

USE OF OXALIC ACID FOR SCREENING INTACT SUNFLOWER PLANTS FOR RESISTANCE TO *Sclerotinia* *IN VITRO*

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SUMMARY

Three sunflower inbred lines differing in resistance to *Sclerotinia* were grown on nutritive medium supplemented with different concentrations of oxalic acid. After two weeks of culture, plant height, fresh and dry weight of above-ground part, length of first pair of leaves, root length, and fresh and dry weight of root were measured. Data obtained were analysed using ANOVA and LSD test. Out of the tested parameters, plant height and root length were found to be the most reliable indicators of plant resistance/susceptibility to *Sclerotinia*. Based on the data obtained, oxalic acid concentrations of 4 mM, 3 mM and 2 mM were chosen for further work in which test would be done on a larger number of genotypes.

Key words: sunflower, *Sclerotinia* resistance, *in vitro* screening, oxalic acid

INTRODUCTION

The white rot disease caused by the fungus *Sclerotinia sclerotiorum* is the major disease of sunflower in countries with the humid climate, while in countries with the moderate climate, it causes yield loss in rainy years (Škorić and Rajčan, 1992). It is a facultative polyphagous parasite that attacks a variety of wild plants and agricultural crops. There are no suitable cultural control methods against it (Lumsden, 1979) and no resistant genotypes of cultivated sunflower have yet been found or developed. Breeding work is complicated since there are several forms of disease and the mechanisms of tolerance to the various forms are controlled by different genetic and other processes (Roberts *et al.*, 1987).

De Bary was the first who associated oxalic acid with *Sclerotinia* infection (Lumsden, 1979). Later, Noyes and Hancock (1981) demonstrated its importance as a factor in the pathogenicity of this fungus, while Hartman *et al.* (1988) found

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correlation between oxalic acid production and virulence of different isolates of *Sclerotinia*.

For an early selection for the disease resistance, a laboratory method is highly desirable. The larger number of individuals that can be screened and the limited space that is needed make the application of *in vitro* selection an attractive approach. 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; Vasić *et al.*, 1999). Whole plants or their parts were used, and correlation was found between field susceptibility/resistance of genotypes tested to *Sclerotinia* and reaction of the explants of the same genotypes when grown on a medium to which oxalic acid was added.

In this paper we describe a method for *in vitro* screening for resistance to *Sclerotinia* by culture of intact sunflower plants in the presence of oxalic acid.

MATERIAL AND METHODS

Inbred lines PR-ST-3B, tolerant to *Sclerotinia* on the head, CMS₁-90A, susceptible to *Sclerotinia* on the root, and OD-3369A, susceptible to *Sclerotinia* on the stem, were used in the experiment (Škorić, personal communication). Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia, provided seeds of all lines.

Seeds were surface sterilized by soaking in 5% solution of commercial bleach for 20 minutes, washed with distilled water and dehulled. Dehulled seeds were sterilized again by dipping in 70% ethanol for one minute followed by soaking in commercial bleach for ten minutes. They were then rinsed three times with sterile distilled water and placed in 250 ml Erlenmeyer flasks with 80 ml of MS medium (Murashige and Skoog, 1962) supplemented with 5 g l⁻¹ of sucrose and 6 g l⁻¹ of agar, pH 5.7, and different concentrations of oxalic acid (Tables 1, 2 and 3). Seeds were further cultured in the light of intensity of 34 μE m⁻¹, photoperiod 16:8 (light:dark) and temperature 25°C.

After two weeks of culture following parameters were measured: plant height, fresh and dry weight of above-ground part, length of first pair of leaves, root length, fresh and dry weight of root. The experiment was set in four repetitions, and three cycles. In each cycle different set of oxalic acid concentrations was tested. For statistical analysis, the data obtained for each parameter were expressed as percentage of the respective mean of control (100%). Data were analyzed using ANOVA and LSD test.

RESULTS AND DISCUSSION

Primary choice of oxalic acid concentrations was made based on the research of Vasić *et al.* (1999) done on sunflower protoplasts (Table 1). In the work of Vasić *et al.* (1999), the concentration of 1 mM of oxalic acid discriminated well between the resistant and susceptible genotypes. This was not the case in our study, where concentrations between 1.2 and 1.8 mM did not have negative effect on the tested parameters of either resistant or susceptible genotypes. In some cases, even stimulating effect on some parameters was observed (Table 1). Higher selectivity of given concentration of oxalic acid in the case of protoplasts can be explained by their greater sensitivity to osmotic shocks, which oxalic acid as a very strong chelator of calcium and other cations (Lumsden, 1979) provokes. Stimulating effect of small concentrations of stress agents in *in vitro* culture was observed also in the experiments with herbicides (Olofsdotter *et al.*, 1994), and is thought to be a consequence of phenomenon that stress agents when present in small concentrations act as nutrients (Vasić *et al.*, 1997).

Table 1: Reaction of tested genotypes on treatment with different concentrations of oxalic acid. Within the column and the genotype means followed by different letter differ. Legend for traits: h - plant height, fm - fresh weight of above-ground part, dm - dry weight of above-ground part, l - length of first pair of leaves, rfm - fresh weight of root, rdm - dry weight of root, rl - root length.

