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Photography: *Spanish landrace of confectionary sunflower collected by L. Velasco and B. Pérez-Vich in Villarta de San Juan, Ciudad Real, Spain, on October 10, 2007.*

Foreword

The proceedings of the *17th International Sunflower Conference* contain 142 contributions from scientists of 24 countries. They include plenary lectures in several disciplines and regular communications presented in posters during the conference and discussed in the corresponding workshops. The manuscripts are classified by disciplines. They offer a good picture of the current state of the art of sunflower research and cultivation around the world.

The manuscripts in the *Proceedings* have been reviewed by an editorial committee with the main objective of helping the authors to improve their manuscripts through a critical reading. The authors received the edited manuscripts together with the comments of the reviewers and then went on to draft their final version. All the manuscripts received have been published in the *Proceedings*. The contents of the manuscripts are the responsibility of the authors. They should be considered as being privileged communications that require the express consent of the authors to be reprinted in part or as a whole. We wish to thank both the members of the Editorial Committee for their dedication to the task of editing such a large number of manuscripts, as well as all the authors for their collaboration throughout the whole edition process.

The Organizing Committee would also like to thank Diana Badder and José A. Palacios for their excellent editorial assistance in the preparation of these *Proceedings*. We are indebted to the Spanish Association of Sunflower Breeders (Asociación Española de Mejoradores de Girasol), which collaborated actively in the organization of the conference, and, very especially, to Juan Parejo, who was in charge of the financial side.

Finally, we would like to thank all the participants in the conference, who have contributed to its success by a careful preparation and revision of manuscripts and posters, presentation of their research in the workshops, and stimulating discussions throughout the conference on the scientific and technical aspects of sunflower research and cultivation in the world.

The Organizing Committee
17th International Sunflower Conference
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CROP PRODUCTION – MANAGEMENT

Towards *Sclerotinia* resistance – *In vitro* screening of wild sunflower species

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ABSTRACT

This paper presents the work on testing the possibility of the use of *in vitro* screening for determination of wild *Helianthus* species resistance to *Sclerotinia.* For this purpose, micropropagated plants of different accessions of *H. maximiliani*, *H. mollis*, *H. rigidus* and *H. tuberosus* were grown on MS medium supplemented with 0, 0.5, 1 and 2 mM of oxalic acid. Fresh and dry weight of above-ground part, and dry weight of root could be considered as the potential parameters of wild species resistance/susceptibility to *Sclerotinia* in *in vitro* tests, as they were not affected by treatment in resistant (100%) accessions and were significantly decreased in susceptible (25%) ones in the presence of 2 mM of oxalic acid.

Key words: oxalic acid – *in vitro* screening – resistance – *Sclerotinia sclerotiorum* – wild sunflower.

INTRODUCTION

White rot caused by the fungus *Sclerotinia sclerotiorum* Lib. (de Bary) is the major disease of sunflower (*Helianthus annuus* L.) in countries with a humid climate, while in countries with moderate climate, it causes yield losses in rainy years (Škorić and Rajcan, 1992). Wild sunflowers (*Helianthus* spp.) constitute an important source of resistance against several major sunflower diseases including *Sclerotinia* (Georgieva-Todorova, 1993). Populations of several wild sunflower species were found to be tolerant to white rot (Škorić and Rajcan, 1992; Henn et al., 1997; Tavoljanski et al., 2002; Cerboncini et al., 2002; Vasic et al., 2004). Resistance screening was done either by observing naturally occurring infection (Tavoljanski et al., 2002) or by using different artificial inoculation methods (Henn et al., 1997; Cerboncini et al., 2002; Vasic et al., 2002; 2004).

De Bary was the first researcher to associate oxalic acid with *Sclerotinia* infection (Lumsden, 1979). Later, Noyes and Hankock (1981) demonstrated its importance as a factor in the pathogenicity of this fungus, while Hartman et al. (1988) found a correlation between oxalic acid production and virulence of different *Sclerotinia* isolates. There have been several attempts to create a bioassay in which resistance to oxalic acid would be used as an indicator of resistance to *Sclerotinia* (Hartman et al., 1988; Noyes and Hancock, 1981; Raducanu and Soare, 1992; Tu, 1985; Vasic et al., 1999; 2002). Whole plants or their parts were used, and correlation was found between field susceptibility/resistance of tested genotypes to *Sclerotinia* and reaction of the explants of the same genotypes when grown on a medium into which oxalic acid was added.

