Зборник Матице српске за природне науке / Proceedings for Natural Sciences, Matica Srpska Novi Sad, № 102, 29—33, 2002

UDC 633.854.78:631.547.1

Dragana M. Vasić, Ksenija J. Taški, Sreten Z. Terzić, Slavko E. Kevrešan[1], *Dragan M. Škorić*

Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Yugoslavia
[1] Poljoprivredni fakultet, Trg Dositeja Obradovića 8, 21000 Novi Sad

TRANSFERRING OF *SCLEROTINIA* RESISTANCE FROM WILD INTO CULTIVATED SUNFLOWER — COMBINING OF CONVENTIONAL AND LABORATORY TECHNIQUES

ABSTRACT: Five populations of each *H. molis*, *H. maximiliani*, *H. rigidus* and *H. tuberosus* were screened for resistance to stem form of *Sclerotinia*. On the basis of the results obtained by screening, nine crosses of resistant populations with either other wild species populations or with cultivated sunflower were made. As in some crosses a small quantity of seed was produced and the seeds germinated poorly, modified tissue culture methods were used to enhance germination and produce clones of interesting plants. These methods were found to be efficient both for seed germination and plant production and multiplication.

KEY WORDS: wild sunflower, *Sclerotinia* resistance, tissue culture

INTRODUCTION

White rot caused by the fungus *Sclerotinia sclerotiorum* is the major disease of sunflower (*Helianthus annuus* L.) in countries with a humid climate, while in countries with moderate climate, it causes the yield loss in rainy years (Škorić and Rajčan, 1992). This parasite usually attacks all parts of the plant: roots, stalks, 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 yet been found or developed.

Wild sunflowers constitute an important source of resistance against several major sunflower diseases including *Sclerotinia* (Georgieva-Todorova, 1993). In some cases, transfer of these traits into cultivated sunflower genome using conventional methods is difficult because of a high interspecific incompatibility. In sunflower, embryo rescue technique has been used to overcome this problem.

Micropropagation is interesting for wild sunflower species whose seeds germinate poorly. Imhoff et al. (1996) described a method for efficient propagation of three wild sunflower species using sterile rhizomes. Vasić et al. (2001) have used shoot tips and nodal segments for multiplication of *H. maximiliani*.

In this paper an integrated approach to the *Sclerotinia* resistance screening and breeding, combining conventional and modified tissue culture methods, is described.

MATERIAL AND METHODS

Wild sunflower accessions were grown in quarantine plot of Institute of Field and Vegetable Crops, Novi Sad.

Five populations of each *H. molis*, *H. maximiliani*, *H. rigidus* and *H. tuberosus* (Table 1) were screened for resistance to stem form of *Sclerotinia*. Four plants per population were artificially inoculated by incorporation of sclerotias in the stems in the phasis of butonisation. Wounds with sclerotia were covered with wet cotton and aluminium foil. Plot was regularly irrigated. Screening was done two weeks after full flowering, using the scale 1—5. Resistance was determined as percentage of healthy plants.

Plants of populations found to be resistant were crossed with either other wild sunflower populations or cultivated sunflower, using classical method.

Seeds of progenies of crosses were germinated in liquid MS medium (Murashige and Skoog, 1962) and planted in Jiffy pots. Well-grown plants were transferred into growth chamber. Further multiplication of plants was done by rooting side branches in sand, with watering with distilled water or water solutions of natural naphthenic acids isolated from lower fractions of "Velebit" oil.

RESULTS AND DISCUSSION

Two populations of *H. molis* were found to be 100% resistant to *Sclerotinia* attack on stem (Table 1). In all other tested species some highly tolerant populations were found, which confirms the notion that wild species could be valuable sources of if not resistance than tolerance to *Sclerotinia* (Škorić, 1988). In contrast to the results of Škorić and Rajčan (1992), population 1631 of *H. maximiliani* was only tolerant to stem *Sclerotinia* (Table 1). This could be explained by high variability that exists within the populations of wild sunflower species.

On the basis of the results obtained by screening, nine crosses of resistant populations with either other wild species populations or with cultivated sunflower were made (Table 2). Crosses with other wild species were done in cases where it was not possible to cross them with cultivated sunflower. Crosses were made with wild species crossable with cultivated sunflower with the hope that hybrids will be crossable with it as well.

Table 1. — Resistance of tested populations to artificial *Sclerotinia* infection on stem. Resistance is given as a percentage of healthy plants

Genotype	Resistance (%)	Genotype	Resistance (%)
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 1702	75

Table 2. — Seeds and plant from interspecific crosses obtained by conventional and laboratory methods

Cross	Total seeds obtained	Number of germinated seeds	Percentage of regenerated plants	Percentage of plants obtained from side branches
CMS1-17A x max 2007	1	—	—	—
Ha-48 x tub 6	4	—	—	—
Ha-48 x tub 7	1	1	100%	100%
arg 1805 x max 1631	50	10	30%	100%
gig 1605 x max 1631	46	19	32%	—
tub 1700 x max 1631	42	10	30	—
max M x gig 2115	4	—	—	—
gro 1685 x molx	35	8	12%	—
mol 1298 x ann 2197	7	—	—	—

As in some crosses a small quantity of seed was produced (Table 2) and the seeds germinated poorly, modified tissue culture methods were used to enhance germination and produce clones of interesting plants.

