

Wild annual *Helianthus* species as sources for resistance to some foliar pathogens on sunflower

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Abstract

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Phytopathological investigations of hybrid progenies, originated from wild annual *Helianthus annuus* L. and *Helianthus argophyllus* Torr. & Gray, were carried out. Cultivated sunflower was represented by five male sterile lines, developed in DAI, General Toshevo. Presence of resistance to grey (*Phomopsis helianthi*), brown (*Alternaria* sp.) and black (*Phoma macdonaldi*) spots on sunflower was established for hybrid plants in F₂ generation. The hybrid material was grouped according to the type of infection and its reaction to the different pathogens. Four groups of hybrid crosses were selected – the first group included materials, resistant to all three pathogens, the second group included materials, resistant to pathogens caused grey and black spots, the third – resistant to pathogens caused black and brown spots, and the fourth – resistant to pathogens caused grey and brown spots on sunflower. The seed oil content and the vegetation period were determined for all studied hybrid forms. The obtained resistant hybrid forms with proved transfer of Rf genes and increased seed oil content will be an important initial material in sunflower breeding program in DAI for developing new R lines.

Keywords: Phomopsis; Phoma; Alternaria; resistance; Helianthus annuus; Helianthus argophyllus

Introduction

Sunflower is a major oilseed crop for the consumers in many countries and can be successfully grown on marginal soils and in semiarid conditions. Sunflower is more resistant to abiotic stresses, than other field crops. Unfortunately, it is very sensitive to biotic stresses (Skoric, 2016). Diseases are one of the important limiting factors of sunflower production worldwide. The sunflower plant is a known host of over three dozen infectious microorganisms, mostly fungi, which may, under certain climatic conditions, significantly reduce yield and quality (Gulya et al., 1997).

Reducing the harmfulness of disease attacks on a plot in a given year is the main objective of crop protection. Reducing injuries (sporulating lesions), even in the absence of detrimental effects on annual crop production, is a way to

reduce the production of primary inoculum and thus future epidemics. Therefore, the reasoning of disease control methods must consider both temporal and spatial dimensions as soon as the fungus is able to spread widely. During the two previous decades, genetic control made a lot of progress for controlling sunflower diseases (Vear, 2016). One of the possible control strategies is to avoid or limit the crop injuries, losses of seed yield and quality of product (Debaeke et al., 2017).

Breeding on resistance was always been a priority in solving the problems connected to the sunflower pathogens and its role will increase in the near future more seriously. It has been a constant challenge for the breeders to identify durable sources of resistance in cultivated and wild *Helianthus* species. Applying of resistant sunflower cultivars and hybrids is one of the main methods and it was the most effective mean

for overcoming the foliar diseases, such as grey (*Phomopsis helianthi*), brown (*Alternaria* sp.) and black (*Phoma macdonaldi*) spots on sunflower (Skoric, 1992; Vear & de Labrouhe, 1997; Hahn & Degener, 1999; Roustae et al., 2000). Breeding on resistance solved the problems for years ahead and spared the nature in maximum decreasing the use of chemical agents (Poehlman & 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 (Morris et al., 1983; Treitz, 2003; Boerema et al., 2004; Georgiev, 2018; Georgiev & Piskov, 2019; Neno, 2019). Genetic variability of the cultivated sunflower and its biotic resistance could be increased by interspecific hybridization with wild *Helianthus* species. Wild relatives of crop plants are genetically much more diverse than related cultivated lineages. Genetic diversity contributes 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 biochemical characters, as well for their reaction to disease pathogens and insects. Foliar diseases of sunflower are caused by various pathogens. Some of them are *Phomopsis helianthi*, *Phoma macdonaldi*, *Alternaria* sp. These diseases cause various symptoms on foliage which may decrease sunflower yields in seasons conducive to the development of these diseases. Environmental conditions for the development of epidemics of these diseases vary and it is therefore important that correct disease identification is critical in developing appropriate disease management strategies for each.