As maintenance of wild species collection and field screening are costly and labour-intensive, we have tested the possibility of the use of *in vitro* screening for determination of wild sunflower species resistance to *Sclerotinia*.

MATERIALS AND METHODS

Accessions of *H. maximiliani* Schrader (max), *Helianthus mollis* Lam. (mol), *H. rigidus* (Cass.) Desf. (rig) and *H. tuberosus* L. (tub) were obtained from wild *Helianthus* species collection of Institute of Fields and Vegetable Crops in Novi Sad, Serbia (Table 1). The accessions were pre-screened for *Sclerotinia* resistance by measuring sclerotia infection on stem (Vasic et al., 2004). Their resistance was determined as the percentage of healthy plants (Table 1).

The plants were propagated *in vitro* using culture of apical shoots (Vasic et al., 2001). Prior to transfer to a propagation medium, shoots were dipped into 0.1% indolebuteric acid (IBA) solution for 4 min. For the resistance screening, apical shoots of *in vitro* grown plants were placed in 250 ml Erlenmeyer flasks with 80 ml of MS medium (Murashige and Skoog, 1962), pH 5.7, supplemented with 5 g l^{-1} of sucrose, 6 g l^{-1} of agar, and different concentrations of oxalic acid (Table 1). Control plants were grown on MS medium without oxalic acid. There were four Erlenmeyer flasks with four shoots per accession for each oxalic acid concentration. One Erlenmeyer flask was treated as one replication in the data analysis. The shoots were grown at 24ºC with a photoperiod of 16 h (light)/8 h (dark).

After six weeks of culture, the following parameters were measured: plant height, fresh and dry weight of above-ground part, root length, fresh and dry weight of root. The data were analysed using ANOVA and LSD test.

RESULTS AND DISCUSSION

Analysis of variance showed that both genotype and treatment had significant effect on the measured parameters.

Table 1. Reaction of tested wild sunflower accessions on treatment with different concentrations of oxalic acid[1,2].

Genotype	Resistance (%)	Concentration mM	h	rl	fm	dm	rfm	rdm
		Control	10.875a	3.925a	0.476a	0.049a	0.478a	0.039a
mol x	100	0.5	4.625b	5.225a	0.208b	0.0185b	0.132b	0.011c
		1	3.550b	3.650a	0.183b	0.019b	0.340ab	0.011c
		2	4.075b	5.075a	0.395a	0.040a	0.296ab	0.025b
		LSD$_{0.05}$	2.750	2.022	0.151	0.014	0.342	0.009
		LSD$_{0.01}$	3.855	2.835	0.212	0.020	0.479	0.014
		control	15.200a	11.200a	0.293ab	0.030b	0.088ab	0.006b
mol 1298	100	0.5	9.325c	2.750c	0.153c	0.022b	0.059b	0.004b
		1	13.675ab	7.525b	0.253bc	0.026b	0.117ab	0.007ab
		2	10.950bc	8.525ab	0.387a	0.042a	0.146a	0.010a
		LSD$_{0.05}$	3.328	2.732	0.113	0.011	0.062	0.003
		LSD$_{0.01}$	4.666	3.829	0.159	0.015	0.087	0.005
		control	14.700a	11.875a	0.492a	0.048a	0.183a	0.013a
max 34	75	0.5	12.425ab	3.550c	0.299b	0.025b	0.048b	0.004b
		1	11.775b	3.575c	0.277b	0.030b	0.074b	0.006b
		2	4.800c	6.550b	0.338b	0.048a	0.245a	0.017a
		LSD$_{0.05}$	2.862	2.812	0.139	0.016	0.067	0.004
		LSD$_{0.01}$	4.012	3.942	0.195	0.023	0.094	0.006
		control	15.400a	18.775ab	1.493a	0.105a	0.749a	0.048a
max 1631	50	0.5	16.225ab	14.650b	0.945a	0.066a	0.274b	0.016b
		1	14.100ab	22.325a	1.503a	0.104a	0.884a	0.051a
		2	11.825b	18.675ab	1.058a	0.077a	0.416ab	0.025ab
		LSD$_{0.05}$	3.836	4.436	0.737	0.047	0.473	0.028
		LSD$_{0.01}$	5.377	6.219	1.034	0.066	0.664	0.039
		control	12.600a	11.325a	0.690b	0.025c	0.257b	0.016a
tub 675	50	0.5	10.425a	11.175a	1.018a	0.054b	0.364a	0.021a
		1	8.600a	10.700a	0.512b	0.078a	0.201bc	0.029a
		2	2.600b	4.450b	0.134c	0.043bc	0.154c	0.048a
		LSD$_{0.05}$	4.535	4.291	0.259	0.021	0.101	0.048
		LSD$_{0.01}$	6.358	6.016	0.363	0.030	0.142	0.067
		control	13.625a	12.850a	0.312b	0.030b	0.249b	0.020b
rig 1692	50	0.5	14.750a	12.025a	0.486a	0.053a	0.380a	0.034a
		1	14.375a	10.700a	0.257b	0.025b	0.197b	0.014b
		2	11.500a	11.675a	0.259b	0.026b	0.173b	0.015b
		LSD$_{0.05}$	5.003	5.082	0.108	0.009	0.056	0.006
		LSD$_{0.01}$	6.697	5.880	0.159	0.015	0.085	0.008
		control	12.175a	4.150a	0.678b	0.048b	0.215b	0.016b
mol 1530	25	0.5	12.350a	3.475a	0.728b	0.070ab	0.497ab	0.042ab
		1	13.525a	4.050a	1.359a	0.115a	0.926a	0.067a
		2	5.350b	3.925a	0.626b	0.055b	0.314b	0.031ab
		LSD$_{0.05}$	2.239	1.290	0.623	0.050	0.483	0.038
		LSD$_{0.01}$	3.140	1.809	0.873	0.070	0.677	0.053
		control	11.125a	6.575ab	0.529b	0.062b	0.473b	0.047b
rig 1843	25	0.5	8.975a	6.350ab	0.448b	0.059b	0.372b	0.041b
		1	7.950ab	7.500a	0.781a	0.089a	0.835a	0.083a
		2	4.800b	4.825b	0.530b	0.063b	0.346b	0.038b
		LSD$_{0.05}$	3.466	LSD$_{0.05}$	0.180	0.022	0.258	0.025
		LSD$_{0.01}$	4.860	LSD$_{0.01}$	0.252	0.031	0.362	0.035

