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TRANSFERRING *Plasmopara halstedii* RESISTANCE FROM ANNUAL WILD INTO CULTIVATED SUNFLOWER

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SUMMARY

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 the whole seed immersion method. Resistant populations were then crossed with cultivated sunflower. Analysis of meiosis and pollen viability in parent and F_1 populations was used for characterization of F_1 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 the F_1 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*. The obtained results suggest that 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, an economically important disease of cultivated sunflower, is caused by the parasitic fungus *Plasmopara halstedii*. At present, this disease can be found in majority of the sunflower growing countries. Its proliferation depends on the rainfall and it is most often found in regions with the moderate climate (Gulya *et al.*, 1991). Extent of yield damage depends on the type of infection. Primary infection (seed infection) causes significant yield loss while secondary infec-

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tion may not influence the yield significantly (A_imovi_, 1998; Gulya *et al*., 1997). In the process of selection, cultivated sunflower has lost the negative traits of its wild predecessor, but some positive traits such as disease resistance have been lost too. Wild sunflower species are a source of resistance to many pathogens of the cultivated sunflower, including the parasitic fungus *Plasmopara halstedii* (Georgieva-Todorova, 1993). Because of that, wild species are used as a source of resistance genes in sunflower breeding programs. Vranceanu and Stoenescu (1971) concluded that the resistance to *Plasmopara halstedii* is controlled by a single dominant gene (*Pl*). Several years after the introduction of the *Pl* gene into the cultivated sunflower, typical symptoms of Plasmopara were again found on cultivated plants. Extensive search for new sources of Plasmopara resistance was started after research results showed that a new race of the pathogen had developed (Maširevi_, 1992; Gulya *et al*., 1997). Tests showed that resistance genes can be found mostly in perennial species while the annual species are as a rule not resistant (Pustovojt and Ilatovskij, 1972). Annual species are nevertheless still used in the search for disease resistance because they are much easier to cross with the cultivated sunflower and because 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 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 Plasmopara pathotype 730 (earlier designated as race 4). As the infection is most likely to occur between germination and the stage of 3-4 pairs of leaves, the inoculation was done by dipping sunflower 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 then dipped into a zoospore solution for 4h at 18°C in the dark. They were replanted and grown in an air conditioned chamber at 18°C and in constant light. Resistance was assessed at the stage of the first pair of leaves, and expressed as the percent of healthy plants. The susceptible line OCMS-44 was used as a positive control and the resistant line JM-8 as a negative one. After the evaluation, the resistant plants were transferred to the field and subsequently crossed with HA26, a commercial cultivated sunflower line, using the classical method. Secondary inoculation was also performed to check the results of the first test.

Self-fertilization may occur in interspecific crosses. The analysis of meiosis and pollen viability in parent and F_1 populations was used for characterization of the F_1 interspecific hybrids. The acetocarmine method was used to check the regularity of chromosome pairing, and chromosome number and regularity at 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). Reduced pollen viability and irregularities in meiosis were used as evidence of success in interspecific crossing.

RESULTS AND DISCUSSION

Seedlings were not obtained in 6 out of 29 populations because the seeds failed to germinate, so 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 the other *H. annuus* populations, the percent of resistant plants was 9.09-50%. Except in *H. annuus*, resistance was found only in *H. argophyllus* populations, with 25-57.14% of resistant plants (Table 1).

Table 1: The total number of plants tested 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 line HA26 and the wild populations was higher then 95% and the analysis of meiosis showed no irregularities. Branching and leaf shape suggested the hybrid nature of the F_1 plants. The reduction in pollen viability was not significant in all F_1 hybrid combinations. On the other hand, irregularities were found in all phases of meiosis in 0-20.83% of the meiocytes (Table 2).

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

It seems that the obtained results can be explained by the fact that even though the irregularities in meiosis are the main cause of sterility, they do not have to influ-

ence it directly (Atlagic, 1991). Lowered pollen viability and irregularities in meiosis confirmed the interspecific hybrid nature of F_1 plants.

