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BREEDING OF SUNFLOWER (*HELIANTHUS ANNUUS L.)* AT DOBRUDZHA AGRICULTURAL INSTITUTE – GENERAL TOSHEVO

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Abstract

Dobrudzha Agricultural Institute – General Toshevo is the only breeding center of sunflower in Bulgaria. Since its establishement in 1951, over 50 varieties and hybrids of this crop have been developed here. More than 15 joint hybrids have been registered abroad, the result from cooperation with our European partners. The institute has a rich and variable collection of initial breeding materials. The methods we use in our breeding work are intraspecific, interlinear, interspecific and intergeneric hybridization, experimental mutagenesis, embryoculture, somaclonal variation, *in vitro* screening and selection. The methods for evaluation of resistance to economically important diseases and the parasite *Orobanche* have been adapted to the working conditions of the Institute. Morphological, biological, technological, biochemical and phytopathological characterizations of the released and registered hybrids and their parental lines have been made. Many new materials have been developed during the last decade, which possess valuable breeding properties. Over 6000 inbred lines are involved in the breeding work. Annually, 1400 new hybrid combinations are being tested in Bulgaria and abroad. Hybrids have been developed, which possess very good productivity and adaptability potential, and which have been registered in Bulgaria, EU and other foreign contries. Some foreign companies included our new hybrids (Velko, Veleka, Yana, Divna, Valin, etc.) in their catalogs and are successfully organizing their seed production and marketing in the respective countries. A new direction of our breeding is the devlopment of sunflower hybrids resistant to herbicides. Several hybrids - Enigma, Sunny imi, Desi, Viyani, Danaya, etc., are now within the system of official testing in Bulgaria and abroad. Their registration is forthcoming. The aim of this investigation was to present the current status and achievements of the breeding work on sunflower at DAI-General Toshevo during the last decade.

Keywords: Hybrids, lines, productivity, resistance.

Introduction

Sunflower breeding in Bulgaria began as early as the 1920's. It was initially carried out only as individual family selection breeding method with the aim to develop varietal populations (Petrov et al., 1994). Thus the first large-seeded cultivar Stadion was developed, which has high protein content, low percent of oil and is suitable for direct consumation. This cultivar was followed by another large-seeded variety Favorit, which is currently widely distributed in Bulgaria and abroad. The improvement and breeding work by the method of interlinear hybridization for development of hybrids started in 1963. Distant hybridiuzation, experimental mutagenesis and biotechnology have been used as auxiliary methods. One of the first breeding tasks was increasing the oil content and reducing the percent of husk. Later, with the occurrence and wide distribution of broomrape, breeding for resistance to this parasite also began. During 1970 – 2000, the breeding work by the method of heterosis gradually increased. Significant results were obtained both in the breeding of inbred lines and in the development of hybrids. This became possible after the discovery of the first CMS source in sunflower by Leclercq (1969) and of effective restorers of fertility (Enns et al., 1970; Kinman, 1970; Leclercq, 1971; Vranceanu and Stoenescu, 1971). The testing of the first Bulgarian

hybrids began in 1973 (Stoyanova et al., 1977; Ivanov et al., 1988). The first Bulgarian hybrid Start was released and distributed on the entire teritory of the country in 1979. It was resistant to downy mildew and exceeded variety Peredovik, its contemporary standard of highest mass distribution, with more than 12 % (Gotsov et al., 1981). In the 1980s, a new group of early hybrids was developed, among them Albena, Super Start, Dobrich and SantaFe. They gradually occupied over 90 % of the areas sown with sunflower in Bulgaria. In 1988, hybrid Albena was registered and distributed in France, and in 1993 it became the main hybrid there, with 40 % of the sunflower areas. Albena was also acknowledged as a world standard from the group of the early maturing hybrids. The fast spreading of the parasite *Orobanche* became the main reason for the development of a new group of hybrids at the end of the last century and the beginning of the new one, which possessed parallel resistance to downy mildew and *Orobanche* (Christov et al., 2009). Hybrids San Luka, Maritsa, Mussala, Rada, Yana, Merkuriy, Perfekt, etc., were released and distributed. The new Bulgarian hybrids occupied at that time more than 80 % of the sunflower areas in Bulgaria. Unfortunately, in the last decade the percent of the own hybrids has decreased dramatically. The major reason for this is the flexible, aggressive and financially well supported policy of the foreign companies. Nowadays over 50 sunflower hybrids and varieties are registered in Bulgaria, which have been developed at DAI – General Toshevo. The aim of this investigation was to present the current status of sunflower breeding at DAI – General Toshevo and its achievements during the last decade.

