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SOURCES OF RESISTANCE TO THE LEAVES PATOGENS CAUSED GREY (*Phomopsis helianthi*), BROWN (*Alternaria* sp.) AND BLACK (*Phoma macdonaldi*) SPOTS ON SUNFLOWER

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Abstract

The presented results are from phytopathological investigations carried out with the wild species Helianthus argophyllus and its hybrid progenies, obtained from crosses with five male sterile lines of cultivated sunflower, created in DAI. It was established the presence of resistance to grey (*Phomopsis helianthi*), brown, (*Alternaria* sp.) and black (*Phoma macdonaldi*) spots on sunflower. The investigations were carried out in the infection field of Dobroudja Agricultural Institute during 2008 – 2010. As a result from self-pollination and purposeful selection some new hybrid forms were developed; they combined high resistance to some pathogens with valuable economic traits. Some morphological, phenological and biochemical characters were also studied. The obtained materials possess resistant genes and can therefore be either successfully included in the breeding programs of DAI for developing the new genetic material or be used as donors for developing new hybrid combinations.

Key words: sunflower, resistance, *Phomopsis helianthi*, *Alternaria* sp., *Phoma macdonaldi*

INTRODUCTION

Sunflower cultivation is of great economical importance for our country. However, this crop is exposed to a risk of certain factors – climatic conditions, cultivar structure, fertilization, sowing density, presence of pathogens caused fungal, viral, bacterial and other infections. In the group of economically important diseases, which decrease seed yield and worsen sunflower production quality are grey, brown and black spots on sunflower. For decreasing of negative effects of these diseases, different chemical and agro-technical methods were used. Emphatically is proved that using of resistant sunflower cultivars and hybrids is the most effective way for overcoming fungal diseasese including grey, brown and black spots on sunflower (Nikolova et al., 2001; Roustaee et al., 2000; Hahn and Degener, 1999; Vear and de Labrouhe, 1997; Skoric, 1985). Purposeful breeding for creating of new hybrids with high productive potential and high resistance to economically important sunflower diseases was carried out by many researchers (Boerema et al., 2004; Treitz, 2003), Morris et al., 1983. Their developing is unthinkable without including of wild sunflower species. Their use as donors for resistance is one of the most popular and favorable ways for developing new sunflower cultivars and hybrids. Breeding on resistance solve the problems for many years ahead and spare nature at the most, diminishing use of chemical agents to its minimum (Poehlman and Sleper, 1995). The wild species *Helianthus argophyllus* T. & G. represented some specific adaptations that play an important role in the study of its genetic potential. The silver-leaf sunflower became increasingly attractive to breeders because of existence of many natural hybrids and the opportunities to introduce desirable genes into cultivated sunflower. These genes determined either resistance to diseases and stress factors, or cytoplasmic male sterility and fertility restoration. This wild annual species has been reported to show resistance to some diseases such as Phomopsis (Skoric, 1992), Puccinia (Seiler et al., 1992; Quresh et al., 1993), Sclerotinia (Christov, 1996; Christov et al., 1997), downy mildew (Christov, 1990; Seiler, 1991; Seiler et al., 2007).

The aim of this study was to present the characteristics of hybrid combinations, which showed resistance to caused agents of grey spots (*Phomopsis helianth* Munt.-Cvet. *et al./Diaporthe he-*

lianthi Munt.-Cvet. et al.), brown (*Alternaria* sp.) and black spots (*Phoma macdonaldi*/*Leptosphaeria lindquistii*), with a view to continue their using in the breeding process.

MATERIAL AND METHODS

Investigations were carried out in Dobroudzha Agricultural Institute near General Toshevo in artificial infection plot during the period 2008 – 2010.

Plant material: Seven different accessions from the wild sunflower species collection of Dobroudzha Agricultural Institute General Toshevo were studied – GT-E-006, GT-E-007, GT-E-008, GT-E-091, GT-E-130, GT-E-131 and GT-E-132 of wild *H. argophyllus*. *H. argophyllus* (2n = 34) is an annual diploid species (Table 1).

Infection plot and evaluation of accessions reaction: Sunflower accessions were sown in artificial infection plots. Annually, infected plant remainders (stems) distinguished with symptoms of the three pathogens have been supplemented in the experimental plots. Stems were collected previous year and were left to pass the winter at the open air. After sunflower germinating they were spread out in chess-board order between the rows.

