TRANSFERRING OF *PLASMOPARA HALSTEDII* RESISTANCE FROM ANNUAL WILD INTO CULTIVATED SUNFLOWER

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Abstract

Twenty-nine populations of five wild annual sunflower species (*H. annuus, H. petiolaris, H. argophyllus, H. praecox, H. debilis* and *H. neglectus*) were screened for resistance to *Plasmopara halstedii* by whole seed immersion method. Crosses of resistant populations were made with cultivated sunflower, on the basis of the results obtained by screening. Analysis of meiosis and pollen viability in parent and F1 populations was used for characterization of F1 interspecific hybrids, as self-fertilization can also occur.

Resistant plants were found in the populations of the species *H. annuus* and *H. argophyllus*. The percent of resistant plants in *H. annuus* populations was 9,09-100% and in *H. argophyllus* 50,00-57,14%. Irregular chromosome pairing in diakinesis was found in 0-20,83% of meiocytes of F1 interspecific hybrids, with quadri- and univalents present. Pollen viability of male fertile interspecific hybrid plants was 10,21-98,85% in *H. annuus* and 39,90-52,47% in *H. argophyllus*. Obtained results suggest that the annual wild sunflower species can be used to obtain resistance, or at least to increase the tolerance of cultivated lines to *Plasmopara halstedii*.

Key words: wild sunflower, *Plasmopara* resistance, interspecific hybridization, meiosis, pollen viability

Introduction

Downy mildew is one of the economically important diseases of cultivated sunflower. A parasitic fungus *Plasmopara halstedii* causes it. At present, this disease can be found in majority of the sunflower growing countries. Its growth depends on the rainfall and it is most often found in the regions with moderate climate (Gulya et all. 1991). The yield loss depends on the type of infection. The primary infection (seed infection) causes significant yield loss while the secondary does not have to influence the yield significantly (Acimovic, 1998; Gulya et all. 1997). Through the selection process cultivated sunflower lost the negative, but also some positive traits of it's wild relative. Wild species of sunflower represent the source of resistance to many pathogens that attack cultivated sunflower, including the parasitic fungus

Plasmopara halstedii (Georgieva-Todorova, 1993). Because of that, it is reasonable to use the wild species as a source of resistance genes in sunflower breeding programs through interspecies crosses. Vranceanu and Stoenescu (1971) concluded that the resistance to *Plasmopara halstedii* is under the control of one dominant gene (Pl). Several years after the introduction of the Pl gene into cultivated sunflower, typical symptoms of *Plasmopara* were again found on the cultivated plants. The search for the new sources of plasmopara resistance was intensified after the results showed that a new race of pathogen was found (Masirevic, 1992; Gulya et all. 1997). Tests showed that the resistance genes are mostly in perennial species while the annual species are mostly not resistant (Pustovojt and Ilatovskij, 1972). Annual species are still being used because they are much easier to cross with cultivated sunflower and it has been shown that they can also carry Pl genes (Miller and Gulya, 1991).

Materials and Methods

Wild species were grown in the collection of the Institute of Field and Vegetable Crops in Novi Sad. Twenty-nine populations of five wild annual sunflower species (*H. annuus, H. petiolaris, H. argophyllus, H. praecox, H. debilis* and *H. neglectus*) were tested for resistance to pathotype 730 of *Plasmopara* (earlier designated as race 4). The infection is most likely to occur between germination and the stage of 3-4 pairs leaves, so that the inoculation was done by dipping the seedlings in a *Plasmopara* zoospore solution (Tourvieille de Labrouhe et al., 2000). Fifty surface sterilized seeds per population were germinated on filter paper. The seedlings were than dipped into a zoospore solution for 4h on 18°C in dark. They were than planted in prepared substrate and grown in air conditioned chamber with 18°C and constant light. The resistance was observed at the phase of first pair of leaves, as the percent of healthy plants. Susceptible line OCMS-44 was used as a positive control and the resistant line JM-8 as a negative. After the evaluation, the resistant plants were transferred into the field and crossed using classical method with a commercial cultivated sunflower line HA26. Secondary inoculation was also performed to check the results of the first test.

