

## PRODUCTION POTENTIAL OF NEW SUNFLOWER HYBRIDS DEVELOPED AT DOBRUDZHA AGRICULTURAL INSTITUTE – GENERAL TOSHEVO

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### ABSTRACT

Sunflower breeding at Dobrudzha Agricultural Institute – General Toshevo (DAI) is traditionally carried out at a high level showing very good results. The working collection includes over 6 000 inbred lines. In the past decade, many new materials with very good combining ability and valuable properties were developed here. The new released hybrids possess high production potential. Over 1400 hybrid combinations are annually being tested in Bulgaria and abroad. Work is primarily focused on two-linear simple hybrids with full fertility restoration. A large part of the new genotypes were included in official varietal testing networks in Bulgaria and abroad; having demonstrated high performance, they were registered in the varietal lists of EU and other countries. These are hybrids Alpin, Veleka, Vokil, Velko, Gabi, Mihaela, Dea, Sevar. A number of foreign companies included our hybrids in their catalogs and are now promoting them. The testing of already released hybrids is ongoing with a view of their distribution on larger territories under variable environments. The developed sunflower lines and hybrids are the result from the efforts of the entire research team of the Sunflower Breeding Department at DAI who are working on several important projects aimed at higher productivity and oil content, variable chemical composition of oil, early maturity, drought tolerance and resistance to economically important diseases and the parasite *Orobanche*. The aim of this investigation was to present a brief characterization of the new DAI hybrids, demonstrating their productivity and adaptability potential.

**Key words:** Sunflower, New hybrids and lines, Production potential, Official varietal testing

### INTRODUCTION

Sunflower is an oil seed crop of primary importance in Bulgaria. The country is one of the greatest exporters of sunflower worldwide (Christov et al., 2009). In 2013, the export reached a peak – over 1 000 000 tons, which was 17.8 % of the global export. Almost two thirds of the production from this crop are being annually exported.

During the last decade the volume of the produce increased twice, from 788 000 tons in 2003 to 1 697 000 tons in 2015. This is a result from both the larger areas sown with sunflower and the higher mean yield, which was about 1200 kg/ha in 2003, and 2120 kg/ha in 2015. Annually 700 000 – 800 000 ha of sunflower are sown in Bulgaria. It has been noted that during the recent years the normal crop rotation is not being observed and sunflower is sown at an interval of 2-3 years, some times even only one year. Although profitable, this practice should be limited because it can cause the distribution of a number of diseases and pests, which, on its turn, can compromise irreversibly the sunflower crops.

Under the contemporary conditions characterized with certain variations of the abiotic and biotic environmental factors, adequate response is needed; such a response requires development of new

breeding materials with enhanced productivity and resistance to stress influences of various nature (Ivanova and Mihova, 2012, Marinković et al, 2011; Mihova, 2011; Gonzáles et al, 2013; Khan et al., 2013). Specific methods for evaluation of the genetic variability and selection of genotypes with high adaptability potential are applied (Mihova, 2013). The year 1917 can be considered the beginning of sunflower breeding in Bulgaria (Stoyanova et al., 1977). In 1963, the breeding and improvement work on development of hybrids started, using the method of inter linear hybridization (Petrov et al., 1994). The practical application of the methods for heterosis breeding became possible after the discovery of a stable CMS source by Leclercq (1969) and fertility restorer genes for this type of CMS (Enns et al.; Kinman, 1970; Leclercq, 1971; Vranceanu and Stoenesko, 1971). There are several schemes for hybrid seed production developed at DAI (Velkov and Stoyanova, 1974), but only one of them is used in practice – a simple hybrid with full restoration of fertility.

In 1979, the first Bulgarian hybrid Start was released and distributed on the territory of the entire country (Gotsov et al., 1981; Ivanov et al., 1988). A new page of Bulgarian sunflower breeding was opened in 1988, when the new hybrid Albena was registered in France, followed by registration in Bulgaria as well on the next year. During the years to come it became the most widely distributed hybrid in Bulgaria, while in France it occupied as much as 42 % of the sunflower production areas. The hybrid was grown very successfully in other European countries as well. The high results of hybrid Albena in Bulgaria and abroad made it a world standard. More than 20 own and joint hybrids involving the mother line of Albena have been registered. Up to now mainly early hybrids have been developed at DAI. The best known among them are Super Start, Dobrich, Mussala, Maritsa, Rada, Merkurji, Perfekt, Diamant, and the large-seeded variety Favorit.

