HELIA, **19**, Nr. 25, p.p. 87-94, (1996) UDC 631.524.86:633.854.78

TRANSFERRING STEM CANKER RESISTANCE FROM *Helianthus tuberosus* L. INTO INBRED LINE OF SUNFLOWER BY EMBRYO RESCUE TECHNIQUE

Dozet, B., Jovanka Atlagić and Dragana Vasić

Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Yugoslavia

Received: March 1,1996.
Accepted: November 18, 1996.

SUMMARY

Several populations of wild sunflower species have been tested for resistance to stem canker by inoculating with the mycelium of the fungus. The highest degree of resistance was exhibited in *Helianthus tuberosus* L. By isolation of 5-days old embryos, we obtained 21 hybrid plants out of more than 200 embryos isolated. All F_1 plants were found resistant. A modified B_5 medium gave good results in production of healthy, enlarged embryos from excised young hybrid embryos. The F_1 hybrids had a lower pollen viability. Meiotic irregularities occurred in all F_1 interspecific hybrids.

Key words: *Helianthus tuberosus*, stem canker, interspecific hybridisation, embryo rescue

INTRODUCTION

Cultivated sunflower has a narrow genetic base and it is deficient in genes for disease resistance. Stem canker caused by *Diaporthe/Phomopsis helianthi* Munt.-Cvet. et al., is one of the most widely distributed diseases of cultivated sunflower. New Yugoslav lines SRB-77, SRB-82, SRB-169 and S-1-184 are field resistant to stem canker (Škorić, 1992). Several wild sunflower species have been identified as sources of resistance to stem canker (Škorić et al., Griveau, 1992). *Helianthus tuberosus* L. is the best source of resistance to stem canker (Škorić et al., 1989). Such high degree of resistance was not found in the cultivated lines.

Wild sunflower species differ in ploidy level and direct crossing in order to transfer genes for resistance from wild species into cultivated sunflower results in a high rate of embryo abortion. This problem can be efficiently overcome by embryo rescue techniques (Chandler and Beard, 1983; Li Yong-Hung, 1988; Kräuter et al., 1991).

This report describes the results obtained by using a mycelium test to evaluate the stem canker infection on five wild sunflower species and the successful transfer into susceptible inbred line by embryo rescue technique.

MATERIALS AND METHODS

The experiment was carried out in 1991 and 1992 in cage which was covered with plastic foil. The following wild species were evaluated in the test: *Helianthus annuus* L. population 1985, *Helianthus maximiliani* Schrader population 1631, *Helianthus tuberosus* L. populations 1700, 1704 and NS-2, *Helianthus nuttallii* ssp. *nuttallii* Torrey and Gray population 1996, *Helianthus petiolaris* ssp. *petiolaris* Nuttall population 1910, inbred lines V-8931-3-4, Ha-74 and NS-B.

A mycelium test on petiole was used to determine the level of resistance. The inoculum was a fungus isolate taken from the experimental field and cultivated on liquid potato dextrose medium and shaken at 80 rpm. Inoculation was performed with 14-day-old isolate by injection into leaf petiole. Inoculations were performed twice in each cycle because of the differences in vegetation period among plants. Intensity of infection was rated on the scale 0 - 4 (0 and 1 were considered resistant, mid-resistant was rated 2, mid-susceptible was rated 3, and susceptible was rated 4).

Heads of all wild species were emasculated in the morning and pollen was washed from the stigmas with a spray of tap water. During the day (between nine and eleven a.m.) pollen from inbred line was applied. The main substrate for embryo rescue technique was B_5 medium (Gamborg et al., 1968). Modification for sunflower were made by Chandler and Beard (1983) and Jan (personal com-