Embryo culture in *in vitro* conditions can sometimes lead to decreased plant vigour (P e l l e t i e r et al., 1992). That is why, as suggested by P a u l and B a r t h o u (1994), for improvement of seed germination a non-sterile technique was used. Germination percentage varied from 80—100% (data not shown). Plants obtained were normal and vigorous. This is in accordance with the results obtained by P a u l and B a r t h o u (1994).

Micropropagation of interspecific progenies was done in non-sterile conditions as well. The sterile technique that was found to be efficient in propagation of *H. maximiliani* shoot tips (V a s i ć et al., 2001) was not efficient here. The same stands for growing branches in water or water solution of tested compounds. No matter which of the substances was used, no root formation was observed. The most efficient was growing branches in the sand. Rooting

was induced in all the variants, but the intensity of root formation was different among the variants.

The obtained results showed that wild sunflower species could be a potential source of genes for *Sclerotinia* resistance. Combination of classical crossing with embryo rescue and micropropagation was found to be a good method for obtention and multiplication of progenies of interesting interspecific crosses. Further studies on wild sunflower relatives regarding *Sclerotinia* resistance are in progress. The techniques described in this paper are going to be applied in the new interspecific crosses. All this will hopefully lead us to production of cultivated sunflower genotype with at least high tolerance to *Sclerotinia*.

LITERATURE

Georgieva-Todorova, J. (1993): *Interspecific hybridization and its application in sunflower breeding*, Biotechnol & Biotechnol Eq 7: 153-157.

Imhoff, U., Wingender R., Dresen, B., Schnabl, H. (1996): *Micropropagation of three Helianthus wild perennial species*, Angew. Bot. 70: 137—139.

Lumsden, R. D. (1979): *Histology and physiology of pathogenesis in plant diseases caused by Sclerotinia species*, Phytopathology 69:890—896.

Murashige, T., Skoog, F. (1962): *A revised medium for growth and bioassays with tobacco tissue cultures*, Physiol. Plant. 15: 473—497.

Paul, M. H., Barthou, H. (1994): *Technique simplifiee et non sterile pour la culture d'embryons immatures de tournesol (Helianthus annuus)*, Agronomie 14: 281—284.

Pelletier, C., Tourvielle, D., Vear, F. (1992): *The effect of in vitro culture of immature sunflower embryos on some morphological and agronomical characteristics*, Proc 13th Inter Sunflower Conf, Pisa, Italy, p. 1517—1522.

Škorić, D. (1988): *Sunflower breeding*, Uljarstvo 25: 1—90.

Škorić, D., Rajčan, I. (1992): *Breeding for Sclerotinia resistance in sunflower*, Proc. 13th Int. Sunflower Conf., Pisa, Italija, p. 1257—1262.

Vasić, D., Škorić, D., Alibert, G., Miklič, V. (2001): *Micropropagation of Helianthus maximiliani (Schrader) by shoot apex culture*, Helia 24, 34: 63—68.

Zimmer, D., Hoes, J. (1978): *Diseases*. U: *Sunflower Science and Technology*. J. Carter (Ed.), American Society of Agronomy, Madison, USA, p. 225—262.

ПРЕНОШЕЊЕ ОТПОРНОСТИ ПРЕМА *SCLEROTINIA* ИЗ ДИВЉЕГ У ГАЈЕНИ СУНЦОКРЕТ — КОМБИНОВАЊЕ КОНВЕНЦИОНАЛНИХ И ЛАБОРАТОРИЈСКИХ ТЕХНИКА

Драгана М. Васић, Ксенија Ј. Ташки, Сретен З. Терзић,
Славко Е. Кеврешан[1], Драган М. Шкорић
Институт за ратарство и повртарство, Максима Горког 30,
21000 Нови Сад, Југославија
[1] Пољопривредни факултет, Трг Доситеја Обрадовића 8, 21000 Нови Сад

Резиме

Тестирано је по пет популација *H. molis, H. maximiliani, H. rigidus* и *H. tuberosus* на отпорност према *Sclerotinia* стабла. На основу добијених резултата извршено је девет укрштања отпорних популација са другим дивљим врстама или гајеним сунцокретом. Како је у неким случајевима добијена мала количина семена која су слабо клијала, коришћене су модификоване методе културе ткива да би се повећала клијавост и произвели клонови интересантних биљака. Ове методе су се показале ефикаснима и за наклијавање семена и за производњу и умножавање биљака.