[1]Within each column, genotype means followed by different letter differ significantly at the level p=0.05.

[2]Legends for traits: h - plant height, rl - root length, fm - fresh weight of above-ground part, dm – dry weight of above-ground part, rfm - fresh weight of root, rdm - dry weight of root.

The choice of oxalic acid concentrations was made based on the research done on cultivated sunflower protoplasts (Vasic et al., 1999) and intact plants grown *in vitro* (Vasic et al., 2002). Results obtained with 0.5 and 1 mM concentrations of oxalic acid were not conclusive as there was neither any difference between resistant and susceptible accessions nor a regular pattern in measured parameter variation (Table 1). This is in accordance with the results obtained on the sunflower plants grown in the presence of oxalic acid (Vasic et al., 2002). The same applies in the reaction of tolerant accessions (*H. maximiliani* 1631, *H. tuberosus* 675 and *H. rigidus* 1692) to oxalic acid treatment, and is probably the consequence of differences in morphology and biochemistry between wild sunflower species (Heiser et al., 1969).

Concentration of 1 mM of oxalic acid had a stimulant effect on the most susceptible accessions – *H. mollis* 1530 and H. r*igidus* 1843 (Table 1). Stimulant effect of non-selective concentrations of stress agents in *in vitro* culture was also observed in the experiments with herbicides, and is thought to be a consequence of the phenomenon that stress agents when present in small concentrations act as nutrients (Olofsdotter et al., 1994).

Similarly to the work of Vasic et al. (2002), concentration of 2 mM of oxalic acid discriminated between resistant and susceptible genotypes. This oxalic acid concentration only affected plant height in the resistant accessions (*H. mollis* x and 1298) and almost all the traits in susceptible ones, except for the root length in *H. mollis* 1530 (Table 1). This is in disagreement with the results of Mouly (1989) who found that concentrations of oxalic acid lower than 4.44 mM were not selective in a bioassay with sunflower leaves. The same author recommended a concentration of 8.88 mM as optimal.