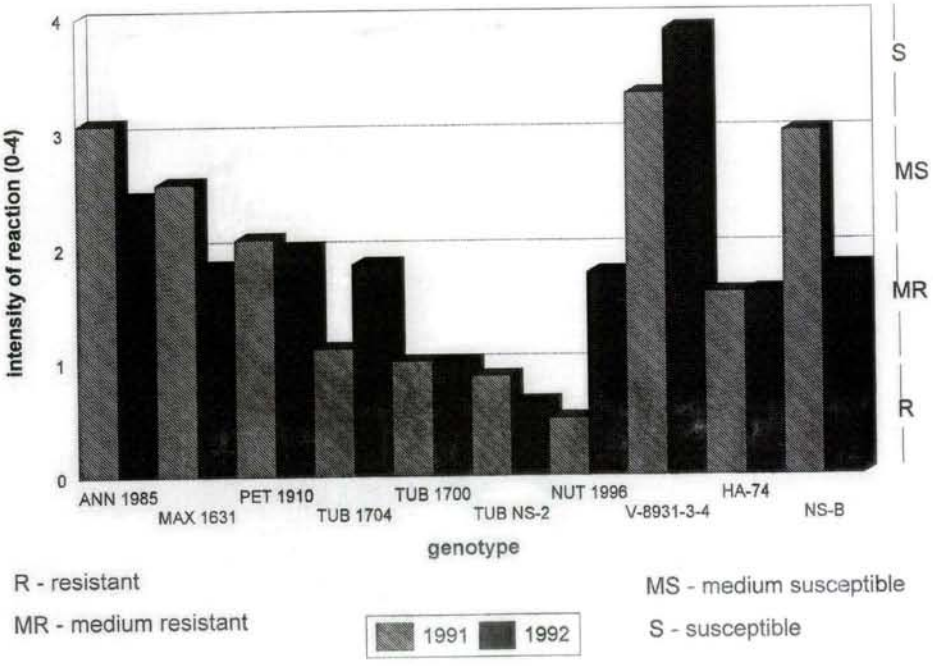

Figure 1. Reaction of sunflower germplasm on infection by mycelium of Phomopsis

Table 3: Composition of embryo culture medium

Component	Embryo growth medium	Germination medium
Inorganic minerals mg.l^{-1}	B_5 salts	B_5 salts
Vitamins		
Nicotinic acid	1.00	-
Thiamine HCl	10.00	-
Pyridoxine HCl	1.00	-
Myo-inositol	100.00	100.00
Amino acids mg.l^{-1}		
L-alanine	500.00	-
L-glutamine	400.00	-
L-serine	80.00	-
L-tryptophan	25.00	-
L-cysteine	5.00	-
Hormones		
NAA	0.005	-
Sucrose g.l^{-1}	100.00	-
Agar	7.00	7.00
pH	5.8	5.8

Figure 2. Plant from H. tuberosus x H. annuus (inbred line V-8931-3-4)

munication). We modified medium I by reducing the amino acids by half (Table 1). Six to ten embryos were placed in each Petri dish. Interspecific hybrids were produced by a two step embryo culture in which two different medium were used: 1) initial growth medium (medium I), and 2) germination medium (medium II). The dark achenes were surface sterilized in 70% ethanol for 1 minute and rinsed in sterile distilled water. After that, the achenes were sterilized in solution of commercial bleach for 10 minutes. Then the surface-sterilized achenes were rinsed twice in sterile distilled water. Five-day-old embryos were placed in Petri dishes which were sealed with paraffin to prevent moisture loss and incubated at 24±2°C in the dark. After 10 days embryos larger than 3.5 mm were transferred to medium II and were incubated under light at approximately 55 $\mu Em^{-2} \cdot sec^{-1}$. After the embryo developed

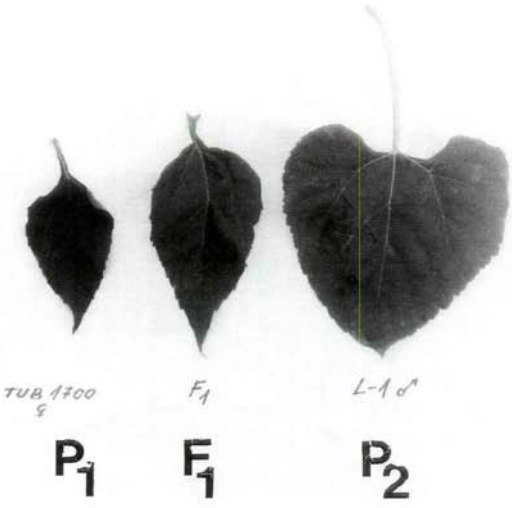

Figure 3. Leaf morphology of H. tuberosus (left). H. tuberosus x H. annuus (centre) and H. annuus (inbred line V-8931-3-4) (right).