Disease control generally means genetic resistance of the host plant. The resistance to *Phomopsis*, *Botrytis*, *Alternaria* and *Sclerotinia* is partial, non-race-specific, horizontal and polygenic (Vrânceanu et al., 1983; Škoric, 1985; Hahn & Degener, 1999; Oliveira et al., 2004)

Number of wild *Helianthus* species was reported to have resistance to *P. helianthi*. Škoric (1985) found tolerance in four inbred lines: two based on perennial *H. tuberosus*, and one each based on annual *H. annuus* and *H. argophyllus* and reported that cultivated hybrids developed from *H. tuberosus* and *H. argophyllus* had high field tolerance to *Phomopsis* stem canker (PhSC). Griveau et al. (1992) obtained tolerant interspecific hybrids using annuals *H. argophyllus*, *H. debilis* and *H. praecox*. Field resistance to *Phomopsis* was reported in interspecific hybrids derived from *H. argophyllus*, *H. deserticola*, *H. tuberosus* and *Helianthus x laeiflorus* (Degener et al., 1999). Nikolova & Encheva (1994) observed field resistance to PhSC in progenies of interspecific hybrids derived from annual *Helianthus* species. Christov (2008) and Encheva et al. (2006) identified annual species *H. annuus*, *H. argophyllus*

and *H. debilis* from DAI, General Toshevo collection as potential sources of PhSC resistance in some half-sib families based on field screening in Bulgaria. It has been noted that resistance to drought, *Macrophomina phaseolina* (Tassi) Goidanich and *Ph. macdonaldii* in sunflower has been correlated positively with resistance to *D. helianthi* however, it is unknown whether or not linked genes are the source of resistance to these pathogens (Skoric, 1988).

Skoric (1988) concluded that most cultivated sunflower accessions examined had insufficient levels of resistance to *Alternaria* leaf and stem spot, but he did identify accessions of wild *H. annuus* that had only necrotic flecks or very small lesions in field tests. Several wild annual species *H. praecox*, *H. debilis* ssp. *cucumerifolius* T. & G., and *H. debilis* ssp. *silvestris* Heiser had high levels of resistance to *Alternaria* leaf spot and also to *Septoria* leaf spot (caused by *Septoria helianthi* Ell. & Kell.) in field evaluations (Block, 1992). Reddy et al., (2006) identified resistant genotypes and concluded that genes for resistance to *alternaria* blight are dispersed differently in genotypes and in hybrid combinations, the favorable alleles are accumulated to give resistant disease reaction. According to them, the resistance to *alternaria* blight was polygenically controlled and dominance phenomenon was expressed in hybrids. Venkata et al. (2014) indicated that it was possible to synthesize hybrids with reasonable degree of tolerance, by involving disease tolerant parents. The extent of resistance could be enhanced, when allelic differences existed between parents.

Genetic variability for partial resistance to phoma black stem in sunflower was reported in both field (Peres et al., 1994) and controlled conditions (Bert et al., 2004). Using parental genotypes and their F₁ hybrids, Roustae et al. (2000) showed that the variation for disease severity score among genotypes studied resulted from the additive and dominant effects of genes controlling black stem partial resistance.

The present study was carried out to assess the hybrid combinations with wild *Helianthus annuus* and *Helianthus argophyllus* for their resistance/tolerance to the agents caused grey (*Phomopsis helianthi* Munt.-Cvet. et al. /*Diaporthe helianthi* Munt.-Cvet. et al.), brown (*Alternaria* sp.) and black spots (*Phoma macdonaldi* / *Leptosphaeria lindquistii*), under field conditions with aim for their further including in the breeding program on sunflower.

Material and Methods

The investigations were carried out during the period 2016-2019 in Dobrudzha agricultural institute under artificial infection conditions, which were annually maintained. In the experimental rows were spread infected plant residues (stems)

with typical lesions caused by the three pathogens. The infected stems were collected previous year and were remained outdoor the whole winter. They were scattered and staggered in rows spacing after sunflower plants germination. Field screening can result in a wide distribution of resistance or susceptible reactions (Langar et al., 2002) and discrimination among genotypes (Degener et al., 1999).

Plant material. The plant material includes forms, obtained from interspecific crosses originated from wild *Helianthus annuus* (35 accessions) and *Helianthus argophyllus* (3 accessions), showed field resistance to the pathogens (Encheva et al., 2006) Cultivated sunflower was presented by five CMS lines used as maternal parents in the crosses. Hybrid forms in F₂ generation were included in the study. Presence of Rf genes was previously confirmed. As a result of preliminary breeding evaluation, 85 hybrid combinations were selected and planted in the artificial infection plot for further detailed observations and investigation. The beginning and end of symptoms appearance was checked.

Infection background and determination of accessions reaction. Hybrid materials were planted under artificial infection field. Plants were inoculated at the stage beginning of button formation, applying the method for phomopsis inoculation, developed by Encheva & Kiryakov (2002). The inoculum was prepared under laboratory conditions immediately before the inoculation. The isolates of pathogens were collected in the region of Dobrudzha agricultural institute. The plants reaction was checked twice – 10 days after inoculation and at the beginning of plants flowering on five-degree scale (Encheva & Kiryakov, 2002). For the other two pathogens – phoma and alternaria, the inoculum was placing between rows (infected plant residues) of the test materials. The assessment is carried out in the yellow-brown ripeness phase on the following scale:

- 0 – no symptoms
- 1 – necrotic spot localized around the leaf handle;
- 2 – several merge necrotic spots on the stem;
- 3 – the stem covered with necrotic spots.