During the 1990's, hybrid San Luka was released, which occupied about 90 % of the sunflower areas in Bulgaria till 2008. For more than a decade now, the invasion of foreign hybrids in Bulgaria has increased considerably (Georgiev et al., 2009). Almost all major sunflower seed companies introduced new products in the domestic market and aided by their enormous financial potential logically displaced our own hybrids from the sunflower production areas. We were unprepared economically for this situation. However, efforts were made and opportunities were found for distribution of our products abroad. Now our hybrids are successfully produced and traded on the Ukrainian, Russian, Romanian, Moldovian and other markets. The testing of our hybrids with the aim of their registration is ongoing.

At DAI, the breeding of sunflower is carried out in three main directions:

Higher productivity;

Higher resistance to the economically important diseases, the parasite *Orobanche*, some herbicides, soil and air drought; Higher oil percent in seed and higher variability of its chemical composition.

The work in these directions is in accordance with the present reality of sunflower production, the scientific achievements in this field, the conventional and bio technology methods applied in breeding, the human resources and last, but not least, the financial means available in our system. Our work is focused on the use of heterosis by developing and investigating inbred lines, testing of experimental hybrids and production of seeds from parental lines of already developed and registered hybrids (Petrov et al., 1994).

The aim of this investigation was to present a brief characterization of the new developed and registered hybrids of DAI – General Toshevo, revealing their production and adaptability potential.

## MATERIAL AND METHODS

The main investigations related to this study were carried out at DAI – General Toshevo. They are the result from the implementation of a long-term program which encompasses several 4-year periods. The main purpose of this program is to develop sunflower hybrids with enhanced production potential, resistant to economically important diseases and the parasite *Orobanche* by combining conventional and biotechnology methods.

Breeding material was used, which includes Bulgarian and foreign direct and hybrid varieties, landraces and foreign populations, our old sterility maintainer lines, their sterile analogues and fertility restorers, wild species of genus *Helianthus*, and species from other genera of *Compositae* family. To obtain new forms, lines and hybrids, the following methods are applied: hybridization (interspecific, interlinear, intraspecific and intergeneric), experimental mutagenesis, selection, gamma-induced parthenogenesis, embryo culture, somaclonal variation, combined use of *in vitro* methods and physical mutagenesis.

Eight new male fertile two-linear sunflower hybrids were developed through the method of interlinear hybridization. These are Alpin, Veleka, Vokil, Velko, Gabi, Mihaela, Dea and Sevar. The mother components of these hybrids are lines 2607, 217, 3607 and 807. They possess very good general and specific combining ability and resistance to economically important diseases; with the exception of line 2607, they also possess resistance to the parasite *Orobanche*, races A-F.

Using these mother lines, other new hybrids registered abroad have also been developed, as well as hybrids which are now in the process of official testing. The fertility restorers involved in the new hybrids are 10681R, 166R, 340R, 105R, 127R, 10671R, 509R and 626R. They are all resistant to the above diseases and the parasite *Orobanche* and have excellent combining ability. All are branched and rich in pollen.

Each new sunflower hybrid goes through three-year testing in the trial fields of DAI according to a growing technology approved for this crop (Georgiev et al., 1997). The standards used in this testing were the most widely distributed hybrids in Bulgaria – San Luka and Maritsa, as well as the most highly productive and most marketed foreign hybrids Brio, Diabolo, Meldimi, Clarica, PR64F50, LG5665, PRLE19, PRLE25, etc.

Having demonstrated very good results, the new hybrids were provided to our partners from Saaten Union – Romania to produce and distribute them. Following one more year of testing in their experimental fields, they were subjected to a three-year official testing at 10 locations within the system of the State Institute for Variety Testing and Registration. Having once again demonstrated very good results, the new sunflower hybrids were officially registered. They are enlisted in the European catalog of field and vegetable crops. The trait seed yield kg/ha was read. The observations and the evaluation of the morphological characters were made in accordance with the UPOV protocol (2002). The phytopathological characterization of the hybrids was made at DAI – General Toshevo. The resistance to downy mildew (*Plasmopara halstedii*) was determined according to the standard methodology (Vear and Tourvieille, 1987) adapted to the working conditions of the institute. The response of the hybrid to races 700 and 731 of the pathogen was presented as percent of resistance.