Figure 4. Leaves of F_1 H. tuberosus x H. annuus without symptoms of the stem canker.

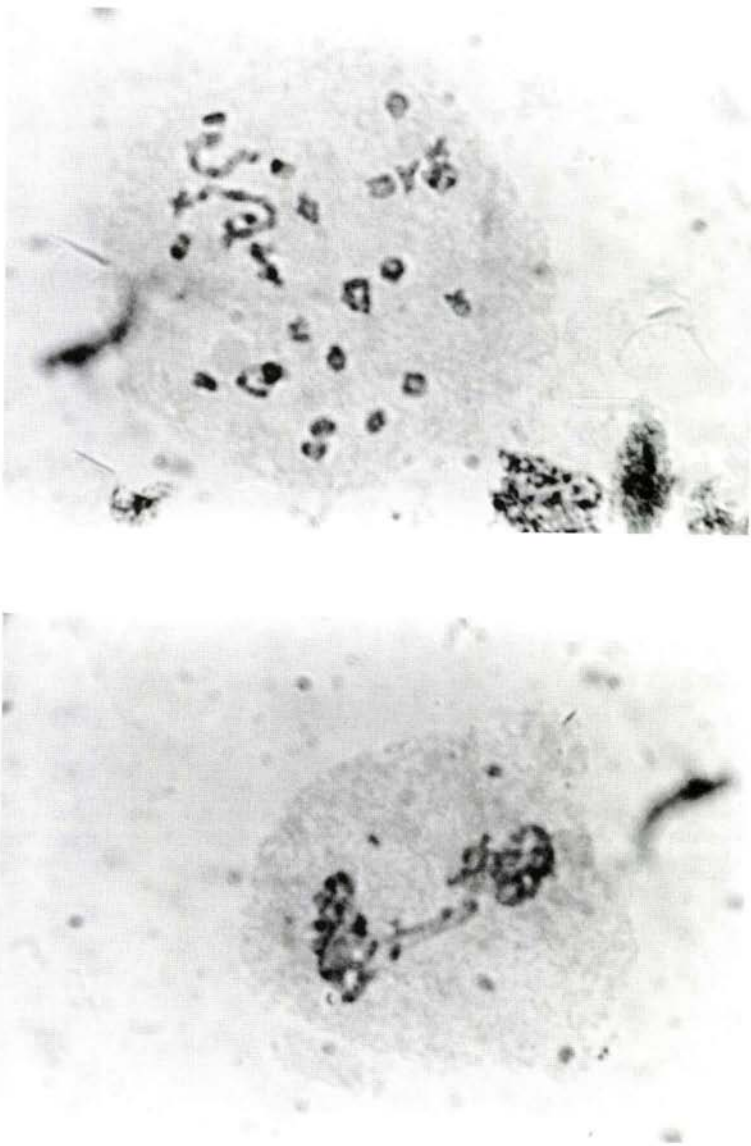

Figure 5. Irregular meiosis in F_1 hybrids H. tuberosus x H. annuus
Legend:
a) diakinesis with bivalents, univalents and multivalents
b) anaphase I with chromosome bridges

Figure 6. Plant of the F_2 generation produced by embryo rescue technique.

roots and a shoot it was transferred to a Jiffy-7 bag in the greenhouse. The plants were covered with plastic foil for a few days in order to increase the humidity. After 15 days plants were transferred to the field.

Pollen viability was studied by the method of Alexander (1969). Meiosis was analysed in diakinesis by the acetrocarmine method (Georgieva-Todorova, 1976).

RESULTS AND DISCUSSION

Test results confirmed those of other authors about sources of resistance to the stem canker (Marić et al., 1988; Škorić et al., 1989). According to the examinations, which were done in 1991 and 1992, two populations of *Helianthus tuberosus* (NS-2 and 1700) were resistant to stem canker. There was no statistically significant difference between the two analysed years (Figure 1). Artificial inoculation in controlled conditions in the greenhouse was found very convenient because microclimate factors can be controlled in that way. But in order to test higher number of genotypes higher number of cages have to be used. That is why *in vitro* methods of testing are more convenient (Maširević et al., 1988; Dozet et al., 1991; Vasić et al., 1994).