The category was determined on the type and degree of attacks (Van Schoonhoven & Pastor-Corales, 1987).

Breeding characters observed. The seed oil content and vegetation period of investigated materials were defined. Seed oil content was determined using nuclear magnetic resonance.

Results and Discussion

The observed reactions of hybrid combinations, originated from wild *Helianthus annuus* and *Helianthus argophyllus*, varied in wide range – from free of disease incidence to fully susceptible to the three diseases. This was the reason to categorize them on the type of infection and their reaction to

the different pathogens. Four groups of hybrid combinations were formed. The first group included materials resistant to the three pathogens, the second – resistant to grey and black spots, the third – resistant to black and brown spots and the fourth – resistant to grey and brown spots. In Table 1 were presented the materials, which after inoculation showed resistant type of reaction to the three pathogens. This group of materials was of great importance because it gave 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 21 hybrid forms. The plants from these hybrid combinations were free of disease incidence or the fungal infection was stopped at the petiole base or next to the stem. The hybrid materials, included in this group, were valuable also because the seed oil content varied from 43.20% to 46.50% for the crosses, originated from wild *Helianthus annuus* L. The percent of oil content was comparatively higher for such interspecific crosses and was of interest for the breeding of high oil content hybrids. The seed oil content of hybrid combinations, originated from wild *Helianthus argophyllus* was less and varied from 39.25% to 40.75%. The variation in vegetation period of studied accessions was not significant. For the crosses, originated from wild *Helianthus annuus*, it was 118-123 days. Only the cross 617 A x GT-E-093 was characterized with shorter vegetation period – 110 days. This distinctive character was very important in breeding respect for obtaining of lines with shorter vegetation period. The hybrid combinations, originated from wild *Helianthus argophyllus* accessions, were characterized with longer vegetation period. It varied from 128 days for crosses, originated from accession GT-E-130 to 132 days – for crosses with accession GT-E-132. A lot of differences among plants were observed in morphological, phenological, biochemical point of view and just a few characters were discussed in this investigation.

In Table 2 was presented the second group of hybrid materials, which were resistant to the pathogens – phoma and phomopsis. The seed oil content of materials from this group was satisfied and gave the possibility these crosses to be used in the future breeding research. Two hybrids, originated from wild *Helianthus annuus* – GT-059 and GT-E-126 were distinguished with higher seed oil content and shorter vegetation. These hybrid combinations were included in the breeding program for obtaining of new restorer lines. The vegetation period was longer for the hybrid, originated from *H. argophyllus* accession GT-E-130.

The largest was the group of resistant materials to the fungal diseases phoma and alternaria. It included 32 hybrid combinations. In Table 3 were presented those with higher

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

№	Hybrid combination	Category	Seed oil content, %	Vegetation period, days
1	631 A x GT-E-088	R	45.10	118
2	631 A x GT-E-111	R	43.25	118
3	631 A x GT-E-115	R	43.55	120
4	631 A x GT-E-119	R	45.10	122
5	631 A x GT-E-130	R	40.75	128
6	617 A x GT-E-093	R	46.50	110
7	617 A x GT-E-109	R	44.20	120
8	617 A x GT-E-117	R	43.75	118
9	617 A x GT-E-130	R	40.50	128
10	617 A x GT-E-131	R	39.75	130
11	617 A x GT-E-132	R	39.25	132
12	699 A x GT-E-113	R	45.10	120
13	699 A x GT-E-120	R	43.50	122
14	699 A x GT-E-125	R	43.00	123
15	699 A x GT-E-130	R	40.10	128
16	700 A x GT-E-127	R	45.50	120
17	700 A x GT-E-129	R	44.75	120
18	700 A x GT-E-130	R	40.15	128
19	705 A x GT-E-114	R	44.40	118
20	705 A x GT-E-126	R	43.20	120
21	705 A x GT-E-131	R	39.40	130

