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Sunflower hybrids and lines resistant to pathogens economically important for Bulgaria, developed by conventional and biotechnological methods

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Abstract

The diseases on sunflower are a limiting factor for sunflower production not only in Bulgaria but also in all countries where the crop is grown. This is the reason for the great number of researchers who have directed their efforts toward solving the problem. A priority in the research work carried out at DAI is the problem not only of seed quality and quantity, but also the phyto sanitary condition of sunflower. The results from the past ten years and more show that success has been achieved in the resistance to leaf fungal pathogens (Phomopsis helianthi; Phoma macdonaldi; Alter nar ia sp.), downy mildew (Plasmopara halstedii) and the parasite broomrape (Orobanche *c*umana). The result from the activity of the researchers working at DAI are the released hybrids San Luka, Mousala, Mercuriy, Maritsa, Mesta, Moura, Magoura, Biotsvet, Rada, Yana, Valin, Dobrotsvet, Alpin, Mihaela, Vokil, Veleka, Velko, Gabi, etc. A part of these hybrids were developed by conventional methods, others - by using bio technology methods, while the development of third hybrids involved a combination of both. Many of them are now in production demonstrating good results, and others are to be approved by the national agencies. Each of these hybrids possesses complex resistance to some of the leaf fungal pathogens, as well as obligatory resistance to downy mildew and broomrape. The genetic stock center of DAI maintains a great number of lines derived mainly from the wild annual and perennial species. They are used as donors of resistance in the breeding program of the institute.

Keywords: leaf pathogens, broomrape, downy mildew, resistance

Introduction

The diseases on sunflower are a limiting factor for sunflower production not only in Bulgaria but also in all countries where the crop is grown. More than thirty pathogens are known which cause economically important damages. A large part of them have already been investigated, others, which occurred later and became a problem for the crop, are being investigated now. In all cases the knowledge collected on the pathogens up to now contributes to solving the problem with them. Economically most important pathogens in Bulgaria are becoming downy mildew (***Plasmopara halstedii***), the parasite broomrape (***Orobanche cumana***), the leaf pathogens – black (***Phoma macdonaldi***), grey (***Phomopsis helianthi***) and brown (***Alternaria* sp.)** spots on sunflower. This publication is aimed at presenting the achievements of the Sunflower Breeding Department of DAI in the investigation on the pathogens, finding of resistant lines and developing of hybrids with complex resistance to the economically important diseases on sunflower in Bulgaria.

Material and methods

The investigations were carried out at Dobrudzha Agricultural Institute – General Toshevo in the infection field for testing of leaf pathogens, and under greenhouse conditions for testing the resistance of the same materials to downy mildew and the parasite broomrape. Breeding materials obtained through distant hybridization, wild species from genus *Helianthus*, fertility restorer lines in different generations, B lines, F_1 hybrids, materials produced by mutagenesis and biotechnology methods were investigated for their response to the cause agents of downy mildew, the parasite broomrape and grey, black and brown spots on sunflower.

Standards methods were used:

- For testing the breeding materials to the fungal pathogen downy mildew, the methodology of Gulya, T. J. et al. 1991 was applied;

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For testing the breeding materials to the parasite broomrape, the methodology of Pancenko, A. N., 1973 was used;

-For testing the breeding materials to the leaf pathogens on sunflower, the method of Encheva V. and Kiryakov I., 2002 was used. -

Results

Resistance of the sunflower breeding materials and hybrids to downy mildew (*Plasmopara halstedii* Farl. Berlese et de Toni)

In the last 10 years, the races of the fungal pathogen *Plasmopara halstedii* have been occuring in Bulgaria rather dynamically. Just two races (1 and 2) were widespread till 1997, race 2 being only sporadically identified in the north-east part of Bulgaria (Shindrova, 2000). During this period the first more significant achievements of the Sunflower Breeding Department at DAI were made with the development of the first joint hybrids with French companies: San Luka, Alinka, Arte, Allianz, Florello, Gala, Altesse, Aria and Allstar. In the following years the race variability in Bulgaria changed more dynamically.

