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Transferring of *Sclerotinia* Resistance from Wild into Cultivated Sunflower - Evaluation of Wild Sunflower Species

- Original scientific paper -

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Abstract: Accessions of *H. mollis*, *H. maximiliani*, *H. rigidus* and *H. tuberosus* were screened for resistance to *Sclerotinia*, during the two-year period (2001-2002). Artificial inoculation methods were used for that purpose. In the first year, only a stem resistance to *Sclerotinia* infection was observed, while in the second year, heads and stems of tested plants were infected by mycelia and sclerotia of this pathogen. Some differences in the response to mycelium and sclerotia infection within the same species were observed, as well as, differences in the response to sclerotial stem infection in different years. The *H. mollis* accession 1298 was found to be of a special interest for breeding as it was resistant to both head and stem infections with sclerotia.

Key words: Artificial inoculation, *Sclerotinia* resistance, wild sunflower.

Introduction

White rot caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary is the major disease of sunflower (*Helianthus annuus* L.) in countries with a humid climate, while in countries with the moderate climate, it causes the yield loss in rainy years, ***Škorić*** and ***Rajčan***, 1992. This parasite usually attacks all parts of the plant: roots, stems, leaves, flower buttons and heads, ***Zimmer*** and ***Hoes***, 1978. There are no suitable cultural control methods, ***Lumsden***, 1979, and no immune genotypes of cultivated sunflower have been found or developed yet.

Wild sunflower species are important sources of resistance against several major sunflower diseases including *Sclerotinia*, ***Georgieva-Todorova***, 1993. Accessions of several wild sunflower species were found to be tolerant to white rot, ***Škorić*** and ***Rajčan***, 1992, ***Henn et al.***, 1997, ***Tavoljanski et al.***, 2002, ***Cerboncini et al.***, 2002, ***Vasić et al.***, 2002. Resistance screening was done either by observing

infection occurring naturally, ***Tavoljanski et al.***, 2002, or by using different artificial inoculation methods, ***Henn et al***., 1997, ***Cerboncini et al***., 2002, ***Vasić et al.,*** 2002. Some authors also used different laboratory techniques i.e. the culture filtrate and the oxalic acid treatment for this purpose, ***Vasić et al***., 1999, ***Cerboncini et al***., 2002.

The two-year results obtained by screening several wild sunflower accessions of our wild species collection for *Sclerotinia* resistance, are presented in this paper. Accessions were screened by applying different artificial inoculation methods, with the aim to use them for interspecific crosses with cultivated sunflower, either by using conventional or tissue culture methods, i.e. embryo rescue and somatic hybridisation.

Material and Methods

Wild sunflower accessions were grown in the quarantine plot of the Institute of Field and Vegetable Crops, Novi Sad, in the period 2001-2002.

In 2001, five accessions of each *H. mollis*, *H. maximiliani*, *H. rigidus* and *H. tuberosus* (Table 1) were screened for resistance to stem form of *Sclerotinia*. Four plants per accession were artificially inoculated by the incorporation of sclerotia into stems in the phase of budding. Wounds with sclerotia were covered with wet cotton and aluminium foil. The plot was regularly irrigated. Screening was done two weeks after full flowering, using the 1-5 scale. Resistance was determined as a percentage of healthy plants.

In 2002, heads and stems of plants of accessions found to be either tolerant or resistant to *Sclerotinia* (50-100% of resistant plants) (Table 2), were inoculated by using either mycelia or sclerotia. Stems were inoculated as described above. Heads were inoculated at the beginning of flowering, and screened at the end of vegetation, using the 1-6 scale. For each variant, ten plants per accession were screened.

Results and Discussion

In 2001, two accessions of *H. mollis* were found to be 100% resistant to the *Sclerotinia* stem infection (Table 1). In all other tested species some highly tolerant accessions were found, confirming the concept that wild species could be important sources of tolerance, if not of resistance, to *Sclerotinia*, ***Škorić***, 1988. In contrast to the results of ***Škorić*** and ***Rajčan***, 1992, the *H. maximiliani* accession 1631 was only tolerant to stem *Sclerotinia* infection (Table 1). This could be explained by a high variability existing within the accessions of wild sunflower species.

The *H. mollis* accession 1298 was again found to be 100% resistant to both, head and stem *Sclerotinia* infection in 2002 (Table 2). Beside this accession, the accession 285 of the same species expressed a high level of tolerance to these two types of infection. Although ***Degener et al***., 1999, developed interspecific hybrids resistant to *Sclerotinia* by crossing *H. tuberosus* to cultivated sunflower, accessions

*Table 1. Resistance of Tested Accessions to Artificial **Sclerotinia** Infection on Stem in 2001 (Resistance is Given as a Percentage of Healthy Plants)*
*Otpornost ispitivanih populacija na veštačku inokulaciju stabla **Sclerotinia**-om u 2001. godini (otpornost je izražena kao procenat zdravih biljaka)*

