Sources for resistance to the leaves pathogenes caused grey (*Phomopsis helianthi*), brown (*Alternaria* sp.) and black (*Phoma macdonaldi*) spots on sunflower originated from the wild species *Helianthus annuus* L.

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ABSTRACT

- The aim of this study is to be characterized the hybrid combinations with wild *Heliathus annuus* presented resistance to the agents caused grey (*Phomopsis helianth* Munt.-Cvet. et al/*Diaporthe helianthi* Munt.-Cvet. Et al.), brown *(Alternaria sp.)* and black spots (*Phoma macdonaldi*/*Leptosphaeria lindquistii*).
- The investigation was carried out in Dobrudzha Agricultural Institute near General Toshevo during the period 2008-2010 using artificial inoculation of plants. Phytopathological investigations of wild *Helianthus annuus* L and its hybrid progenies obtained from crossing with cultivated sunflower were carried out. Cultivated sunflower was represented by five male sterile lines, developed in DAI, General Toshevo and the wild species – by 52 accessions from the collection of wild species in DAI. The obtained fertile hybrid plants were tested. Presence of resistance to grey (*Phomopsis helianthi*), brown (*Alternaria* sp.) and black (*Phoma macdonaldi*) spots on sunflower was established.
- As a result of self-pollination and purposeful selection some new hybrid forms were obtained. They combined high resistance to the studied pathogens and valuable breeding characters. Presence of Rf genes was established and on this base the new hybrid forms could be included in sunflower breeding for developing new R lines to increase the genetic variability of the cultivated sunflower.

Key words: black spots (*Phoma macdonaldi*) - brown spots (*Alternaria* sp.) - grey spots (*Phomopsis helianthi*) - resistance - wild *Helianthus annuus* L.

INTRODUCTION

Breeding on resistance was always been a priority in solving the problems connected to the sunflower pathogens. Its role will increase in the future more seriously as using of good plant protected methods in growing of agricultural crops got expanded. Applying of resistant sunflower cultivars and hybrids was the most effective mean for overcoming the fungal diseases, such as grey (*Phomopsis helianthi*), brown (*Alternaria sp*.) and black (*Phoma macdonaldi*) spots on sunflower (Николова и др., 2001; Roustaee et al., 2000; Hahn and Degener, 1999; Vear and de Labrouhe, 1997; Scoric, 1992). Breeding on resistance solved the problems for years ahead and spared the nature in maximum decreasing the use of chemical agents to their minimum (Poehlman and Sleper, 1995). Creating of new hybrids with high productive potential and high resistance to the economically important diseases on sunflower was carried out by many researchers (Boerema et al., 2004; Treitz, 2003), Morris,J.B. et al 1983. Studies showed that wild sunflower *Helianthus annuus* could be used as donor for resistance which conducted to increasing seed yield and its quality (Lipps and Herr, 1986). As sources for resistance very often were used wild sunflower species. Wild relatives of crop plants were genetically much more diverse than related cultivated crops. Genetic diversity contributed to long-term survival of species by allowing them to adapt quickly to changes in their environment. The wild sunflower species are adapted to a wide range of habitats, characterized with high genetic diversity and possess considerable variability for most agronomic and achene quality characters, and for their reaction to insects and disease pathogens. Wild *Helianthus* species have been important sources of genes for disease resistance, cytoplasmic male-sterility (CMS), drought tolerance, and etc. Genetic variability of cultivated sunflower and its biotic resistance could be increased by interspecific hybridization with wild *Helianthus annuus*, its closest relative. 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 *et al*., 2007).

The aim of this study is to be characterized the obtained hybrid combinations with wild *Heliathus annuus* accessions, which presented resistance to the agents, caused grey (*Phomopsis helianth* Munt.-Cvet. et al/*Diaporthe helianthi* Munt.-Cvet. Et al.), brown *(Alternaria sp.)* and black spots (*Phoma macdonaldi*/*Leptosphaeria lindquistii*), with view their further including in the breeding process.

MATERIAL AND METHODS

The investigations were carried out in Dobrudzha Agricultural Institute near General Toshevo during the period 2008-2010 on artificial infection plot. This special plot was maintained annually as there were supplemented infected plant stems with symptoms of the pathogens. Stems were collected previous year and were left in an open ear during the whole winter. After sunflower germination they were spread out in chess-board order between the rows.

Plant material: Hybrid combinations with wild *Helianthus annuus* - 292 accessions were studied and 52 of them were selected for more detail investigation.

Infection plot and evaluation of accessions reaction: Sunflower accessions were sown in artificial infection plots. Plants were inoculated at phase of button formation on the method of Encheva and Kiryakov (2002). The inoculum was prepared in laboratory conditions immediately before the inoculation. The isolate used was from the region of DAI. The reaction to pathogens was twice evaluated – 10 days after inoculation and at the end of flowering phase on five levels scale (Encheva and Kiryakov, 2002).) The category was determined on the base of type and degree of infection (Van Schoonhoven and Pastor-Corales, 1987). |

Breeding characters: vegetation period and seed oil content using nuclear magnetic resonance.