The resistance to grey spots on sunflower (*Phomopsis helianthi*) was done according to the method of Encheva & Kiryakov (2002) under field conditions against artificial infection background. The type of attack was read one week after full flowering and at stage milk maturity according to the following scale: 0 – no symptoms; 1 – necrotic spot up to 5 cm in diameter; 2 – necrotic spot with diameter more than 5 cm; 3 – several merged necrotic spots on stem; 4 – stem broken at the place of infection.

The testing for black spots on sunflower (*Phoma macdonaldii*) was carried out under field conditions in an artificial infection field. Inoculation was done at stage budding – beginning of flowering according to the method of Maric et al. (1981). The response of the plants was read at stage yellow-brown maturity according to a 4-degree scale: 0 – no symptoms; 1 – necrotic spot localized around the petiole; 2 – several merged necrotic spots on stem; 3 – entire stem covered with necrotic spots or broken.

The resistance to the parasite broomrape (*Orobanche cumana*) was determined by the method of Panchenko (1975). The evaluation was made under greenhouse conditions using the index percent of resistance. The experimental data were analyzed by ANOVA 3. The applied statistical model was:

$$Y_{ijk} = Y.. + G_i + Y_j + R_k + (GY)_{ij} + (GR)_{ik} + (YR)_{jk} + (GYR)_{ijk} + E_{ijk}$$

where  $G_i$  is the factor genotype,  $Y_j$  is the factor climatical conditions, and  $R_k$  - the factor location.

The Additive Main Effects and Multiplicative Interaction (AMMI) model has developed a new statistical method for analyzing the genotype by environment interaction. In the AMMI method, first the main additive effects of genotype and environment are considered by variance analysis, then are analyzed by principal characteristics of remain value from variance analysis model (Gauch et al, 1996; Dias et al., 2003, Lee, 2004). Totally, AMMI follows three basic purposes: first, this is an appropriate method for primary analysis of performance tests. Second, it explains the effect of the genotype  $\times$  environment interaction. Third, performance estimate is done with greater accuracy. This method is applied to estimate the ecological stability and plasticity of the hybrids. Data were analyzed with the help of the software SPSS, version 19.0.

## RESULTS AND DISCUSSION

Following the official testing, all new hybrids underwent a two-year test for distinctness, uniformity and stability at the State Institute for Variety Testing and Registration – Romania. They were acknowledged as distinct, uniform and stable. Their morphological descriptions were done using the methodology of UPOV (2002).

## BIOLOGICAL AND ECONOMIC PROPERTIES

The eight new Bulgaria hybrids presented here are from the group of the medium early varieties, with growth season 110 – 120 days; however, the earliest among them are Alpin, Mihaela and Sevar. They all have linoleic type of oil, with oil percent within the range 46-50 %. The number of seeds per plant can reach up to 1200 – 1300, which makes their production potential very high, exceeding sometimes 4300 kg/ha. The stems are medium high and resistant to lodging. All hybrids possess very high plasticity with regard to the growing conditions. Hybrids Velko and Dea demonstrated highest drought resistance.

The possibility of sowing both parental forms simultaneously is a great advantage of all presented hybrids. The father lines are strongly branched and very rich in pollen, which, on its part, allows planting design of 10:2 female to male lines. This design should naturally be provided with at least 3-4 good bee hives per ha.

## PRODUCTIVITY

All eight new hybrids were subjected to three-year official testing at the State Institute for Variety Testing and Registration – Romania. The testing was carried out at 10 locations representative for regions with various soil and climatic conditions from all over the country. The first three, Alpin, Veleka and Vokil were tested during 2009 – 2011 (Table 1).

The three years of investigation were with comparatively similar climatic conditions as evident from the given mean yields, which were with similar values and low variation. Averaged for the investigated period, all new hybrids exceeded the standard. Hybrid Alpin demonstrated highest mean results with an average yield for the entire period of testing 3572 kg/ha, followed by Vokil and Veleka.