The new races 100, 300 and 700 occurred, the latter being most widespread in the country (Shindrova, 2005, 2006). As a result from purposeful breeding, a new group of hybrids were developed: Penka, Stozher, Nelly and Perfect. They were all completely resistant to the races of the pathogen widespread in Bulgaria. In a later investigation of the above author, which encompassed the period 2004 – 2006, the occurrence of two new aggressive races was reported, 721 and 731; at the same time race 700 continued to be the most widespread race in Bulgaria. During that time a new group of hybrids were released by the Sunflower Breeding Department: Maritsa, Moura, Mesta, Magoura and Selena. Again, the policy of the Department to release in production only hybrids and varieties which are completely resistant to the fungal pathogen downy mildew was followed. Till the present moment race 731 is still the most virulent in Bulgaria, and once again the hybrids developed in the last few years (Vokil, Veleka, Gabi and Velko) are completely resistant to the pathogen.

Besides the already developed and released hybrids, DAI has at its disposal a large number of breeding materials at different stages of heterosis breeding, distant hybridization, biotechnology and seed production.

Table 1 gives data for the last three years and demonstrates the results from the analysis carried out on the development of resistant breeding materials that can be included in the next stages of breeding. It has been found out that the percent of the resistant materials is increasing in each successive year: from 21.2 % in 2011 to 45.3 % in 2013. This shows that purposeful selection has been carried out by this index during the years of investigation and as a result among all breeding materials of DAI there are some which are resistant to race 731.

Table 1. Breeding materials with different resistance to downy mildew (race 731) during 2011-2013

Year	Resistance to downy mildew, %									
	0		1 - 50		51 - 75		76 - 99		100	
2011	1973	52,5	81	2,1	147	3,9	182	4,8	801	21,2
2012	1428	50,0	46	1,6	101	3,5	157	5,5	852	29,8
2013	858	31,0	9	0,4	62	2,5	120	5,0	1057	45,3
2011-2013	**4259**	**47,0**	**136**	**1,5**	**310**	**3,4**	**459**	**5,1**	**2710**	**29,9**

Resistance of breeding materials and sunflower hybrids to broomrape (*Orobanche cumana* Wallr.)

Broomrape (*Orobanche cumana* Wallr.) on sunflower is a serious problem for sunflower production in Bulgaria. Since its first occurrence in 1935 it has been a constant companion of the crop. The morphogenesis of genus *Orobanche* is still going on, evidence for which is the occurrence of increasing number of new *Orobanche cumana* Wallr. races overcoming the resistance of the recently developed sunflower varieties and hybrids. According to the differential analysis, 5 *Orobanche cumana* races existed in Bulgaria till 2000 (A, B, C, D and E). Five years later two new races occurred (G and F). According to the researches of Shindrova, a new more aggressive race H occurred in the recent years, which has been identified only in 2007 on a very small area. The most widespread race in the main north-east sunflower production region is race E, while in the southern part of the country the most widespread race is G. This race is also widespread in central north Bulgaria. According to the above author, race F has lost its economic significance and occurs very rarely. In all these years the breeding of varieties and hybrids has been going on in parallel with the occurrence of the new races and all hybrids developed at DAI (mentioned above) are resistant

to them. Furthermore, in the last three years new breeding materials have also been produced which are resistant to the most aggressive broomrape races in Bulgaria. During 2011 – 2013, breeding samples including various breeding materials were analyzed (Table 2). These were mainly completed A, B and R lines, materials from distant hybridization, samples for the needs of seed production, and completed varieties and hybrids. The obtained results showed that the volume of the materials resistant to broomrape was considerably lower than the volume of materials resistant to downy mildew.

Averaged for the reported period, the materials resistant to the parasite were 6.6 %. The explanation for this is one the one hand the occurrence of new and more virulent races of the parasite, and on the other – the heritability mechanisms of the resistance to broomrape.

Table 2. Breeding materials with different resistance to broomrape during 2011-2013

Year	Resistance to broomrape, %									
	0		1 - 50		51 - 75		76 - 99		100	
	No	%	No	%	No	%	No	%	No	%
2011	1874	68,8	306	11,2	142	5,2	83	3,1	116	4,3
2012	1599	42,6	399	10,6	543	14,5	577	15,4	437	11,6
2013	2982	70,8	536	12,7	270	6,5	127	3,0	139	3,3
2011-2013	**6455**	**60,4**	**1241**	**11,6**	**955**	**8,9**	**787**	**7,4**	**692**	**6,6**

Resistance of sunflower breeding materials and hybrids to the leaf pathogens *Phomopsis helianthi Munt.-Cvet et al., Phoma macdonaldii Boerema* and *Alternaria spp*.

Each year a test for resistance is applied to check the breeding materials derived from distant hybridization, the wild species of genus *Helianthus*, the fertility restorer lines at different generations, B lines, F_1 hybrids, and materials obtained through mutagenesis and bio technologies. Hybrids resistant to all three pathogens have not been developed till now although many of them are resistant either to one or two of them. There are no materials resistant to *Alternaria spp*.

