SOURCES OF RESISTANCE TO *DIAPORTHE/PHOMOPSIS HELIANTHI* MUNT.-CVET. ET AL. AND THEIR USE IN SUNFLOWER BREEDING APPLYING THE *IN VITRO* EMBRYO CULTURE

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Summary

Populations of 11 wild sunflower species have been tested for resistance to *Phomopsis/Helianthi* using the method of inoculation with the mycelium of the fungus (three variants of the method).

The highest degree of resistance to *Phomopsis/Helianthi* was exhibited by the *H. tuberosus* populations 1700, 1704, and 1705.

As it is difficult to successfully cross cultivated sunflower to hexaploid *H. tuberosus*, we resorted to the method of *in vitro* embryo culture (Chandler, 1983; C.C. Jan, personal communication).

By isolating 5- and 9-day old embryos, we obtained 18 plants from the cross *H. tuberosus* 1700 x L-1, nine plants from the cross *H. tuberosus* 1705 x L-1, and three plants from the cross *H. tuberosus* 1704 x L-1.

Introduction

Wild sunflowers differ in ploidy level. Some are diploids, like the cultivated sunflower, and some are tetraploids or hexaploids. Direct crossing to transfer

genes for resistance and other biological characteristics from wild species into the cultivated sunflower results in a high rate of embryo abortiveness. This is efficiently overcome by embryo culture.

Applicability of embryo culture has been studied in a number of crops. Regarding sunflower, significant contribution to the use of embryo culture was made by Chandler and Beard (1983). According to Finer (1987), 7- and 14-day old embryos extracted from a cross of 2 sunflower lines showed no growth on 3% sugar combined with any of a number of auxin concentrations used. Embryos did continue to grow on 6% and 12% sugar. Li Yong-Hung et al., (1988) used embryo culture to prevent embryo abortiveness.

The objective of the investigation discussed in this paper was:
- to establish variability for *Phomopsis/Diaporthe helianthi* resistance,
- to find differences in the level of resistance to three mycelial tests (inoculation of the lamina, the petiole, and the stem).
- to identify wild species resistant to *Phomopsis*,
- to make successful crosses between resistant and susceptible sunflower genotypes using embryo culture.

Material and Method

The experiment discussed was carried in 1988/89 and 1990 at the Institute of Field and Vegetable Crops in Novi Sad. In the first part of the experiment, 16 populations from 11 wild species and the cultivated line L-1 were tested. The mycelium of the fungus was used to inoculate the lamina and the petiole of the plants tested. Inoculation of the stem was used with the genotypes which had a specific leaf structure. The inoculum was a fungus isolate taken from the experiment field and cultivated on PDA. Inoculation was done with the 7-day isolate. Inoculations were performed 2 times in each cycle because of the

differences in vegetation period among the plants. Infection intensity was scored on the scale from 0 to 4 (0-immune, 1-resistant, 2-moderately resistant, 3-moderately susceptible, 4-highly susceptible).

The main substrate for embryo culture was E5 (Gamborg et al., 1968). We used a two-phase system with substrates I and II. Substrate I had first been modified by Dvorak (according to Chandler and Beard, 1983), for wheat embryo culture. Modifications for sunflower were made by Chandler and Beard (1983) and by Jan (personal communication). We modified substrate I by reducing the amino acids by a half. Young embryos were extracted with a surgical needle under a magnifying glass. Six to 10 embryos were placed in each Petri dish. Embryos were checked 5, 7, 14 and 21 days after placement and those larger than 3.5 mm were transferred to substrate II. After the development of the first pair of true leaves, when they became intensively green, the plants were transferred to Jiffy-7 bags. These were covered with plastic bags in order to maintain a high relative air humidity of the micro-climate. Nutrient solution was added at 3-day intervals.

Results and Discussion

The mycelial test applied to the petiole was found to be most reliable for wild sunflower species. *H.tuberosus* 1704 was identified as immune, *H.tuberosus* 1700 and *H.nuttallii* 1886 as resistant. *H. resinosus*, *H.tuberosus* 1702 and 17005, *H. laevigatus*, *H. nutt* 1986 and *H. occidentalis* were medium resistant. *H. nutt* 1517, *Hm. maximiliani* and L-1 were medium susceptible, and *H. pumilis* was susceptible. Over 400 achenes were checked. The embryo extracted 5 days after pollination differed in length. Embryo length was measured again 5 and 14 days after placement on the substrate. The Petri dishes showing fungal or bacterial infection were discarded. The former infection was more frequent, the latter less frequent. The young embryo is highly vulnerable. At the moment

of extraction, the husk is still soft and a highly concentrated sterilizing liquid or a long treatment may cause permanent damage of the embryo. The injured embryo changes colore and perishes in 2 days. This is why the extraction should be performed with utmost care.

The first group placed on substrate I comprised 48 embryos shorter than 1 mm on average. After 5 days of culturing, the average embryo length was 0.66 mm, after 14 days 0.89 mm or 34.8% larger. Callus forming occurred in 12.5% of the cases. The 2nd group comprised 48 embryos which were 1.1 to 2 mm long. The average embryo lengths after 5 and 14 days were 1.75 and 2.75 mm, respectively. The increase was 57.14%. The third group comprised 36 embryos which were 2.1 to 3.0 mm long. The average embryo lengths after 5 and 14 days were 2.63 and 5.17 mm, respectively. The increase was 96.8%. Callus forming occurred in 13.7% of the cases. The 4th group comprised 19 embryos which were longer than 3.1 mm. The average length after 5 days was 3.92 mm. After 14 days, most of these embryos were transferred to substrate II. Callus formation occurred in 26.5% of the cases (Table 1).

The rate of growth was largest in groups three and four, the lowest in group one. Of the 156 embryos tested, 63.6% were in groups one and two. The most critical period were the 7 days after the transfer to Jiffy bags. Up to 50% of tested plants may perish during that period.

Conclusion

The mycelial test applied to the petiole was most reliable for testing wild sunflower species. This test was used as the criterion for selecting the genotypes to be crossed. The test was also reliable for selecting individual plants within the populations. *H. tuberosus populations* 1700, 1704 and 1705 were selected for crossing.

Tab. 1 Main characteristics of *in vitro* embryo culture

	5-day old embryos								9-day old embryos
	<1.0		1.1-2.0		2.1-3.0		>3.1		
	5	14	5	14	5	14	5	14	
Average embryo length (mm)	0.66	0.89	1.75	2.75	2.63	5.17	3.92	Transferred	3.92
Increase (%)	34.8		57.14		96.38				
Callus (%)	12.5		35.4		13.7		26.5		9.14
No. of embryos	48		48		36		19		85

The embryos extracted 5 days after pollination differed in length and stage of development (from globular to cordate). Of the 156 embryos tested, 63.6% came from the 1st and 2nd group in which the smallest growth was registered, 34.8 and 57.14%, respectively. The growth was most intensive in the 3rd and 4th group. The highest rate of callus forming was found in the 2nd group. Regarding callus development, the solid substrate was better than the liquid one. The extraction of normal embryos at the torpedo stage and 9 days after pollination indicated that we did not deal with fully incompatible crosses. It is assumed that the embryos would develop normally even without the *in vitro* technique used. The crossings of *H.tuberosus* 1700 x L-1, *H.tuberosus* 1705 x L-1, and *H.tuberosus* 1704 x L-1 produced 18.9 and 3 plants, respectively.

References

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