Fresh and dry weight of above-ground parts and dry weight of root could be considered the potential parameters of wild sunflower resistance/susceptibility to *Sclerotinia* in *in vitro* tests, as they were not affected by treatment in resistant accessions and they were significantly decreased in susceptible ones in the presence of 2 mM of oxalic acid (Table 1). In contrast to the results obtained in cultivated sunflower (Vasic et al., 2002), plant height and root length were not good indicators of wild sunflower resistance/susceptibility to *Sclerotinia*. This may be due to structural differences between cultivated and wild sunflowers and their biochemical reaction to *Sclerotinia*, as previously observed in *H. resinosus* Small (Mondolot-Cosson and Andary, 1994).

The results obtained in our study showed that there is potential for the use of oxalic acid bioassays for screening wild sunflower species for resistance to *Sclerotinia*. Fresh and dry weight of above-ground parts and dry weight of root were found to be good morphological parameters for discrimination between resistant and susceptible accessions, in combination with an oxalic acid concentration of 2 mM. However, more work should be done in determining the optimal oxalic acid concentration. Also, the morphological and biochemical differences between different sunflower species should be taken into account in further studies.

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REFERENCES

Cerboncini, C., G. Beine, P.C. Binsfeld, B. Dresen, H. Peisker, A. Zerwas, and H. Schnabl. 2002. Sources of resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary in a natural *Helianthus* gene pool. Helia 25:167-176.

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

Hartman, C.L., P.A. Donald, G.A. Secor, and J.F. Miller. 1988. Sunflower tissue culture and use in selection for resistance to *Phoma macdonaldii* and white mold (*Sclerotinia sclerotiorum*). p. 347-351. In: Proc. 12th Int. Sunflower Conf., Novi Sad, Yugoslavia.

Heiser, C.B., D. Smith, S. Clevenger, and W. Martin. 1969. The North American sunflower (*Helianthus*). Mem. Torrey Bot. Club 22:1-218.

Henn, H.J., U. Steiner, R. Wingender, and H. Schnabl. 1997. Wildtype sunflower clones: Source of resistance against *Sclerotinia sclerotiorum* (Lib.) de Bary stem infection. Angew. Bot. 71:5-9.

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

Mondolot-Cosson, L., and C. Andary. 1994. Resistance factors of a wild species of sunflower, *Helianthus resinosus*, to *Sclerotinia sclerotiorum*. Acta Hortic. 381:642-645.

Mouly, A. 1989. Recherche de marqueurs moleculaires de la tolerance du tournesol a *Sclerotinia sclerotiorum*. PhD Thesis, UPS Toulouse, France.

Murashige, T., and F. Skoog. 1962. A revised medium for growth and bioassays with tobecco tissue cultures. Physiol. Plant. 15:473-497.

Noyes R.D., and J.G. Hancock. 1981. Role of oxalic acid in the *Sclerotinia* wilt of sunflower. Physiol. Plant Pathol. 18:123-132.

Olofsdotter, M., A. Olesen, S.B. Andersen, and J.C. Streibig. 1994. A comparison of herbicide bioassays in cell cultures and whole plants. Weed Res. 34:387-394.

Raducanu, F., and G. Soare. 1994. An approach of *in vitro* testing sunflower for resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary. Rom. Agric. Res. 2:35-39.

Škorić, D., and I. Rajcan. 1992. Breeding for *Sclerotinia* resistance in sunflower. p. 1257-1262. In: Proc. 13th Int. Sunflower Conf., Pisa, Italy.

Tavoljanski, N., A. Yesaev, V. Yakutkin, E. Akhtulova, and V. Tikhomirov. 2002. Using the collection of wild species in sunflower breeding. Helia 25:65-78.

Tu, J.C. 1985. Tolerance of white bean (*Phaseolus vulgaris*) to white mold (*Sclerotinia sclerotiorum*) associated with tolerance to oxalic acid. Physiol. Plant Pathol. 26:111-117.

Vasic, D., G. Alibert, and D. Škorić. 1999. *In vitro* screening of sunflower for resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary. Helia 22:95-104.

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

Vasic, D., K. Taski, S. Terzic, S. Kevresan, and D. Škorić. 2002. Transferring of *Sclerotinia* resistance from wild into cultivated sunflower - combining of conventional and laboratory techniques. Proc. Nat. Sci., Matica Srpska 102:29-33.

Vasic, D., N. Dusanic, V. Miklič, and D. Škorić. 2004. Transferring of *Sclerotinia* resistance from wild into cultivated sunflower – Evaluation of wild sunflower species. Acta Agronom. Serb. 65:5-10.