Self-fertilization can occur in interspecific crosses. The analysis of meiosis and pollen viability in parent and F_1 populations was used for characterization of F_1 interspecific hybrids. Acetocarmin method was used to check the regularity of chromosome pairing, their number and regularity of diakinesis, metaphase I, anaphase I and telophase II (Georgieva-Todorova, 1976). Pollen viability was determined by differential staining of viable and abortive pollen grains (Alexander, 1969). Lowered pollen viability and irregularities in meiosis were used as an evidence of a successful interspecific cross.

Results and Discussion

Seedlings were not obtained in 6 out of 29 populations because the seeds failed to germinate, so that the test could not be performed. Of the tested populations, only ANN2157 showed 100% resistance but only one plant was screened so that the result should be interpreted with caution. Among other *H. annuus* populations the percent of resistant plants was 9,09-50%. This kind of result can be explained by population variability of the same specie that includes variability in resistance. Except in *H. annuus*, resistance was found only in populations of *H. argophyllus* with 25-57,14% of resistant plants (Tab 1.).

Table 1. The total number of tested plants and the percentage of resistant plants per population of wild *Helianthus*

Population	Number of tested plants	Resistant plants (%)	Population	Number of tested plants	Resistant plants (%)
PRA1342	3	0	PET2011	8	0
PRA1145	7	0	PET2119	17	0
DEB1810	2	0	PET2164	5	0
ANN2138	0	-	PET2167	9	0
ANN2165	4	50.00	PET2122	9	0
ANN2168	0	-	PET2145	4	0
ANN2197	0	-	PET2146	0	-
ANN2159	11	9.09	PET2203	12	0
ANN2157	1	100.00	NEG1181	14	0
ANN2141	13	38.41	ARG1812	20	50.00
ANN2144	0	-	ARG1677	9	0
PET71	9	0	ARG1805	7	57.14
PET74	3	0	ARG1807	4	25.00
PET722	0	-			
PET1910	13	0	OCMS-44	30	3.33
PET2004	11	0	JM-8	30	100.00

Pollen viability of the HA26 line and the wild populations was higher then 95% and the analysis of meiosis showed no irregularities. Branching and the shape of the leaves suggested the hybrid nature of F1 plants. The reduction in pollen viability was not significant in all F1 hybrid combinations. On the other hand, irregularities were found in all phases of meiosis in 0-20,83% of meiocytes (Tab. 2).

Table 2. The characteristics of meiosis in F1 hybrids with cultivated sunflower

Phase	Characteristics	F1 ANN 2165	F1 ANN 2159	F1 ANN 2157	F1 ANN 2141	F1 ARG 1805	F1 ARG 1812	F1 ARG 1812
Diakinesis	Number of bivalents per cell	17.00(48)	16.98 (94)	17.00 (14)	17.00 (72)	16.42 (24)	16.46 (28)	17.00 (48)
	Meiocytes with:							
Metaphase I	Fast chromosomes	7/139	24/270	1/70	18/234	8/63	13/114	7/116
Anaphase I	Lagging chromosomes	2/105	5/215	1/113	3/189	3/46	6/144	0/99
	Chrom. bridges	2	7	0	7	1	10	4
Telophase II	Lagging chromosomes	0/93	4/270	0/88	1/154	5/51	14/79	1/68
Pollen viability (%)		10.21 - 99.52	65.85 - 98.85	95.43 - 99.05	94.31 - 97.29	39,90	6,19	98,95 - 98.53
Number of male sterile plants		1/24	0/22	3/35	1/23	4/12	4/7	3/11

Populations F1ANN2159, F1ARG1805 and F1ARG1812 were found to have meiocytes with less than 17 bivalents accompanied with uni- and quadrivalents.

The explanation of the obtained results can be made by the fact that even though the irregularities in meiosis are the basic cause of sterility, they do not have to influence it directly (Atlagic, 1991). Lowered pollen viability and irregularities in meiosis confirmed the interspecific hybrid nature of F1 plants.

Conclusions

The obtained results indicate that the annual species can be used as a potential source of resistance genes for *Plasmopara*. The classical method of hybridization is sufficient for obtaining the interspecies hybrids with annual species and thus will be used in the new interspecific crosses. Further studies on F1 and back cross relatives are in progress. These results are obtained on DM race 730, but other DM pathotypes are possible to be included.

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