The next three tables (3, 4 and 5) present the results for resistance to the three pathogens (2011 – 2013). The results show that a sufficient number of resistant materials have been produced that can be involved in the breeding program of DAI. During calendar year 2011, the materials immune and resistant to phomopsis were 28.3 % and 24.3 %, which amounted to half of all materials tested. This was a good result for the Sunflower Breeding Department. In the next year 2012, the immune and resistant materials were again about a half from the tested materials, the higher percent being in favor of the resistant materials – 39.7 %, while the immune ones were 17.1 %.

A lower percent of immune materials were read in 2013 – 7.5 %. The percent of the resistant materials was also lower in comparison to the previous years. The reason for this may be both the climatic conditions and the genotype of the tested materials.

Table 3. Response of the sunflower breeding materials to grey spots caused by *Diaporthe/Phomopsis helianthi* Munt. Cvet. *et al*., under artificial infection field during 2011 - 2013

Years	Type of infection									
	0		1		2		3		4	
	No	%	No	%	No	%	No	%	No	%
2011	479	28.3	441	24.3	511	30.1	244	14.4	40	2.4
2012	225	17.1	523	39.7	479	36.4	262	19.9	0	0.0
2013	85	7.5	288	25.4	388	34.2	339	29.9	31	2.7

The summarized results for 2011 – 2013 on the resistance of the breeding materials to black spots caused by the fungal pathogen */Phoma macdonaldii* Boerema/ are given in Table 4. Lowest percent of resistant materials was found in 2011: 36.6 %. The highest percent was registered in 2012: 92.0 %. This high value was due to the extremely high air temperatures in that year which were the reason for the ceased development of

the pathogen. The next year 2013 was characterized with temperature and precipitation normal for the development of the pathogen, but the percent of resistant materials was again high. This was most probably due to the lower amount of inoculum that had been accumulated in the previous year. Regardless of the fact that inoculum is annually taken out to the infection field with the infected plant residues, the air-borne spores of the pathogens also have their effect on the infection process.

Table 4. Response of the sunflower breeding materials to the cause agent of black spots */Phoma macdonaldii* Boerema/ under artificial infection field during 2011 - 2013

Year	Attacking rate							
	0		1		2		3	
	No	%	No	%	No	%	No	%
2011	620	36.6	761	44.9	291	17.2	13	0.8
2012	1211	92.0	60	4.6	28	2.1	1	0.1
2013	1005	88.5	113	10.0	9	1.5	0	0.0

The tendency observed in the occurrence of black spots on sunflower was valid in the case of occurrence of brown spots, too. Only 2.6 % were the resistant materials in 2011. A sharp increase of the percent of resistant materials was observed in the next year (2012) – 49.6 %. The following calendar year 2013 was characterized with climatic conditions normal for this region, but again the percent of resistant materials was high – 63.0 %. In previous investigations it has been found that this pathogen occurs annually regardless of the climatic conditions and causes particularly heavy damages in years with cool and humid summers. The pathogen is transferred from the parenchymal part of the inflorescence to the seeds of the sunflower plants, considerably worsening the quality of the obtained oil.

Table 5. Response of the sunflower breeding materials to the cause agent of brown spots *Alternaria spp*. under artificial infection field during 2011 -2013

Year	Attacking rate							
	0		1		2		3	
	No	%	No	%	No	%	No	%
2011	43	2.6	231	14.0	693	42.1	680	41.3
2012	630	49.6	277	21.8	219	17.3	163	12.8
2013	693	63.0	198	18.4	188	17.2	9	0.8

Conclusion

Success has been achieved in the resistance to downy mildew ***Plasmopara halstedii***, the parasite broomrape ***Orobanche cumana*** and the leaf fungal pathogens ***Phomopsis helianthi, Phoma macdonaldi*** and ***Alternaria sp***. As a result from the activity of the Sunflower Breeding Department at DAI the following hybrids were developed: San Luka, Mousala, Mercuriy, Maritsa, Mesta, Moura, Magoura, Biotsvet, Rada, Yana, Valin, Dobrotsvet, Alpin, Michaela, Vokil, Veleka, Velko, Gabi, etc.

Each of these hybrids possesses complex resistance to some of the leaf fungal pathogens and obligatory resistance to downy mildew and broomrape.

In the genetic stock center of DAI there are an enormous number of lines that can be used as donors of resistance in the breeding program of the institute.

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