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

№	Hybrid combination	Category	Seed oil content, %	Vegetation period, days
1	631 A x GT-E-042	R	44.10	124
2	631 A x GT-E-045	R	45.30	125
3	631 A x GT-E-053	R	44.70	124
4	631 A x GT-E-056	R	44.10	122
5	631 A x GT-E-058	R	45.60	124
6	631 A x GT-E-060	R	46.75	123
7	631 A x GT-E-062	R	45.05	123
8	631 A x GT-E-077	R	44.50	120
9	631 A x GT-E-081	R	42.85	122
10	700 A x GT-E-088	R	44.20	120
11	700 A x GT-E-093	R	45.10	120
12	700 A x GT-E-111	R	44.75	122
13	700 A x GT-E-124	R	46.80	122
14	700 A x GT-E-126	R	47.55	110
15	705 A x GT-E-059	R	47.50	118
16	705 A x GT-E-063	R	45.75	124
17	705 A x GT-E-078	R	47.55	118
18	705 A x GT-E-130	R	40.55	132

seed oil content. The vegetation period of hybrid combinations in this group varied from 116 to 124 days. The hybrids, originated from *Helianthus argophyllus* accessions, included in this group, were characterized with lower seed oil content and longer vegetation period. Great diversity was observed in

their morphological traits and after selection and self-pollination they could be included in a program for obtaining of new initial material for sunflower breeding.

The smallest was the group of hybrid combinations, resistant to both diseases phomopsis and alternaria. Hybrid

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

№	Hybrid combination	Category	Seed oil content, %	Vegetation period, days
1	631 A x GT-E-109	R	45.50	122
2	631 A x GT-E-110	R	45.30	122
3	631 A x GT-E-118	R	45.10	120
4	631 A x GT-E-123	R	46.75	116
5	631 A x GT-E-125	R	44.25	118
6	617 A x GT-E-115	R	43.75	122
7	617 A x GT-E-116	R	43.25	118
8	699 A x GT-E-110	R	45.10	120
9	699 A x GT-E-118	R	43.30	124
10	699 A x GT-E-119	R	44.25	120
11	699 A x GT-E-123	R	46.50	116
12	700 A x GT-E-116	R	45.50	118
13	700 A x GT-E-117	R	43.30	124
14	700 A x GT-E-120	R	44.20	120
15	705 A x GT-E-109	R	42.50	124
16	705 A x GT-E-110	R	44.55	120
17	705 A x GT-E-118	R	44.20	124
18	705 A x GT-E-123	R	46.10	116
19	705 A x GT-E-124	R	45.50	118

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

№	Hybrid combination	Category	Seed oil content	Vegetation period, days
1	631 A x GT-E-131	R	39.50	128
2	631 A x GT-E-132	R	39.70	128
3	617 A x GT-E-061	R	42.30	120
4	617 A x GT-E-112	R	45.75	120
5	699 A x GT-E-061	R	44.30	120
6	699 A x GT-E-131	R	39.30	128
7	699 A x GT-E-132	R	39.50	128
8	700 A x GT-E-081	R	44.30	118
9	700 A x GT-E-131	R	38.50	128
10	700 A x GT-E-132	R	39.30	128
11	705 A x GT-E-057	R	44.30	110

combinations in this group were not with indicative seed oil content, which varied from 38.50% to 39.70% for materials, originated from wild *Helianthus argophyllus* accessions, to 42.30% – 45.75% for materials, originated from wild *Helianthus annuus*. The variation of vegetation period for the hybrid combinations in this group was 118-127 days. The combination 705 A x GT-E-057 was distinguished with 44.3% seed oil content and vegetation 110 days (Table 4).

Conclusion

Scoring the damage caused by the pathogen in genotypes under artificial infection field can be reliable, but it is not always possible to expose plants to the pathogen evenly and

so achieve uniform infection (Darvishzadeh et al., 2007). Some additional investigations will be carried out in the next generations of hybrid forms, derived from crosses with wild *Helianthus annuus* and *H. argophyllus*. New sunflower genotypes will be selected on the basis of their agricultural characteristics and levels of resistance to these diseases. New restorer lines could be developed by self-pollination and evaluation of their resistance on each stage of breeding.

The presented results showed, that including of wild *Helianthus annuus* and *H. argophyllus* accessions from the collection of DAI in the hybridization with cultivated sunflower is of great importance for the transfer of genes for resistance to the fungal diseases caused grey (*Phomopsis helianth* Munt.-Cvet. et al/*Diaporthe helianthi* Munt.-Cvet. et al.), brown

(*Alternaria* sp.) and black spots (*Phoma macdonaldi/Leptosphaeria lindquistii*) on sunflower. The obtained hybrid combinations were characterized with comparatively high seed oil content and varied vegetation period. This could be the base to develop new initial breeding material with valuable agronomical characters and proved transfer of Rf genes.

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