Sexual crosses between resistant *H. tuberosus* and susceptible inbred line V-8931-3-4 by embryo rescue technique resulted in the recovery of 21 hybrid plants out of more than 200 isolated embryos (Figure 2). All F_1 plants were found resistant to the stem canker. Characterisation of hybrids by comparison of the morphological traits was not possible on that basis alone (Figure 3). But the hybrids could easily be distinguished by their morphological characteristics, reduced pollen fertility and meiotic abnormalities. The leaf morphology of the most of hybrid plants between *H. tuberosus* and inbred line is intermediate, but some hybrid plants could not be easy classified on the basis of leaf morphology (Atlagić, 1986; Dozet, 1988) (Figure 3). Pollen viability of the tested species and inbred lines was high (over 90%). The F_1 hybrids had a lower pollen viability (from the 30.9 to 75.79%). Low pollen viability in the F_1 generation makes interspecific hybridization of sunflower difficult. Meiotic irregularities occurred in all F_1 hybrids (Figures 5a and 5b). A high percentage of meiocytes with chromosome bridges in the crosses with *H. tuberosus* was a cause of

the low pollen viability. According to Atlagić et al., (1993) a high percentage of multivalent configurations in diakinesis and chromosome bridges in anaphase I indicates the occurrence of translocations and inversion, i.e., differences between *Helianthus tuberosus* and the cultivated sunflower. The meiotic irregularities seem to indicate a different chromosome structure between *H. tuberosus* and the cultivated line.

The high sucrose and low auxin embryo growth medium gave good results in production of healthy enlarged embryos from 5-days old embryos from early heart or heart stage. A two step culture procedure was very successful especially for cultivation of F_2 generation embryos. According to Kräuter et al., (1991) but in contrast to the results of Chandler and Beard (1983) we did not find it necessary to add amino acids to the initial medium. 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 to the embryo. The injured embryo changes colour and perishes in 2 days or forms a callus. That is why the extraction should be performed very carefully.

REFERENCES

Alexander, M.P., 1969. Differential staining of aborted and non-aborted pollen. Stain Technology, 11 (3), 117-123.

Atlagić J., B. Dozet and D. Škorić, 1993. Meiosis and pollen variability in *Helianthus tuberosus* L. and its hybrids with cultivated sunflower. Plant Breeding, 111, 318-324.

Atlagić J., 1986. Biological characteristics of some species of genus *Helianthus* and their hybrids with cultivated sunflower in F_1 generation. Ms. thesis (in Serbian), University of Novi Sad, Yugoslavia.

Chandler, J.M. and B.H. Beard, 1983. Embryo culture of *Helianthus* hybrids. Crop Science, Vol. 23, 1004-1007.

Dozet, B., 1988. The inheritance of plant height, number of side branches, length of leaf petiole and head diameter in some F_1 interspecific sunflower hybrids. VI Youth Conference of Genetics, Albena, Bulgaria.

Dozet, B., 1990. Resistance to *Diaporthe/Phomopsis helianthi* Munt.-Cvet. et al., in wild sunflower species. Sunflower Research Workshop, Fargo, ND, USA, 86-88.

Dozet, B., Atlagić J., Lj. Vasiljević, 1991. Callus induction and maintaince of sunflower germplasm (*Helianthus annuus* L.). II. Selection of sunflower callus to culture filtrate of grey stem spot (*Diaporthe/Phomopsis helianthi* Munt.-Cvet. et al.). Journal of Yugoslav Oil Industry, 3-4, 47-49.

Gamborg, O.L. and K. Oima, 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res., 50, 151-158.

Georgieva-Todorova J., 1976. Interspecies relationship in the genus *Helianthus*. Bulgarian Academy of Science, Sofia.

Griveau, Y., H. Serieys and E. Balhassen, 1992. Resistance evaluation in interspecific and cultivated progenies of sunflower infected by *Diaporthe helianthi*. XIII-th International Sunflower Conference, Vol. II, 1056-1058.