Table 1. Mean results from 10 locations of official testing of hybrids Alpin, Veleka and Vokil

Hybrids	Yield kg/ha	% from standard	Yield kg/ha	% from standard	Yield kg/ha	% from standard	Averaged for 3 years kg/ha	Relative yield according to the standard, averaged for 3 years
	2009		2010		2011			
Standard	3108	100	3240	100	3659	100	3336	100
Alpin	3489	112	3619	112	3609	99	3572	108
Veleka	3272	105	3368	104	3485	95	3375	101
Vokil	3259	105	3356	104	3635	99	3417	103

The group of hybrids including Velko, Mihaela and Gabi were tested during 2011 – 2013 (Table 2). In this case, again, the mean results of the three hybrids were above the mean standard of the entire investigated period. The exceeding was within the range 4 – 9 %, hybrid Velko being the highest yielding. It was also the hybrid with highest mean results out of all eight according to the index seed yield during the entire official testing - 3578 kg/ha. Hybrid Velko also gave best results during the less favorable year 2012.

Table 2. Mean results from 10 locations of official testing of hybrids Velko, Mihaela and Gabi

Hybrids	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Averaged for 3 years kg/ha	Relative yield according to the standard, averaged for 3 years
	2011		2012		2013			
Standard	3659	100	2648	100	3642	100	3316	100
Velko	3694	101	3072	116	3969	109	3578	109
Mihaela	3618	99	2934	111	3757	103	3436	104
Gabi	3689	101	2879	109	3947	108	3505	106

The last two hybrids, Sevar and Dea, were officially tested during 2012 – 2014 (Table 3). They also exceeded the standard, averaged for 3 years. Dea showed better results, especially in 2012, the year with lowest precipitation, which defines this hybrid as drought resistant.

Table 3. Mean results from 10 locations of official testing of hybrids Sevar and Dea

Hybrids	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Averaged for 3 years kg/ha	Relative yield according to the standard, averaged for 3 years
	2012		2013		2014			
Standard	2648	100	3642	100	3234	100	3175	100
Sevar	2650	100	3959	109	3210	99	3273	103
Dea	2954	112	3783	104	3424	106	3387	107

The results from the dispersion analysis are presented in Table 4.

Table 4. Dispersion analysis for the index *productivity*

	Mean of square	df
G	11089,8 <sup>c</sup>	8
Y	15793 <sup>c</sup>	2
R	7726,6 <sup>c</sup>	7
G x Y	1416,1 <sup>c</sup>	16
G x R	438,1 <sup>a</sup>	56
Y x R	4061,7 <sup>c</sup>	14
G x Y x R	474,3	112
Error	221,4	278

The applied dispersion analysis proved with a high degree of significance ( $p=0.001$ ) the differences in the genetic potential of the selected hybrids according to the index *productivity*. The effects of the factors climatic *conditions* and *location* were with highest significance.

The interaction between the indices *genotype x climatic conditions* and *genotype x location* was also determined statistically. The lower significance of /G x R/ showed that the predominant part of the genotypes responded in a similar way to the various locations of testing. Their response to the year conditions /G x Y/ was specific. This fact allowed applying AMMI (1, 2) models for evaluation of the ecological plasticity and stability of the investigated hybrids with regard to the index productivity (Table 5).

Five of the investigated Bulgarian hybrids had  $ASV < 0.200$  indicative of high ecological stability and plasticity. Hybrids Alpin, Veleka and Vokil showed lower response to the changeable environments with regard to the index productivity. Hybrids Sevar, Dea and Gaby had more susceptible reaction.