Material and methods

The investigations subject to this publication were carried out at DAI – General Toshevo; they are the result from the implementation of several 4-year projecs the Sunflower Breeding Department has been working on. The main aim of these projects was the development of new sunflower hybrids resistant to biotic and abiotic factors through combined use of conventional and biotechnology methods in breeeding. The initial breeding materials were Bulgarian and foreign direct and hybrid sunflower varieties, land races and foreign populations, own old lines – maintainers of sterility, their sterile analogues and fertility restorers, wild species of genus *Helianthus*, species from other genera of *Compositae* family, hybrids, derived through intraspecific, interspecific and intergeneric hybridization, forms obtained by using experimental mutagenesis, lines produced through various bio technology methods applied independently or in combination with induced mutagenesis. A permanen stationary collection of 250 accessions from the perennial species of genus *Helianthus* with official registration at FAO, which is unique for Bulgaria, is being maintained at DAI – General Toshevo under natural conditions. The collection includes also about 200 accessions from 7 annual species. The methodologies for interspecific and intergeneric hybridization are being constantly improved. The methods for evaluation of the resistance to the economically important diseases on sunflower and the parasite broomrape have been adapted to the conditions of the Institute's location (Панченко, 1975; Maric et al., 1981; Vear and Tourvielle, 1987; Encheva and Kiryakov, 2002). A new methodology for testing of the resistance to sclerotiona has been elaborated (Christov et al., 2004), as well as a method for irradiation of immature sunflower embryos with gamma radiation or ultrasonic (Encheva et al., 1997). Oil percent in seed is being determined by improved methodologies of Rushkovskiy,1967, Stoyanova and Ivanov, 1968, Ivanov et al., 1996. Equipment for fast evaluation is also used (Newport Instruments Ltd., 1972). Out of the 4 schemes of hybrid seed production developed at our Institute (Velkov and Stoyanova, 1974), only the method of inter linear hybridization is being used for production of simple two-linear male fertile hybrids with full restoration of fertility. The new hybrid combinations undergo through several-year testing according to a scheme and technology adopted at our Institute; they are then forwarded for official variety testing to our EU partners and other countries. All new released and registered lines and hybrids were given morphological descriptions according to UPOV (2002).

Results and discussion

The higher efficiency of production is a main task of the bereeding programs in the different crops. A key factor for stable yields is the resistance to different types of stress (Mihova et al., 2015; Dimitrova-Doneva et al., 2016). In this relation, the most important task of the project on which the Sunflower Breeding Department is working is the development of high-yielding hybrids resistant to the economically important diseases and the parasite *Orobanche*, which are adaptable to constantly changing environments. This is primarily dependent on the efficiency of the breeding of lines. DAI – General Toshevo has a rich collection of inbred lines derived through conventional methods of breeding, which possess various biological and morphological traits (Table 1).

Table 1. Volume of inbred sunflower lines during the last decade

Inbred lines	Number during 2006-2016 years
R lines – restorers of fertility	3300
B lines – maintainers of sterility	2400
A lines – sterile analogs of B lines	320

Since the above volume of lines is constantly being increased and enriched, and due to the practical impossibility to work with such a great number of materials, the larger part of them are being stored in the genetick stock center of DAI, regularly reproducing the older and more valuable ones.

Breeding of inbred lines with normal cytoplasm

A large part of this group of lines were obtained from old Russian high oleic cultivars, others are with origin from Argentina, USA, Hungary, etc. The percent of the lines obtained through interlinear hybridization of already well established lines, followed by selection, is getting higher. In the past years a large number of lines were also developed by using interspecific hybridization and experimental mutagenesis (Christov, 2002). When developing inbred lines, the selection is carried out for the following traits: productivity, combining ability, duration of vegetative growth, plant height, head diameter, resistance to lodging, 1000 kernel weight, number of seeds per plant, oil and protein content in seed, resistance to diseases, parasites and pests. The new genotypes are tested for combining ability with A-lines; the development of their sterile analog begins simultaneously using citoplasmic male sterility (CMS) type Petiolaris (PET 1). This type of sterility is shown to be stable, it is inherited in the next generaions without being affected by the growing conditions. The sterile analogs obtained on the baisi of this CMS type have normal female fertiliuty. To test the new B-lines, branched sterile fertility restorers of cytoplasmic type ssp. Falax are also used.

Breeding of fertility restorer inbred lines

The first fertility restorers in our institute were obtained in several ways – by selection and selfing of cultivars with established presence of Rf genes; by crossing of two lines, one of which is a carrier of Rf genes; crossing of a line to a cultivar; developing of synthetic populations, etc. In the recent years there are two methods, which are used most frequently – crossing of R lines with valuable traits and selection and selfing of high-yielding hybrids well established on the market. Another less frequent but a valuable way of developing fertility restorer lines is the interspecific and intergeneric hybridization. A large number of R lines are obtained from crosses of male sterile cultural sunflower lines with different *Helianthus* species and species from other genera of *Compositae* family and selfing of the obtained hybrid fertile plants, which is repeated 8-9 times with the aim to render the *Rf* gene in homozygous condition, accompanied with multiple and successive selection. These R lines possess other valuiable properties, as well, inherited from the two parental forms involved in the hybridization (Christov et al., 1996; Christov, 2002). The majority of the new lines possess excellent combining ability, full resistance to downy mildew and the parasite *Orobanche* and high resistance to the diseases phoma, phomopsis and sclerotinia. Significant is the percent of the materials

obtained by using distant hybridization and experimental mutagenesis and by combining distant hybridization with bio technology methods and techniques (Table 2).

