EVALUATION OF NEW INTERSPECIFIC AND INTERGENERIC SUNFLOWER HYBRIDS FOR RESISTANCE TO *SCLEROTINIA SCLEROTIORUM*

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Abstract

New sunflower forms obtained from interspecific and intergeneric hybridization were included in this study. Their evaluation for resistance to *Sclerotinia sclerotiorum* was determined in field conditions. The inoculation was carried out in the phase of button formation of sunflower. The inoculum was placed on the cut leaf petiole using a drinking straw 30 mm by 6 mm containing an agar disk from a culture of *Sclerotinia sclerotiorum* on PDA medium. Results were reported 3, 7, 11, 14 and 30 days after inoculation on a 6 point scale: 0=lack of symptoms; 5=stem breaking. Differences in plant reaction to the pathogen *Sclerotinia sclerotiorum* were registered. It was established that some forms showed resistance to pathogen influence, confirmed after their evaluation in 2002 and 2003.

Introduction

Sunflower (*Helianthus annuus* L.) is one of the main sources for vegetable oil in the world. It is the main oleaginous crop for many countries and occupies large areas of production. This gives the opportunity for the spread of many diseases, parasites and insects which endanger the sunflower production. One of the main factors limiting normal sunflower production are fungal diseases, caused by pathogens *Plasmopara halstedii* (Farl.) Berl de Toni, *Sclerotinia sclerotiorum* (Lib.) de Bary, *Phomopsis helianthi* Munt.-Cvet. et al., *Sclerotium bataticola* Taub., *Botrytis cinerea* Pers., *Alternaria helianthi* (Hansf.) Tub. and Nish., (Mihailova, 1988). *Sclerotinia sclerotiorum* (Lib.) de Bary is a destructive fungus which causes severe economic damage to many crops (Fick and Miller, 1997). The cultivated sunflower is highly sensitive to *Sclerotinia sclerotiorum* whose attack is manifested in several forms: the infection of the root, infection of the medium part of the stem and its leaves, head rot, and the infection of seeds (Skoric, 1988). Resistance against these diseases have been reported in the wild species of the genus *Helianthus* (Schnabl et al., 2002). Our opinion on this statement corresponds to that of Schnabl et al. (2002). In this study we present the results from the evaluation of new interspecific and intergeneric sunflower hybrids studied using the perspective method for resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary.

Materials and Methods

Plant Materials. Cultivated sunflower, cultivar Peredovik from VNIIMK, Krasnodar, Russia and lines 1607 B, 1234 B, 2607 B, 6000 B, 6075 B, 6116 B, 6523B from DAI,General Toshevo, Bulgaria, RHA 356, RHA 367, RHA 368, RHA 373, RHA 374, RHA 386, RHA 397, RHA 400 from the USDA- ARS, NCSL, Fargo, ND, USA. Interspecific hybrids, created in DAI, General Toshevo, Bulgaria-41 numbers. Intergeneric hybrids, created in DAI, General Toshevo, Bulgaria-54 numbers.

Method and Evaluation of Sunflower Plants for Resistance to the Pathogen Sclerotinia sclerotiorum. The evaluation of plants for resistance to the pathogen *Sclerotinia sclerotiorum* was made in 2002 and 2003. The plants were grown in field conditions. The inoculation was carried out for a day at the same growing habit of the plants.

Inoculum Preparation. The isolate Ss-1 of *Sclerotinia sclerotiorum* was cultivated on PDA for 3 days at a temperature of 20 +/- 1C. The inoculum was prepared in a drinking straw, closed on one side with a size of 30 mm/6 mm (Encheva and Kiryakov, 2002). Using straws, a disk of 1.5% water agar was cut consecutively for each straw and from 3 day old fungal culture in a way that left these disks inside the straws. The inoculum prepared in that way was put in the refrigerator at temperature 4C until it was used.

Plant Inoculation. The inoculation was carried out at button growth phase of the sunflower. The leaf petiole was cut 20-25 cm from the leaf base. The straw with the prepared inoculum was placed on the remainder of the leaf petiole so the fungal mycelium was isolated from the outside and but still in direct contact with the tissue. The results were determined 3, 7, 11, 14, and 30 days after inoculation on a 6 point scale (0 to 5): 0=lack of symptoms at the inoculum site, but still a slight fungus growth was observed; 1=the spot extended to the base of petiole; 2=the spot extended to 1/3 of the stem diameter; 3=the spot extended to 1/2 of the stem diameter; 4=the spot extended to the whole stem without breakage; 5=breaking of stem at the infection site.