Table 2: The characteristics of meiosis in the F_1 hybrids with cultivated sunflower

Phase	Characteristic	F_1 ANN2165	F_1 ANN2159	F_1 ANN2157	F_1 ANN2141	F_1 ARG1805	F_1 ARG1812	F_1 ARG1812
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

Conclusions

The obtained results indicate that the annual wild species can be used as a potential source of resistance genes for Plasmopara. The classical method of hybridization is sufficient for obtaining interspecies hybrids with annual wild species and thus it will be used for the development of new interspecific crosses. Further studies on F_1 and back cross relatives are in progress. These results are obtained for downy mildew race 730, but other DM pathotypes will be studied later on.

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TRANSFERENCIA DE LA RESISTENCIA A *Plasmopara halstedii* DEL GIRASOL SILVESTRE ANUAL EN EL GIRASOL CULTIVADO

RESUMEN

Veintinueve poblaciones de cinco especies silvestres anuales de girasol (*H. annuus, H. petiolaris, H. argophyllus, H. praecox, H. debilis* y *H. neglectus*) fueron investigadas en resistencia hacia *Plasmopara halstedii*, por el método de hundir el grano entero. Las poblaciones resistentes son luego cruzadas con el girasol cultivado. El análisis de meiosis de germinación del pólen en progenitores y la población F_1 fue utilizado para la caracterización de las interespecies del híbrido F_1, debido a que puede ocurrir autopolinización.

Las plantas resistentes fueron encontradas en las poblaciones de las especies *H. annuus* y *H. argophyllus*. El porcentaje de las plantas resistentes en las poblaciones de *H. annuus* fue 9,09-100%, y en *H. argophyllus* 50,00-57,14%. Los pares cromosómicos irregulares en diaquinesis fueron encontrados en 0-20,83% de meiocitos del híbrido interespecies F_1, con la presencia de de configuración cuadri- y univalentes. La vitalidad del pólen de las plantas fértiles masculinamente de los híbridos interespecies, fue 10,21-98,85% en *H. annuus*, y 39,90-52,47% en *H. argophyllus*. Los resultados obtenidos indican que las especies silvestres anuales pueden utilizarse para lograr resistencia o por lo menos para incrementar tolerancia de las especies de girasol cultivadas hacia *Plasmopara halstedii*.

TRANSFERT DE RÉSISTANCE AU *Plasmopara halstedii* DU TOURNESOL SAUVAGE ANNUEL AU TOURNESOL DE CULTURE

RÉSUMÉ

La résistance au *Plasmopara halstedii* de vingt-neuf populations de cinq espèces de tournesol sauvage annuel (*H. petiolaris, H. argophyllus, H. praecox, H. debilis* et *H. neglectus)* a été analysée par la méthode d'immersion de la graine complète. Les populations résistantes ont alors été croisées avec du tournesol de culture. L'analyse de la méiose et de la viabilité du pollen dans les populations F1 a été utilisée pour caractériser les hybrides interspécifiques F_1 car l'autofécondation peut aussi intervenir.

Des plantes résistantes ont été trouvées dans les populations de l'espèce *H. annuus* et *H. argophyllus*. Le pourcentage de plantes résistantes dans les populations *H. annuus* était de 9,09-100% et dans les populations *H. argophyllus* de 50,00-57,14%. Des paires irrégulières de chromosomes en diacinèse ont été trouvées dans 0-20,83% des méiocytes de l'hybride

interspécifique F1 avec présence de quadri et univalents. La viabilité du pollen des plantes fertiles mâles de l'hybride interspécifique était de 10,21-98,85% pour *H. annuus* et de 39,90-52% pour *H. argophyllus*. Les résultats obtenus indiquent que les espèces sauvages annuelles peuvent être utilisées pour obtenir la résistance ou au moins pour améliorer la tolérance des espèces de tournesol de culture envers le *Plasmopara halstedii*.