The type and degree of attacks were checked a week after complete flowering and in phase of physiological maturity on the follow scales:

Type of infection of the grey spots: 0 – absence of symptoms; 1 – necrotic spot with diameter not more than 5 cm; 2 – necrotic spot with diameter more than 5 cm; 3 – several necrotic fused spots on the stem; 4 – broken stem at the place of infection.

Type of infection of the brown spots: 0 – absence of symptoms; 1 – necrotic spot localized near the leaf petiole; 2 – several fused necrotic spots on the stem; 3 – whole stem covered with necrotic spots or broken.

Type of infection of the black spots: 0 – absence of symptoms; 1 – necrotic spot localized near the leaf petiole; 2 – several fused necrotic spots on the stem; 3 – whole stem covered with necrotic spots or broken.

Degree of attacks – what part of the stem was covered with spots of the pathogen (1/3, 2/3, 3/3) and in parentheses – number of spots.

Breeding characters. The follow breeding characters were reviewed: vegetation period, days to flowering, 1000 seeds weight, seed oil content on the method of Rushkovskii (1957).

RESULTS AND DISCUSSION

Results for productivity and seed oil content for the accessions of *H. argophyllus* were presented in Table 1. Silvery white plants height varied from 120 to 185 cm. Stems were erect, usually tomentose. Leaves were mostly alternate; petioles 2 – 10 cm; blades ovate to lance-ovate, 15–25 × 10 – 20 cm, bases truncate to subcordate, abaxial faces were usually floccose, sericeous, or tomentose; peduncles 2 – 8 cm Involucres hemispheric, 20 – 30 mm diam. Ray florets were about 15 – 20. Disc florets were about 150; corollas were 6.5 – 7.5 mm and anthers were dark. Weight of 1000 seeds, oil content and the duration of vegetation period till the flowering were established. Accession GT-E-130 was distinguished with the highest 1000 seeds weight. For the rest accessions this character varied from 8.4 to 8.9 g. The lowest value on this index was reviewed for accession GT-E-006. These results correlated closely to the next character – seed oil content. The highest seed oil content was reviewed for accession GT-E-091, and the lowest – for GT-E-006. For the rest accessions this character varied from 29.1 to 31.6%. A certain variation was established in the number of days to flowering for the studied accessions. The earliest among them was accession GT-E-131. Twenty days after it the flowering of accession GT-E-091 began. For the rest accessions days to flowering varied from 80 to 92.

The carefully selected wild sunflower accessions were successfully crossed with 5 cultivated lines-HC 109 A, AK 19 A, AK 42 A, AK 126 A, 383 A. As a result of this, a certain number of fertile hybrid combinations were obtained (Table 2).

In a tabular mode are presented the biometric characteristics of studied hybrid crosses – 1000 seed weight, seed oil content and duration of the vegetation period. It was established that the cross with the lowest seed oil content was HC 109A × GT-E-006. For the other crosses the percentage of oil content varied in many low degrees – 39.1 to 43.4%. The lowest weight of 1000 seeds was again the cross HC 109A × GT-E-006, and the highest – 383 A × GT-E-132. For the other crosses the variation was from 57.8 to 71.5 g. The duration of the vegetation period was included in this study as a factor for fungal diseases progress. Our observations showed that the vegetation period for the hybrid crosses varied from 130 to 168 days. There are extant studies for the appearance and incidence of the disease pho-

Table 1. Seed oil content, 1000 seed weight and days to flowering for *H. argophyllus* accessions

№	Accession	1000 seed weight, g	Seed oil content, %	Days to flowering
1	GT-E-006	8.1	28.7	92
2	GT-E-007	8.6	29.1	90
3	GT-E-008	8.4	30.5	87
4	GT-E-091	9.1	31.9	95
5	GT-E-130	8.8	31.6	89
6	GT-E-131	8.9	32.2	75
7	GT-E-132	8.4	31.8	80

Table 2. Seed oil content, 1000 seed weight and vegetation period of interspecific hybrids in F2 generation