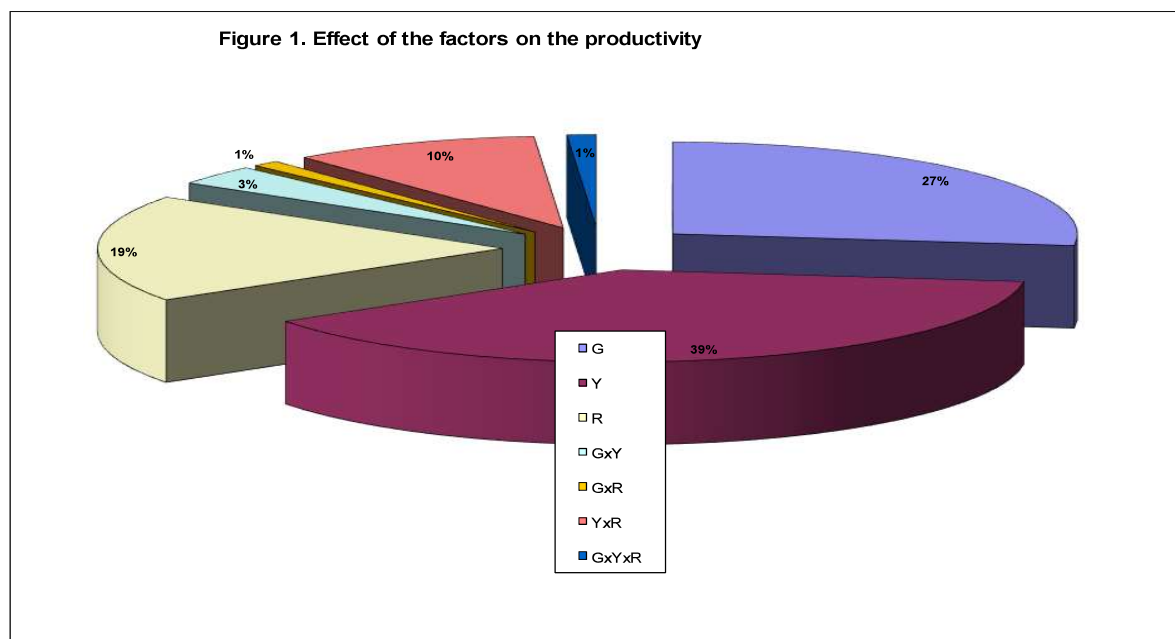


Table 5. Ranking of investigated hybrids by the index *productivity* according to their ecological plasticity and stability

Hybrid	Parameters		
	Rank	ASV	I
Alpin	1	0,022	2,432
Veleka	2	0,084	2,213
Vokil	3	0,111	1,945
Velko	4	0,165	1,929
Standard	5	0,180	1,896
Mihaela	6	0,187	1,854
Gabi	7	0,208	1,805
Dea	8	0,233	1,725
Sevar	9	0,276	1,664

All eight Bulgarian sunflower hybrids were officially registered and included in the European catalog of field and vegetable crops. Because of the demonstrated very good productivity potential, they were included also in the systems of official varietal testing of non-EU countries such as Ukraine, Serbia, Kazakhstan, Russia, etc

In 2013 the testing of our hybrids continued in Hungary as well, at three locations in the central and southern parts of the country (Table 6). Seed yield varied from 3150 до 5304 kg/ha, most of the results being within the range of the higher values and demonstrated a very good level of the index *productivity*. Only hybrids Velko and Dea exceeded the standard at all three locations, and the mean value of hybrid Mihaela also exceeded it. Hybrid Dea demonstrated highest mean results - 4441 kg/da, showing once again that it is hybrid with high plasticity and productivity.

Table 6. Results from testing in Hungary

Hybrids	Bekecsaba		Lanycsok		Cegled		Average	
	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from std
STANDART	4381	100	3892	100	3621	100	3965	100
ALPIN	3679	84	3301	85	3438	95	3473	88
VELEKA	3714	85	3411	88	3435	95	3520	89
VOKIL	3559	81	3458	89	3617	100	3545	89
MIHAELA	4926	112	3800	98	3924	108	4217	106
VELKO	4628	106	3969	102	3772	104	4123	104
GABI	3403	78	3219	83	3503	97	3375	85
SEVAR	4118	94	3554	91	3150	87	3607	91
DEA	5304	121	3922	101	4098	113	4441	112

Table 7. Results from the testing in Ukraine

Hybrids	KHARKIV		ODESSA		ZAPOROGYE		KIROVOGRAD		AVERAGE	
	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from std
STANDARD	3445	100	817	100	1823	100	3075	100	2290	100
Neoma CL	3376	98	670	82	1422	78	3290	107	2190	96
Adagio CL	3796	110	1006	123	1977	108	2970	97	2437	106
ALPIN	3397	98	930	114	1792	98	3670	119	2447	107
VELEKA	3108	90	980	120	1802	99	2520	82	2103	92
VOKIL	3498	101	1007	123	2226	122	3210	104	2485	109
MIHAELA	3671	106	630	77	2029	111	3360	109	2423	106
VELKO	3985	116	500	61	2482	136	3600	117	2642	115
GABI	3434	99	840	103	2039	112	2910	95	2306	101