RESULTS

The accessions of *Helianthus annuus* from the collection of DAI were previously tested for their resistance to the three pathogens (Encheva, 2006). The selected for this study accessions were from the group of immune and resistant to the three pathogens. Their hybrid combinations were also tested and they were the subject of this experiment.

The reaction of hybrid combinations originated from crossing cultivated sunflower x wild species was in a wide range – from immune to highly susceptible to the three diseases. This pointed us to categorize the type of infection as well the reaction to the different pathogens. Four groups of hybrid combinations were distinguished – the first included materials resistant and immune to the three pathogens, the second – resistant and immune to grey and black spots, the third – resistant and immune to black and brown spots and the fourth– resistant and immune to grey and brown spots. In table 1 are presented the materials, which showed after inoculation the resistant and immune type of reaction to the three pathogens. This group of materials is of great importance because it gives the opportunity to select the hybrids with complex resistance to several economically important sunflower diseases. In the group of materials which combined resistance to the three pathogens were selected 26 hybrid combinations. Immune materials were without any symptoms of the diseases and for the resistant ones the fungal infection was stopped at the petiole base or close to the stem. The materials in this group were valuable also because the seed oil content varied from 42.10 for the cross 44 A x GT-E-035 to 47.80 for the cross 217 A x GT-E-128. This oil content is comparatively high for such interspecific crosses and is of interest for the breeding of high seed oil content hybrids. The variation in vegetation period of studied accessions was not significant and varied from 120 to 125 days. Longer vegetation period was reported for the crosses with maternal line 45 A.

Table 1. Hybrid forms F2 generation, tested for resistance to *Phomopsis, Phoma and Alternaria,* (Immune and Resistant).

№	Hybrid combination	Category	Seed oil content,%	Vegetation period, days
1	3 A x GT-E-035	I – R	46.10	122
2	3 A x GT-E-121	I – R	43.75	122
3	3 A x GT-E-122	I – R	43.55	122
4	3 A x GT-E-128	I – R	45.50	122
5	3 A x GT-E-174	I – R	45.75	122
6	10 A x GT-E-035	I – R	46.55	120
7	10 A x GT-E-092	I – R	46.20	120
8	10 A x GT-E-122	I – R	43.75	122
9	10 A x GT-E-128	I – R	45.50	122
10	10 A x GT-E-171	I – R	45.75	122
11	10 A x GT-E-174	I – R	46.55	122
12	44 A x GT-E-035	I – R	42.10	123
13	44 A x GT-E-078	I – R	42.50	123
14	44 A x GT-E-088	I – R	43.00	123
15	44 A x GT-E-092	I – R	46.10	123
16	45 A x GT-E-128	I – R	45.50	125
17	45 A x GT-E-171	I – R	45.75	125
18	45 A x GT-E-174	I – R	45.55	125
19	217 A x GT-E-078	I – R	46.40	120
20	217 A x GT-E-088	I – R	46.40	120
21	217 A x GT-E-092	I – R	47.40	122
22	217 A x GT-E-092	I – R		
23	217 A x GT-E-128	I – R	47.80	120
24	217 A x GT-E-171	I – R	46.55	122
25	217 A x GT-E-171	I – R		
26	217 A x GT-E-174	I – R	46.40	122

In table 2 was presented the second group of hybrid combinations, which were resistant and immune to two pathogens – black and grey spots. The seed oil content of materials from this group was satisfied (41.20 – 46.55 %) and gave the possibility for including of these crosses in future breeding research. The vegetation period was a bit longer comparing to the first group of materials (122 – 127 days). The most valuable was their resistance and the real opportunity for their use in the improving work on sunflower.

Table 2. Hybrid forms F2 generation, tested for resistance to *Phomopsis and Phoma,* (Immune and Resistant)

№	Hybrid combination	Category	Seed oil content	Vegetation period

1	3 A x GT-E-035	I – R	42.50	124
2	3 A x GT-E-042	I – R	42.30	125
3	3 A x GT-E-058	I – R	43.20	124
4	3 A x GT-E-058	I – R		
5	3 A x GT-E-075	I – R	43.50	124
6	3 A x GT-E-078	I – R	43.75	123
7	3 A x GT-E-088	I – R	43.55	123
8	3 A x GT-E-088	I – R		
9	3 A x GT-E-088	I – R		
10	45 A x GT-E-155	I – R	41.20	127
11	45 A x GT-E-172	I – R	44.10	127
12	45 A x GT-E-172	I – R		
13	45 A x GT-E-172	I – R		
14	45 A x GT-E-173	I – R	44.55	127
15	217 A x GT-E-003	I – R	45.50	124
16	217 A x GT-E-049	I – R	45.75	124
17	217 A x GT-E-064	I – R	44.55	122
18	217 A x GT-E-184	I – R	46.55	122

The largest groups of immune and resistant materials to the fungal diseases black and brown spots were presented in table 3. Almost probably it was due to their genome but in this case our investigation was not directed to study the genetic base of the resistance but to characterize the interspecific hybrid combinations for developing sunflower hybrids with complex resistance. In the future investigations we will pay attention to the genetic base of resistance of this group of materials. The obtained hybrid plants were interested also with their comparatively high seed oil content, reached to 47.15 %. The vegetation period of hybrids in this group varied from 120 to 129 days.