Kräuter Regina, A. Steinmetz and W. Friedt, 1991. Efficient interspecific hybridization in the genus *Helianthus* via "embryo rescue" and characterization of the hybrids. Theor. Appl. Genet., 82, 521-525.

Li Yong-hung, Kuo Chung-shen and Wang Fu-hsiung, 1988. *Helianthus* hybrid and observation of somatic embryogenesis in the immature embryo culture of sunflower. XII International Sunflower Conference, Vol. II, 305-308.

Marić, A., D. Čamprag and S. Maširević, 1988. Sunflower diseases and pests and their control. Nolit, Belgrade, 1 - 378.

Maširević, S., G.A. Secor and T.J. Gulya, 1988. Use of cell culture to screen sunflower germplasm for resistance to *Phomopsis* brown/gray stem spot. Plant Cell Report, 7, 528-530.

Mihaljčević, M. and Muntanola-Cvetković M., 1989. The results obtained until now in the investigation of *Phomopsis/Diaporthe helianthi* on sunflowers. II. Estimate of disease intensity and inoculation methods. Plant Protection, Vol. 40 (1), No. 187, 89-100.

Škorić, D., 1992. Achivements and future directions of sunflover breeding. Field Crop Research, 30, 231-270.

Škorić, D., B. Dozet and R. Marinković, 1989. Using wild species in the progress of development of sunflower hybrids. XII-th EUCARPIA Congress, part I, 12-13.

Tourvielle de Labrohue D., Vear F. and C. Pelletier, 1988. Use of two mycelium tests in breeding sunflower resistant to *Phomopsis*. XII-th International Sunflower Conference, Vol. II, 110-114.

Vasić D., Marjanović A. and B. Dozet, 1994. *In vitro* selection of sunflower (*Helianthus annuus* L.) plantlets for resistance to *Diaporthe/Phomopsis helianthi* Munt.-Cvet. et al. First Congress of Genetics of Serbia, p. 190.

Virany, F., T. Sendula, Z. Horvath and F. Nemeth, 1988. A rapid test for evaluating resistance to sunflower stem canker caused by *Diaporthe/Phomopsis helianthi*. XII-th International Sunflower Conference, Vol. II, 115-118.

TRANSFERENCIA DE LA RESISTENCIA DEL CHANCRO DE TALLO DE *Helinthus tuberosus* L. EN LÍNEAS PURAS DE GIRASOL POR LA TÉCNICA DEL CULTIVO DE EMBRIONES

RESUMEN

Varias poblaciones de especies girasol silvestre fueron testadas para resistencia al chancro del tallo usando el método de inoculación con el micelio del hongo. El grado más alto de resistencia fue mostrado por *Helianthus tuberosus* L. Mediante el aislamiento de embriones se obtuvieron 21 plantas híbridas a partir de más de 200 embriones aislados. Todas las plantas F_1 fueron resistentes. Un medio B_5 modificado dio buenos resultados en la producción de embriones sanos agrandados a partir de los jóvenes embriones. Los híbridos F_1 tuvieron una viabilidad del polen más baja. Se encontraron irregularidades meióticas en todas los híbridos interespecificos.

TRANSFERT DE LA RÉSISTANCE AU *Phomopsis d'Helianthus tuberosus* L. DANS LES LIGNÉES FIXÉES DE TOURNESOL PAR LA TECHNIQUE DU SAUVETAGE D'EMBRYONS

RÉSUMÉ

Plusieurs populations sauvages de tournesol ont été testées pour la résistance au *Phomopsis* par inoculation du mycélium du champignon. Le plus fort niveau de résistance a été décelé chez *Helianthus tuberosus* L. Par sauvetage d'embryons âgés de 5 jours, on a obtenu 21 plantes hybrides à partir desquelles plus de 200 embryons ont été isolés. Toutes les plantes F_1 étaient résistantes. A partir de jeunes embryons hybrides, un milieu B_5 modifié a conduit à la production d'embryons bien développés et vigoureux. Les hybrides F_1 avaient une viabilité pollinique plus faible. Des anomalies méiotiques sont apparues chez tous les hybrides F_1 interspécifiques.