Table 2. Species, varieties, forms and lines obtained through distant hybridization, experimental mutagenesis and some bio technology methods and techniques

Species, forms, lines, varieties	Number
Lines obtained through distant hybridization	3500
- R lines	3300
- B lines	200
Lines obtained by combining distant hybridization with bio technology techniques	1100
- R lines	1030
- B lines	70
Lines obtained through experimental mutagenesis	650
Herbicide resistant lines	200
- Distant hybridization + conventional breeding	170
- Biotechnology techniques	30
Initial breeding material	
- Varieties, lines, hybrids and populations	1400
- Accessions from species of genus *Helianthus*	660
- New forms for lines of wild *H. annuus*	50
- Accessions from species of other genera of *Compositae* family	70
- New CMS sources	29
- New sources of Rf genes	230

The results from Table 2 show that a large volume of initial material has been collected and developed, which was multiplied and evaluated for presence of properties important for breeding. Diffeernt methods and techniques were studied and applied in the process of investigation. It is evident that the number of the fertility restorer lines is significantly predominat; this is due to the improved methodology for their development. Furthermore, almost all species from *Helianthus* genus and the species form other genera of *Compositae* family involved in the hybridization are carriers of Rf genes. For developing of herbicide-resistant sunflower lines, six different origins are used at DAI – two from USA, one from Serbia, one from BASF and two own. We are annually working with about 1000 items, including primarily R lines and a smaller number of B lines and their sterile analogs. About 80 % of these materials are imi-tolerant, and 20 % are Express-tolerant. The work on herbicide-resistant materials at DAI started later in comparison to other breeding centers and therefore certain lagging behind is observed in this direction. However work is gong on at accelerated rates and the volume of hybrids is increasing annually, already giving very good results. The main method applied here is the experimental mutagenesis for development of B lines (Christov and Nikolova, 1996). The greater part of the materials were obtained after treatment of seeds, kernels and germs with gamma rays, ultrasonic and EMS. The fertility restorer lines were obtained mainly by selfing of hybrids and selection. All developed and stabilized sunflower lines are tested for their combining ability according to a preliminary approved scheme. The testers used are B lines, R lines or R lines with Falax cytoplasm. Thousands of lines are annually evaluated at DAI, and over 1400 hybrid combinations are tested in Bulgaria and abroad. Table 3 presents the amount of experimental hybrids tested during the past 4 years in Bulgaria and in other countries.

The most important stage from the testing process of the experimental hybrids is the unified varietal trial (UVT), where the best breeding of the Sunflower breeding department is put together (Table 4). The collected information allows determining the yield structure under changeable environment and developing appropriate technological solutions for growing (Baychev and Mihova, 2014). After their testing in this type of trial, the best hybrids are subjected to official testing and registration in Bulgaria, or are provided to foreign partners, who organize their official testing in the respective

countries and eventually enlist them in their catalogs before initiating their seed production and distribution.

Table 3. Volume of experimental hybrids tested in the last 4 years

Stages of testing	Number of hybrids
1. Preliminary varietal trial	1360
2. Competitive varietal trial	4080
3. Unified varietal trial	136
4. Joint hybrids with foreign participation tested at DAI	120
5. Foreign hybrids tested at DAI	110
6. Hybrids under official testing in Bulgaria and abroad	26
7. Bulgarian hybrids tested abroad	90

Table 4. Characteristics of some experimental hybrids under UVT testing in the last 4 years, which exceeded the mean standard with more than 5%.

