Karayvanov, A., Tzvetkova, F., Tzvetkov, S., Velkov, V., Radkov, P., 1981. Achievement and problem of breeding at DAI near General Toshevo. Selection “Sunflower”. Scientific and theoretical conference on the problems of breeding, NAPS, pp 32-36. (in BG)

Ivanov P., Velkov, V., Petrov, P., Georgiev, G., Shindrova, P., Tzvetkova, F., 1988. Directions of contemporary breeding work in sunflower. Agricultural Science, XXVI, No 1, S., pp.40-50. (in BG)

Petrov, P., Tzvetkova, F., Velkov, V., Piskov, Al., Christov, M., Shindrova, P., Petakov, D., Nenov, N., Venkov, V., Nenova, N., Encheva, J., Todorova, M., Nikolova, L., Nikolova, V., 1994. Curent status and problems of sunflower breeding in Bulgaria. Plant breeding sciences, XXXI, No 3-4, pp. 72-77. (in BG)

Petrov, P., 1978. Investigations on the efficiency of the cytoplasm of *H. petiolaris* in the heterosis breeding work in sunflower. Ph.D. thesis, pp. 150. (in BG)

Rushkovskii, C.B., 1975. Methods of investigation in the breeding of oil seed crops for oil content and its quality. M., Pishtempromizsat. (in BG)

Stoyanova, J., Ivanov, P., 1968. Investigation of the preparation of sunflower seeds for laboratory determining of their oil percent. Plant breeding sciences 5(4): 49-57. (in BG)

Stoyanova, J., Simeonov, B., Sabev, G., Petrov, D., Georgiev, I., Dimitrov, I., Georgieva-Todorova, J., Rangelov, L., Petrova, M., Ivanov, P., Palazov, P., Kontev, C., 1977. Sunflower in Bulgaria. BAS. Sofia. (in BG)

Christov, M., 1990. Investigation on wild species of genus *Helianthus* with a view of their using in sunflower breeding. Ph.D. thesis, Sofia. (in BG)

Christov, M., 1995. Development of a new sunflower forms by treating seeds with gamma rays. The first Balkan Symposium on Breeding and Cultivation of Wheat, Sunflower and Legume Crops, June, 26-28, Albena, Bulgaria, pp. 320-323.

Christov, M., 1996a. A new sunflower mutant form. Helia 19(24): 39-46.

Christov, M., Shindrova, P., Encheva. V., 1996b. Transfer of new characters from wild *Helianthus* species to cultivated

sunflower. Genet. a Selech., 32(4): 275-286.

Christov, M., Nikolova, V., 1996c. Increasing of the Sunflower Genetic Diversity by Mutagenesis. In: Proceedings of 14th International Sunflower Conference, Beijing/Shenyang, China, 19-30.

Christov , M., 1996d. Characterization of wild *Helianthus* species as sources of new features for sunflower breeding. In P.d.s. Caligari & D.J.N. Hind (eds). *Compositae*: Biology & Utilization. Proceedings of the International *Compositae* 2, 547-570.

Christov, M., 2002. Results from the use of wide hybridization and Experimental mutagenesis in improvement work on sunflower. Anniversary scientific session-50 year Dopbroudja agricultural institute. Vol. I. 315-335.

Christov, M., Piskov, Al., Encheva, J., Valkova, D., Drumeva, M., Nenova, N., Nikolova, V., Encheva, V., Shindrova, P., Petrov, P., Georgiev, G., 2009. Developing sunflower hybrid cultivars with increased productivity, resistant to disease and broomrape using classical and biotechnological methods. Science-technical bulletin. Institute for oilseed crops. UOSC**,** № 14: 74-87.

Christov, M., 2012**.** Contribution of interspecific hybridization to sunflower breeding. Helia 35(57): 37-46.

Enns H., Dorrell, D. G., Hoes, J. A., Chubb, W.O., 1970. Sunflower research, a progress report, p. 162-167. In: Proc. 4 th Inter. Sunflower Conf., Memphis, Tennessee.

Ivanov, P., Velkov, V., Petrov, P., Georgiev, I., Shindrova, P., Tzvetkova, F., 1996. Study of seed oil and protein quality of some *Bidens tripartita* accessions. Helia 19(25): 79-85.

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.

Miller, J., Al-Khatib, K., 2000. Development of herbiside resistant germplasm in sunflower. Proc. 15th int. sunflower conference, Toulouse, France, June 12-15. Intl. Sunflower Assoc. Paris, France, Vol. 2: 419-423.

Miller, J., Al-Khatib, K., 2004. Registration of two oilseed sunflower genetic stock. SURES 1 and SURES 2 resistant to tribenuron herbicide. Crop Science 39: 301-302.

Nenova, N*.,* Georgiev, G., 2012. Vokil and Veleka- perspective sunflower hybrids. Agricultural science 45 (4): 25-29.

Nikolova, L., Christov, M., 1996. Investigations on Hybrid Combinations between Cultivated Sunflower and the Wild Species *H. neglectus, H. giganteus, H. decapetalus* and *H. strumosus.* In: Proc. Inter. Sunflower Confer., Beijing/ Shenyang, China, 1021-1028.

Sala, C.A., Bulos, M., Echarte, A.M., 2008. Genetic analysis of an induced mutation conferring imidazolinone resistance in sunflower. Crop Science 48:1817-1822.

Skoric D., 1988. Sunflower breeding. Uljarstvo, N 1, Beograd.

Valkova, D., Christov, M., 2004. Characterization of F1 plants obtained from crosses between cultivated sunflower and wild annual *Helianthus annuus. In*: Proceedings of 16th International Sunflower Conference, Fargo, North Dakota, USA, Vol. II, p. 747-750.

Valkova, D., Christova-Cherbadgi, M., Encheva, V., Shindrova, P., Nikolova, V., Christov, M., 2009. Characterization of F1 plants, obtained by crossing of cultivated sunflower and wild annual species (*Helianthus annuus* L.). Field Crops Studies, DAI General Toshevo, Vol. 5(1): 125-129.

Velkov, V., Stoyanova, J., 1974. Biological peculiarities of cytoplasmic male sterility and schemes of its use. Proceedings of the 6th International Sunflower Conference, 22-24 July, Bucharest, Romania, 361-366.

Vranceanu A.V., Stoenescu, F., 1971. Pollen restorer gene from cultivated sunflower (*Helianthus annuus* L.). Euphytica 20(4): 536-541.