№	Interspecific hybrid	Number of infected hybrid forms	Seed oil content, %	1000 seed weight, g	Vegetation period, days
1	HC 109A × GT-E-006	3	38.7	55.4	150
2	AK 126A × GT-E-007	2	39.1	57.8	155
3	AK 19 A × GT-E-008	6	40.5	61.1	155
4	AK 42 A × GT-E-091	8	41.9	71.5	162
5	AK 126A × GT-E-130	5	41.6	61.3	168
6	383 A × GT-E-130	3	40.9	62.2	160
7	HC 109A × GT-E-131	3	42.2	64.9	160
8	AK 126A × GT-E-131	3	41.8	58.5	160
9	HC 109A × GT-E-132	6	42.2	63.3	145
10	AK 126A × GT-E-132	2	42.8	59.2	140
11	383 A × GT-E-132	3	43.4	72.2	130

Table 3. Response of investigated hybrid combinations to the pathogens *Phoma macdonaldi, Alternaria* sp. and *Phomopsis helianthi* during the period of testing

№	Interspecific hybrid forms	*Phomopsis*			*Alternaria*			*Phoma*		
		type	attacking rate	category*	type	attacking rate	category*	type	attacking rate	category*
1	**HC 109A x GT-E-006**	0	0	I	1	1/3(2)	R	0	0	I
2	HC 109A x GT-E-006	1	1/3(1)	R	1	1/3(1)	R	1	1/3(1-2)	R
3	HC 109A x GT-E-006	0	0	I	1	1/3(3)	R	1	1/3(1-2)	R
4	**AK 126A x GT-E-007**	2	2/3 (2)	MR	1	1/3(1)	R	1	1/3(1)	R
5	AK 126A x GT-E-007	2	2/3 (2)	MR	1	1/3(1)	R	1	1/3(1)	R
6	**AK 19 A x GT-E-008**	1	1/3(1)	R	1	1/3(1)	R	1	1/3(1)	R
7	AK 19 A x GT-E-008	0	0	I	1	1/3(2)	R	1	1/3(1-3)	R
8	AK 19 A x GT-E-008	0	0	I	1	1/3(1)	R	1	1/3(1-2)	R
9	AK 19 A x GT-E-008	0	0	I	0	0	I	1	1/3(1-2)	R
10	AK 19 A x GT-E-008	0	0	I	1	1/3(3)	R	1	1/3(1)	R
11	AK 19 A x GT-E-008	0	0	I	1	1/3(3)	R	1	1/3(1)	R

To be continued

12	**AK 42 A x GT-E-091**	1	1/3(1)	R	1	1/3(1)	R	2	2/3 (2)	MR
13	AK 42 A x GT-E-091	3	2/3(2-3)	MS	1	1/3(2)	R	0	0	I
14	AK 42 A x GT-E-091	0	0	I	0	0	I	0	0	I
15	AK 42 A x GT-E-091	1	1/3(1)	R	0	0	I	0	0	I
16	AK 42 A x GT-E-091	1	1/3(1)	R	0	0	I	0	0	I
17	AK 42 A x GT-E-091	1	1/3(1)	R	0	0	I	0	0	I
18	AK 42 A x GT-E-091	2	2/3 2)	MR	2	2/3 (2-3)	MR	2	2/3(2-3)	MR
19	AK 42 A x GT-E-091	2	2/3 (2)	MR	1	1/3(2)	R	2	2/3 (2)	MR
20	**AK 126A x GT-E-130**	1	1/3(1)	R	2	2/3(2-3)	MR	2	2/3 (2)	MR
21	AK 126A x GT-E-130	3	2/3(2-3)	MS	2	2/3 (2-4)	MR	2	2/3 (2-3)	MR
22	AK 126A x GT-E-130	2	2/3 (2)	MR	2	2/3 (2-3)	MR	2	2/3 (2-3)	MR
23	AK 126A x GT-E-130	4	3/3 (4)	S	2	2/3 (2-3)	MR	2	2/3 (2-3)	MR
24	AK 126A x GT-E-130	0	0	I	0	0	I	0	0	I
25	**383 A x GT-E-130**	0	0	I	0	0	I	1	1/3(1)	R
26	383 A x GT-E-130	0	0	I	0	0	I	0	0	I
27	383 A x GT-E-130	1	1/3(1)	R	0	0	I	0	0	I
28	**HC 109A x GT-E-131**	2	2/3(2)0I	MR	0	0	I	0	0	I
29	HC 109A x GT-E-131	0	0	I	0	0	I	0	0	I
30	HC 109A x GT-E-131	1	1/3(1)	R	0	0	I	0	0	I
31	**AK 126A x GT-E-131**	0	0	I	0	0	I	1	1/3(1)	R
32	AK 126A x GT-E-131	0	0	I	0	0	I	0	0	I
33	AK 126A x GT-E-131	1	1/3(1)	R	0	0	I	0	0	I
34	**HC 109A x GT-E-132**	0	0	I	0	0	I	1	1/3(1)	R
35	HC 109A x GT-E-132	0	0	I	0	0	I	1	1/3(1-2)	R
36	HC 109A x GT-E-132	1	1/3(1)	R	0	0	I	1	1/3(1)	R
37	HC 109A x GT-E-132	1	1/3(1)	R	1	1/3(1)	R	1	1/3(1)	R
38	HC 109A x GT-E-132	1	1/3(1)	R	1	1/3(1)	R	1	1/3(1)	R
39	HC 109A x GT-E-132	0	0	I	0	0	I	0	0	I
40	**AK 126A x GT-E-132**	1	1/3(1)	R	1	1/3(2)	R	0	0	I
41	AK 126A x GT-E-132	1	1/3(1)	R	1	1/3(1)	R	1	1/3(1)	R
42	**383 A x GT-E-132**	1	1/3(1)	R	2	2/3 (2-3)	MR	2	2/3 (2)	MR
43	383 A x GT-E-132	2	2/3 (2)	MR	2	2/3 (2)	MR	2	2/3 (2)	MR
44	383 A x GT-E-132	1	1/3(1)	R	2	2/3 (2)	MR	2	2/3 (2-3)	MR