Table 3. Hybrid forms F2 generation, tested for resistance to *Phoma and Alternaria*, (Immune and Resistant)

№	Hybrid combination	Category	Seed oil content	Vegetation period
1	3 A x GT-E-045	I – R	42.50	122
2	3 A x GT-E-092	I – R	42.30	122
3	3 A x GT-E-092	I – R		
4	3 A x GT-E-123	I – R	44.10	122
5	3 A x GT-E-123	I – R		
6	3 A x GT-E-127	I – R	43.75	122
7	3 A x GT-E-127	I – R		
8	3 A x GT-E-127	I – R		
9	3 A x GT-E-129	I – R	43.30	124
10	3 A x GT-E-129	I – R		
11	3 A x GT-E-171	I – R	44.10	124
12	3 A x GT-E-171	I – R		
13	3 A x GT-E-172	I – R	43.30	124
14	3 A x GT-E-172	I – R		
15	3 A x GT-E-173	I – R	42.50	124
16	3 A x GT-E-173	I – R		
17	3 A x GT-E-177	I – R	44.20	124
18	3 A x GT-E-177	I – R		
19	3 A x GT-E-179	I – R	45.50	124
20	3 A x GT-E-179	I – R		
21	3 A x GT-E-180	I – R	43.55	124
22	3 A x GT-E-180	I – R		
23	3 A x GT-E-184	I – R	42.30	124
24	3 A x GT-E-184	I – R		
25	10 A x GT-E-042	I – R	44.10	120
26	10 A x GT-E-042	I – R		
27	10 A x GT-E-045	I – R	46.75	120
28	10 A x GT-E-045	I – R		

29	10 A x GT-E-058	I – R	41.50	120
30	10 A x GT-E-058	I – R		
31	10 A x GT-E-058	I – R		
32	10 A x GT-E-079	I – R	44.10	120
33	10 A x GT-E-088	I – R	42.50	120
34	10 A x GT-E-088	I – R		
35	10 A x GT-E-088	I – R		
36	10 A x GT-E-125	I – R	43.75	122
37	10 A x GT-E-127	I – R	43.55	122
38	10 A x GT-E-127	I – R		
39	44 A x GT-E-059	I – R	43.75	127
40	44 A x GT-E-079	I – R	44.55	127
41	44 A x GT-E-079	I – R		
42	45 A x GT-E-153	I – R	43.25	129
43	45 A x GT-E-155	I – R	43.10	129
44	45 A x GT-E-155	I – R		
45	45 A x GT-E-177	I – R	45.75	129
46	217 A x GT-E-004	I – R	43.55	122
47	217 A x GT-E-042	I – R	44.30	124
48	217 A x GT-E-056	I – R	46.75	122
49	217 A x GT-E-056	I – R		
50	217 A x GT-E-062	I – R	46.55	122
51	217 A x GT-E-178	I – R	47.15	122

The smallest is a group of crosses, immune and resistant to both diseases grey and brown spots. Seed oil content of this group of hybrid forms varied from 42.30 to 44.30 %. Their vegetation period varied from 124 to 127 days.

Table 4. Hybrid forms F2 generation, tested for resistance to *Phomopsis and Alternaria*, (Immune and Resistant)

№	Hybrid combination	Category	Seed oil content	Vegetation period
1	3 A x GT-E-035	I – R	42.50	124
2	3 A x GT-E-035	I – R	42.70	124
3	3 A x GT-E-042	I – R	42.30	125
4	44 A x GT-E-088	I – R	43.0	127
5	217 A x GT-E-042	I – R	44.30	124

DISCUSSION

The presented results showed that including of wild *Helianthus annuus* accessions from the collection of DAI in hybridization with cultivated sunflower lines is of great importance for implementation of genes transfer for resistance to the fungal diseases grey (*Phomopsis helianth* Munt.-Cvet. et al/*Diaporthe helianthi* Munt.-Cvet. et al.), brown (*Alternaria sp*.) and black spots (*Phoma macdonaldi/Leptosphaeria lindquistii*).

In the wild sunflower collection of DAI there were accessions suitable to be included in the breeding program for developing new sunflower hybrids with durable resistance. The obtained hybrid combinations were characterized with comparatively high seed oil content and this could be the base to develop breeding materials with valuable agronomical characters. The transfer of Rf genes was also proved.

As sources for resistance to grey, black and brown spots were selected 4 groups of hybrid crosses with immune to high level of resistance. These crosses were distinguished with their high combining ability, high seed oil content and suitable for the region of Dobrudzha vegetation period. The self-pollinated and selected plants from these crosses could be used successfully in the breeding program of DAI for developing new sunflower lines and hybrids with complex resistance to the economically important for our country diseases.

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