In 2014, at four locations in the central, southern and eastern part of Ukraine, some of the most recent hybrids of DAI – General Toshevo were tested and compared to the most widely distributed



and highly productive commercial hybrids (Table 7). In the regions of Odessa and Zaporogye, where the climate is dryer, the results were naturally lower, while in Kharkiv and Kirovograd the hybrids revealed their true potential. The maximum yields obtained were 3985 kg/ha from Velko in Kharkiv region and 3670 kg/ha from Alpin in Kirovograd. In general, all tested Bulgarian hybrids, with the exception of Veleka, exceeded the standard at the four locations, and Alpin, Vokil, Mihaela and Velko were at the productivity level of the foreign hybrids currently dominant on the market.

### PHYTOPATHOLOGICAL CHARACTERISTICS

The phytopathological characterization was done under laboratory conditions and in the infection field of DAI where all newly developed materials of the Sunflower breeding department are subjected to testing for the economically important diseases and the parasite *Orobanche*. The results from these investigations are given in Table 8.

Table 8. Phytopathological evaluation of sunflower hybrids in artificial infection field at DAI – General Toshevo.

Hybrid	<i>Phomopsis helianthi</i>		<i>Phoma macdonaldi</i>		<i>Plasmopara helianthi</i>		<i>Orobanche cumana</i>
	Attacking rate	#	Attacking rate	#	Resistance to race 700, %	Resistance to race 731, %	Resistance to races A-F, %
San Luka	3/3(3)	3	1/3(1)	1	100.0	92.9	100.0
Perfekt	1/3(1)	1	1/3(1)	1	84.5	-	100.0
Diabolo	2/3(2)	2	1/3(1)	1	100.0	90.5	100.0
Brio	1/3(1)	1	0	0	100.0	100.0	100.0
Meldimi	2/3(2)	2	1/3(1)	1	100.0	90.0	100.0
PR64F50	1/3(1)	1	0	0	100.0	100.0	100.0
Valin	2/3(2)	2	1/3(1)	1	100.0	95.0	100.0
Alpin	2/3(2)	2	1/3(1)	1	100.0	100.0	100.0
Veleka	1/3(1)	1	0	0	100.0	100.0	100.0
Vokil	1/3(1)	1	0	0	100.0	90.0	100.0
Mihaela	2/3(2)	2	1/3(1)	1	100.0	100.0	100.0
Gabi	1/3(1)	1	0	0	100.0	100.0	100.0
Velko	1/3(1)	1	0	0	100.0	100.0	100.0
Dea	1/3(1)	1	0	0	100.0	70.0	100.0
Sevar	1/3(1)	1	0	0	100.0	100.0	100.0

Attacking rate - what part of the plant stem was covered with spots of the pathogen (1/3, 2/3, 3/3). In brackets – number of spots. Rank: 0 – immune; 1 – resistant; 2 – moderately resistant; 3 – moderately susceptible; 4 – susceptible.

Hybrids Veleka, Vokil, Gabi, Velko, Dea and Sevar were resistant to the fungal pathogen *Phomopsis helianthi*, similar to other foreign high-yielding hybrids widely distributed in Bulgaria, such as Brio and PR64F50. In comparison to San Luka and the other hybrids presented in this study, they performed as more tolerant to this disease. With regard to the other important leaf pathogen *Phoma macdonaldi*, these six hybrids demonstrated immune reaction. Although with one degree only, the other hybrids were below their resistance to this disease.

With the exception of Perfekt, the other hybrids demonstrated 100 % resistance to downy mildew (*Plasmopara helianthi*) race 700. To the most recent race 731, full resistance was demonstrated by Brio, PR64F50, Alpin, Veleka, Mihaela, Gabi, Velko and Sevar. In the greater number of Bulgarian hybrids, this resistance came from the male parent. The resistance to the parasite *Orobanche cumana* was 100 % due to the contribution of both parental components.

## CONCLUSION

The new Bulgarian sunflower hybrids were officially registered in Romania and were enlisted in the European catalog of the field and vegetable crop varieties. They are distinct, uniform and stable. They demonstrated high and stable yields under variable environments in Bulgaria and abroad, which makes them hybrids with high adaptability potential. They also possess high field resistance to the economically important diseases and the parasite *Orobanche*.

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