The plants were rated resistant when they were scored 0 and 1, while 2 and 3 ratings were medium resistance/tolerance, and a 4 and 5 were susceptible. All plants, for which no fungus growth was observed, were not included in the evaluation. It was considered in these cases that infection was not successful.

Results and Discussion

The basic method for evaluation of breeding materials for resistance to *Sclerotinia sclerotiorum* using the artificial inoculation at different phases of sunflower vegetation was the invasion into the plant tissue by the fungal culture.

In our previous investigations for evaluation of some wild *Helianthus* species and hybrids obtained from interspecific hybridization, the pure fungal culture grown on barley seeds was placed on the plant roots without wounding the plant (Christov, 1990; Christov et al., 1992; Christov, 1996a; Christov, 1996b; Christov et al., 1996). The mixture of seeds and mycelium was covered with soil to preserve the humidity and to limit the sunlight's influence on the mycelium.

In this study the method used allowed the mycelium to invade the plant tissue quickly and easily since the inoculum was placed on the exposed tissue. A considerably smaller

quantity of pure fungus culture was used for plant infection. The time for infection was significantly shorter. The infection could be made on the different leaves of the plant. The infection, its degree and speed could be read quickly, easily, and correctly.

In the study in 2002, 333 plants from 111 accessions (3 plants per accession) were included with the aim of checking the method and the opportunity of finding resistant/tolerant plants.

All 333 plants showed a reaction to the disease. All plants from 92 accessions were dead. Plants from 19 accessions overcame the infection; three plants from one accession overcame the infection, for 9 accessions, 2 plants, and for another 9 accessions, one plant. After inoculation, seeds were obtained from 21 plants. For two accessions with plants that overcame the infection had an origin from two lines of cultivated sunflower RHA 373, and RHA 397. Two accessions originated from interspecific hybrids and 15 accessions originated from 10 intergeneric hybrids.

In the 2003 investigations we included a total of 62 accessions whose reaction to sclerotinia was rated 0, 1, 2, 3, and 4. Two accessions were crosses of line 6075A with lines 1886 and 1737, rated 0 and 1, and 10 accessions with rating of 5 from cultivar Peredovik and lines 1607 A, 1234 A, 2607 A, 6000 A, 6075 A, 6116 A, 6523A, RHA 356, RHA 368 used as standards.

As the greater part of the sunflower forms were branched plants, seeds from different inflorescences of a plant were used as different numbers. The inoculum was placed on 4 plants from each number on one petiole. Some additional plants from the numbers were inoculated later. The inoculum for these plants was placed on 2 to 4 leaves of each plant. Reaction to the disease occurred in 358 plants. All plants from 23 numbers were dead; plants from 51 numbers overcame the infection, as the infection was fully overcome by all plants from 5 numbers (Table 1). The total number of plants which overcame the infection was 137; 73 of which had ratings of 0 and 1. Forty-six plants had a rating of 2, while 18 had rating of 3. Seeds from isolated plants were obtained from 97 numbers. Some results of this investigation were presented in Table 1. The results from 2002 were fully confirmed for Sc38. Higher results were obtained for Sc 43, Sc 44 and Sc 62. Higher results were obtained also for some other numbers. Probably these results were due to the selection done by the infection. On the basis of all results obtained and analyzed in the investigation, we could conclude that by using the interspecific and intergeneric hybridization some new forms with greater resistance/tolerance to the pathogen *Sclerotinia sclerotiorum* could be obtained.

Such forms were obtained from crosses between cultivated sunflower and the wild annual *Helianthus argophyllus* T. and G. (Sc 5, Sc 58, Sc 59, Sc 62). These and all other forms included in this which investigation showed a certain resistance to *Sclerotinia sclerotiorum* possessed Rf genes. Sc 5, Sc 58, Sc 59 and Sc 62 were in CMS RIG-1 and were restorers of fertility for CMS RIG-1 and CMS PET–1. All other forms were mainly in CMS PET–1 and CMS ARG–3. They were obtained after intergeneric hybridization between cultivated sunflower *H. annuus* and some other species of the Compositae family. Some of them were described by Christov and Vassilevska-Ivanova (1999). Sc 12 was an intergeneric hybrid, obtained with the participation of *Grindelia speciosa* and it was in CMS ARG-3. The

Table 1. Some results from the investigation of different sunflower forms for resistance to the pathogen *Sclerotinia sclerotiorum* (2003).