Hybrid	*Yield kg/ha*	*% from the mean standard*	*Oil %*	*Resistance to downy mildew and Orobanche*
	2013			
3607A x 31R	*4113*	*110.2*	*44.1*	*100+100*
3607A x 240R	*4036*	*108.2*	*44.6*	*100+100*
217A x 242R	*3971*	*106.4*	*46.7*	*100+100*
217A x 12R	*3999*	*107.2*	*48.2*	*100+100*
3607A x 253R	*4275*	*114.6*	*48.2*	*100+100*
Mean standard	*3732*	*100.0*	*47.2*	*100+100*
	2014			
217A x 150R	*4336*	*105.5*	*46.8*	*100+100*
217A x 169R	*4348*	*105.8*	*45.8*	*100+100*
T6A x C60	*4367*	*106.3*	*47.0*	*100+100*
Mean standard	*4110*	*100.0*	*44.5*	*100+100*
	2015			
217A x 10594R	*3724*	*106.4*	*49.2*	*100+100*
217A x 10595R	*3724*	*106.4*	*48.0*	*100+100*
3607A x 10595R	*3764*	*107.6*	*44.4*	*100+100*
T6A x BTI1/1R	*4047*	*115.7*	*48.4*	*100+100*
A5 x 66R	*3882*	*110.9*	*46.1*	*100+100*
Mean standard	*3499*	*100.0*	*45.0*	*100+100*
3607A x 10596R	*4142*	*110.5*	*43.5*	*100+100*
3607A x 509PR	*4008*	*106.9*	*45.1*	*100+100*
846A x 10681R	*3969*	*105.9*	*46.5*	*100+100*
217A x KM85-2	*4236*	*113.0*	*46.0*	*100+100*
Mean standard	*3748*	*100.0*	*44.2*	*100+100*
	2016			
217A x 99R	*4486*	*105.9*	*51.0*	*100+100*
712A x 1216R	*4509*	*106.4*	*50.8*	*100+100*
217A x 278R	*4513*	*106.5*	*52.3*	*100+100*
4645A x 417 x 4151R	*4515*	*106.6*	*49.2*	*100+100*
Mean standard	*4237*	*100.0*	*46.5*	*100+100*
3607A x 99R	*4635*	*117.6*	*45.9*	*100+100*
2017A x PR427	*4184*	*106.1*	*47.8*	*100+100*
813A x 4204R	*4226*	*107.2*	*46.9*	*100+100*
813A x HA335 x 99-392R	*4327*	*109.8*	*44.8*	*100+100*
Mean standard	*3942*	*100.0*	*46.8*	*100+100*

Many of the hybrid combinations from Table 3 are already given names and are now within the systems of official testing in Bulgaria and abroad. Some of them are already registered in official catalogs, while others are currently undergoing the obilatory field tests. Table 5 shows the most recent sunflower hybrids of DAI – General Toshevo developed during the last 10 years, which have been released in the respective country or are currently under official testing.

Table 5. Sunflower hybrids registered in the respective country or under process of official testing

Hybrid	Bulgaria	Romania	Ukraine	Moldova	Russia	Belarus	Serbia	Kazachstan
Alpin		Registered	++	Registered	++	Registered		Registered
Veleka		Registered	Registered	Registered	++		++	
Vokil		Registered	Registered	Registered	++		Registered	
Mihaela		Registered	++		+			
Velko		Registered	Registered		Registered		Registered	
Gabi		Registered	Registered		+			
Divna			Registered		++			
Deya		Registered						
Sevar		Registered	Registered		+			
Yana	Registered			+	++		++	Registered
Valin	Registered		Registered					
Maritsa	Registered		Registered	Registered				
Kameliya					+			
Enigma imi	++							
Dessi imi	+							
Danaya imi	++							
Tedi	Registered							
Deveda	Registered							
Linzi	Registered			+				
Vessi			++					
Sunny imi		++	++					
Giga imi		++						
Vyara				Registered				
Kaliya imi	+			+	+			
Pavlina				+				
Rumyana				+				
Yanitsa				+				
Viliya	+							
Viyani imi	++							
St. George					+			
Rada	Registered				++			
Zhanina					+			
Lyubov					+			
Baikal					+			

+ First year of official testing ++ Second year of official testing

In last few years, the interest of seed production companies from Ukraine and Russia increased considerably; they are intensively testing our materials with the aim to register and promote them. Our comparatively old hybrid San Luka is still successfully grown in Russia; it was one of the first *Orobanche*-resistant hybrids develoiped in collaboration with the company Syngenta. Hybrid Maritsa is recently also being distributed in Russia. The cooperation of DAI with other research centers of sunflower breeding is ongoing. We are constantly exchaning materials which are involved in different breeding programs.

Conclusions

A rich and variable collection of initial breeding material has been built up as a result from the use of different breeding methods. Methods for evaluation of the resistance to the economically important diseases for Bulgaria and the parasite *Orobanche* have been developed and adapted to the working conditions of DAI. New high-yielding sunflower hybrids resistant to biotic and abiotic stress factors were developed. Morphological, biological, technological, bio chemical and phyto pathological

evaluation of the released and registered hybrids and their parental forms were made. The hybrids realized their high production potential not only in Bulgaria, but also in other countries with traditionally large-scale sunflower production and specific soil and climatic conditions. Many of the new hybrids were registered in Bulgaria and abroad, while others are currently involved in official testing in different countries. The joint projects of DAI with other research centers and companies dealing with breeding and production of sunflower are constantly increasing.

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