* I – immune; R – resistant; MR – moderately resistant; MS – moderately susceptible.

mopsis (Vranceanu et al., 1992; Sackston, 1992; Scoric, 1985), where exist the conclusions that some of the hybrids possess so called “green resistance”, which is connected to the vegetation period of the plants. It is interpreted with passing the cycle of growth of the pathogen with that of the plant, i.e. at the time of throwing out of pathogens spores, the plant is not at its susceptible phase for infection. For the grey and black spots on sunflower, the susceptible phase is 6-8 pair of leaves. Probably in this case the hybrid crosses are not so late or so early for these growth cycles to be passed – this the hybrid plant and that of the pathogen. This gives us the reason to accept that in this case it does not concern to “green” resistance, but to presence of genes determined such resistance. In table 3 are presented the results of reaction of selected during the years hybrid crosses to the attacks of grey, brown and black spots on sunflower. F2 generations were tested for resistance to phomopsis, alternaria and phoma (Table 2). Transfer of Rf-genes was proved and the obtained resistant hybrid plants will be included in the future breeding programs for developing resistant R lines.

The obtained results showed that some of the hybrid crosses possessed genes for resistance to the studied three pathogens. Six of them – AK 42 A × GT-E-091, AK 126A × GT-E-130, 383 A × GT-E-130, HC 109A × GT-E-131, AK 126A × GT-E-131 and HC 109A × GT-E-132 were immune to the attacks of *Phomopsis*, *Alternaria* and *Phoma*. This fact made them especially interested for the breeding process. The rest thirty-three crosses were immune or resistant to one, two or three pathogens. They could be successfully used as donors for resistance to the studied three fungal diseases.

It’s obvious from the presented results that including of wild sunflower species *H. argophyllus* in interspecific hybridization with cultivated sunflower is of great importance for transfer of genes for resistance to the fungal diseases grey spots (*Phomopsis helianth* Munt.-Cvet. et al./*Diaporthe helianthi* Munt.-Cvet. Et al.), brown (*Alternaria sp.*) and black spots (*Phoma macdonaldi*/*Leptosphaeria lindquistii*). The investigations indicate that this wild species could be used as donor for resistance and this will lead to increasing the seed yield and its quality. Our results are confirmed by the investigations of Morris et al. (1933) and Lipps and Herr (1986).

The obtained results showed that in the DAI collection of wild species, there are *H. argophyllus* accessions which could be successfully included in the breeding programs for developing new sunflower hybrids with durable resistance. The studied hybrid forms are characterized with comparatively high seed oil content and high 1000 seeds weight which allows together with the resistance to the studied pathogens to be selected lines with valuable and economically important characters.

CONCLUSIONS

As sources for resistance to grey, brown and black spots on sunflower, 19 accessions – hybrid combinations were selected with immune to high level of resistance. These hybrid crosses are characterized with high combining ability, high seed oil content and 1000 seeds weight.

The selected accessions could be successfully included in the breeding program of DAI for developing lines and hybrids with valuable economically important characters and resistance to the important for the country leaves diseases.

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