N: 2003	N: 2002	Cultivar, line, hybrid form	Number of infected plants	Number of plants overcoming infection
Sc 1	1	v. Peredovik	8	0
Sc 2	82	L 2607 B	8	0
Sc 3	309	L 6116 B	8	0
Sc 5	1214	Interspecific hybrid	5	1
Sc 9	1651	L RHA 373	11	9
Sc 10	1660	L RHA 397	5	3
Sc 12	1707	Intergeneric hybrid	5	3
Sc 14	1719	Intergeneric hybrid	4	2
Sc 16	1736	Intergeneric hybrid	4	1
Sc 20	1783	Intergeneric hybrid	5	2
Sc 22	1789	Intergeneric hybrid	4	1
Sc 23	1796	Intergeneric hybrid	6	4
Sc 26	1806	Intergeneric hybrid	8	5
Sc 35	1886	Intergeneric hybrid	8	2
Sc 37	1888	Intergeneric hybrid	4	1
Sc 38	1891	Intergeneric hybrid	**4**	**4**
Sc 39	1899	Intergeneric hybrid	4	3
Sc 41	1901	Intergeneric hybrid	4	3
Sc 42	1902	Intergeneric hybrid	4	3
Sc 43	1916	Intergeneric hybrid	**4**	**4**
Sc 44	1916	Intergeneric hybrid	**5**	**5**
Sc 58	2010	Interspecific hybrid	4	3
Sc 59	2011	Interspecific hybrid	4	3
Sc 62	2012	Interspecific hybrid	**8**	**8**
Sc 65		L 6075 A x 1886	**3**	**3**
Sc 34	1886	Intergeneric hybrid	4	3
Sc 66		L 6075 A x 1737	2	0
Sc 17	1737	Intergeneric hybrid	4	2
Sc 72	1796	Interspecific-Intergeneric hybrid	19	11

hybrid forms Sc 14 and Sc 23 were obtained using *Matricaria chamomila* L.*;* Sc 16, Sc 17, Sc 41 and Sc 42 using *Telekia speciosa* (Schreb.) Baumg*;* Sc 20 using *Inula helenium* L*;* Sc 22 using *Tithonia rotundifolia* (Mills.) S. F. Blake*;* Sc 34, Sc 35 and Sc 37 using *Carduus acanthoides* L.*;* and Sc 39 using *Arctium lappa*. Other forms, included in Table 1 were obtained after more complicated hybridization. In Sc 26, *Inula helenium* and *Tithonia rotundifolia* were included: in Sc 38, *Tithonia rotundifolia* and *Arctium lappa* were included;

and Sc 43 and Sc 44 were obtained with the participation of *Tithonia rotundifolia* and *Verbesina encelioides* (Cav.) Benth. and Hooker f. ex Gray. The last number was obtained from the hybridization between *H. hirsutus* Raf. and *H. annuus,* and sterile F2 plants were crossed with *Matricaria chamomila.* Lines RHA 373 and RHA 397 originated from the USDA- ARS, NCSL, Fargo, ND, USA.

For some numbers variation was observed in the evaluation of different plants. We believe this result was due to the fact that the forms studied were not still genetically uniform for all characters. Perhaps there were differences in cell sap, plant tissues or some other reasons. This lack of knowledge necessitates additional investigation for establishment of the mechanism of resistance and its transfer to other sunflower forms. The results obtained for Sc 65 and Sc 66 prove the necessity of such future investigations.

Conclusions

The results obtained and analyzed in this investigation showed that the interspecific and intergeneric hybridization could be used to obtain new forms of resistance to the pathogen *Sclerotinia sclerotiorum*. Such forms were obtained from crosses between cultivated sunflower and *Helianthus* species, as well some species from other genera from Compositae family. A variation in the evaluation for resistance was observed among plants from several numbers.

These results and the lack of sufficient knowledge in this field necessitate additional investigation for establishment of the mechanism of resistance and its transfer to other sunflower forms.

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