Genotype Genotip	Resistance (%) Otpornost (%)	Genotype Genotip	Resistance (%) Otpornost (%)
mol 1530	25	max 2007	75
mol 1692	25	max 2010	75
mol x	100	max 34	75
mol 1298	100	max m	0
mol 285	50	max 1631	50
rig 2012	75	tub 6	0
rig 1696	25	tub 7	25
rig 1692	50	tub 1699	0
rig 1843	25	tub 675	50
rig 1844	0	tub 1704	75

*Table 2. Resistance of Tested Accessions to Artificial **Sclerotinia** Infection on Stem and Head in 2002 (Resistance is Given as a Percentage of Healthy Plants)*
*Otpornost ispitivanih populacija na veštačku inokulaciju stabla i glave sa **Sclerotinia** u 2002. godini (otpornost je izražena kao procenat zdravih biljaka)*

Genotype Genotip	Resistance (% - Otpornost (%)			
	Infection by mycelium 7 Infekcija micelijom		Infection by sclerotia Infekcija sklerocijama	
	stem - stablo	head - glava	stem - stablo	head - glava
rig 2012	60	100	60	90
max 2007	70	30	50	30
max 2010	70	20	60	10
mol x	90	100	70	100
mol 1298	-	-	100	100
max 34	80	60	50	60
tub 675	-	-	50	0
tub 1704	-	-	40	0
max 1631	*	*	0	*
mol 285	-	-	90	80
rig 1692	80	80	70	40

- no evaluation due to lack of a mycelium development - nije izvršena ocena zbog izostanka razvoja micelije
* no inoculation due to late flowering - nije izvršena inokulacija zbog kasnog cvetanja

of this species, tested in our experiment, showed a low level of resistance to this disease, and could be considered of no interest for breeding programmes. The same refers to *H. maximiliani*, whose various accessions were found to be resistant to sclerotinial infection, in previous research carried out at the Institute of Field and Vegetable Crops by ***Škorić*** and ***Rajčan***, 1992, and the research of other authors, ***Cerboncini et al.***, 2002, ***Schnabl et al.***, 2002.

Some differences in response to mycelium and *Sclerotinia* infections within the same species were observed, as well as, differences in the response to *Sclerotinia* stem infection in different years (Tables 1 and 2). The later phenomenon was observed by other authors, as well, and was contributed to weather conditions, especially temperature and humidity, ***Tourvieille de Laboruhe*** and ***Vear,*** 1984, ***Cerboncini et al***., 2002. This could be the case in our study, since temperatures in the second half of September of 2002 were below average values for that time of the year, and observations of mycelium infection could not be done, due to lack of infection (Table 2). However, sclerotia continued to develop even then, and symptoms of infection could be easily observed in susceptible plants. According to ***Aćimović***, 1998, low temperatures slow down the development of *Sclerotinia sclerotiorum* mycelium, and this slower development of mycelia at lower temperatures could be the cause of absence of infection symptoms at the time of evaluation. Sclerotia are organs for conservation of this fungus and could preserve their vitality even at temperatures as low as -15 ºC, ***Aćimović***, 1998, so their development is not affected that much by weather conditions.

Differences in the response of the same genotype to different infection methods were also observed by other authors, ***Tourvieille de Laboruhe*** and ***Vear,*** 1984, ***Robert et al***., 1987. However, ***Cerboncini et al.,*** 2002, found strong correlation between three types of the mycelium test. In comparison with the ascospores test, this test, especially on the head, is considered to differ so much from the natural infection, hence its results less correlated to results observed in naturally occurring infection, ***Tourvieille de Laboruhe*** and ***Vear,*** 1984. In our experiment, the mycelium test was less convenient as, in contrast to the sclerotia test, its success depended on weather conditions.

Conclusion

The obtained results showed that wild sunflower species could be a potential source of genes for *Sclerotinia* resistance. The accession 1298 of *H. mollis* was found to be of a special interest for breeding as it was resistant to both head and stem *Sclerotinia* infections. This species is included in the experiments with protoplast fusion, as well as, interspecific crosses using the embryo rescue technique. Combining these two techniques with conventional breeding methods, along with further screening of wild species and their utilisation in breeding, will hopefully lead us to the development of a cultivated sunflower genotype with at least high tolerance to *Sclerotinia.*

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Prenošenje otpornosti prema *Sclerotinia* iz divljeg u gajeni suncokret - ocena divljih vrsta suncokreta

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R e z i m e

Populacije *H. mollis*, *H. maximiliani*, *H. rigidus* i *H. tuberosus* su testirane na otpornost prema *Sclerotinia*, tokom dvogodišnjeg perioda, upotrebom metoda veštačke inokulacije. U prvoj godini ispitivana je samo otpornost prema infekciji stabla sklerocijama, dok su u drugoj godini glave i stabla testiranih biljaka zaražavane i micelijom i sklerocijama ovog patogena. Tokom ispitivanja su uočene razlike u reakciji na infekciju micelijom i sklerocijama u okviru iste vrste, kao i razlike u reakciji na zaražavanje sklerocijama u različitim godinama. Populacija 1298 *H. mollis* se pokazala interesantnom za oplemenjivanje, pošto je bila otporna i na infekciju glave i na infekciju stabla sklerocijama.

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