Genotype	Conc mM	Trait (%)						
		h	fm	dm	l	rfm	rdm	rl
PR-ST-3B	Control	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
	1.2	79.7a	97.6a	108.8a	105.8a	87.6a	98.7a	94.5a
	1.4	78.2a	102.9a	105.6a	120.6a	77.0a	74.9a	103.8a
	1.6	90.7a	101.1a	95.8a	138.9a	81.4a	85.6a	119.8a
	1.8	77.4a	98.9a	101.4a	92.9a	72.9a	69.8a	94.4a
	LSD _{0,01}	42.6	22.0	22.9	52.2	33.0	38.2	29.9
CMS ₁ -90A	control	100.0a	100.0bc	100.0a	100.0a	100.0b	100.0bc	100.0a
	1.2	102.1a	127.9a	91.8a	113.9a	149.0a	150.9a	94.8a
	1.4	95.9a	107.0b	104.6a	105.1a	120.4ab	105.4bc	90.3a
	1.6	92.9a	86.5c	81.9a	94.4a	97.1b	93.1c	85.4a
	1.8	92.8a	118.5ab	108.9a	110.1a	146.4a	138.2ab	92.4a
	LSD _{0,01}	29.1	28.4	41.9	33.6	39.6	40.0	19.7
OD-3369A	Control	100.0a	100.0a	100.0a	100.0a	100.0a	100.0ab	100.0a
	1.2	70.5a	70.8ab	80.4ab	65.2b	68.5a	53.9b	53.5a
	1.4	64.8a	63.2b	74.3b	53.9b	63.5a	67.2b	81.7a
	1.6	75.8a	75.4ab	76.9ab	71.6ab	85.6a	73.8b	68.3
	1.8	99.4a	73.9ab	73.4b	74.2ab	129.2a	142.1a	73.2a
	LSD _{0,01}	47.9	30.4	24.5	29.9	78.1	49.3	58.4

In the second cycle of the experiment, a set of oxalic acid concentrations from 2 to 4 mM was tested (Table 2). The reaction of the tested genotypes to these concen-

trations was more similar to the reaction in the field than in the previous set. This especially stands for concentrations of 2, 3 and 4 mM. The resistant genotype did not react to the treatment with the 2 mM of oxalic acid, while this concentration had negative effect on height of both susceptible genotypes (Table 2). The highest concentration (4 mM) had negative effect on all tested parameters of the susceptible genotypes, and height, leaf length and dry and fresh weight of root of the resistant genotype (Table 2). Plant height could be considered as a potential parameter of general resistance/susceptibility to *Sclerotinia* as it was affected in both susceptible genotypes in a similar manner. Another interesting parameter is root length. Beside small coefficient of variability (data not shown), it was not affected by treatment in the resistant genotype and was significantly decreased in the susceptible ones in the presence of the highest concentration of oxalic acid. Concentration of 3 mM was interesting as it probably could be used to discriminate resistance/susceptibility to different forms of *Sclerotinia*, since the reaction to treatment in the two susceptible genotypes differed the most when this concentration was used (Table 2).

Table 2: Reaction of tested genotypes on treatment with different concentrations of oxalic acid. Within the column and the genotype means followed by different letter differ. Legend for traits: h - plant height, fm - fresh weight of above-ground part, dm - dry weight of above-ground part, l - length of first pair of leaves, rfm - fresh weight of root, rdm - dry weight of root, rl - root length.

Genotype	Conc. mM	Trait (%)						
		h	fm	dm	l	rfm	rdm	rl
PR-ST-3B	Control	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
	2.0	75.3ab	97.7a	116.9a	96.6ab	75.9ab	82.0ab	128.9s
	2.5	63.0bc	112.9a	137.5a	116.5a	68.4ab	81.0ab	124.2a
	3.0	58.2bc	98.7a	106.1a	57.9bc	77.9ab	82.3ab	119.4a
	3.5	43.8c	87.4a	93.8a	11.9d	48.9b	54.5b	105.7a
	4.0	52.5c	96.9	102.7a	18.7cd	59.9b	68.8b	103.8a
	LSD _{0.01}	24.7	32.3	45.3	41.1	37.5	29.0	38.34
CMS ₁ -90A	Control	100.0a	100.0a	100.0ab	100.0a	100.0a	100.0a	100.0a
	2.0	81.9b	40.3b	92.2b	88.7a	86.9ab	93.8ab	91.5a
	2.5	81.6b	44.3b	102.4ab	91.7a	86.4ab	87.2ab	95.5a
	3.0	54.5c	35.3b	88.6b	53.1b	67.7b	85.2ab	82.2ab
	3.5	50.9cd	50.1b	88.1b	28.8c	61.5b	72.8b	70.7b
	4.0	37.1d	44.6b	115.2a	5.9c	67.8b	93.8ab	69.5b
	LSD _{0.01}	14.2	33.5	21.7	23.35	27.2	24.9	20.8
OD-3369A	Control	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
	2.0	57.2b	70.2ab	83.8a	63.6bc	79.1ab	65.4ab	65.4b
	2.5	71.1b	87.9ab	101.6a	73.1b	86.5ab	65.4ab	74.0ab
	3.0	58.8b	72.1ab	79.1a	45.6cd	75.6ab	61.6ab	62.1b
	3.5	61.9b	85.5ab	103.2a	54.1bcd	85.4ab	72.7ab	63.2b
	4.0	51.4b	66.6b	90.2a	31.4d	40.1b	41.5b	33.6c
	LSD _{0.01}	25.4	32.2	31.8	24.8	50.82	51.3	27.8

Concentrations of oxalic acid higher than 4 mM were found not to be reliable for the selection for *Sclerotinia* resistance as the results obtained were not in correlation with the field results (Table 3). All tested concentrations affected both resistant and susceptible genotypes. This is in disagreement with the results of Mouly (1989) who found concentrations of oxalic acid lower than 4.44 mM not selective in bioassay with sunflower leaves. The same author recommended a concentration of 8.88 mM as optimal.

Table 3: Reaction of tested genotypes on treatment with different concentrations of oxalic acid. Within the column and the genotype means followed by different letter differ. Legend for traits: h - plant height, fm - fresh weight of above-ground part, dm - dry weight of above-ground part, l - length of first pair of leaves, rfm - fresh weight of root, rdm - dry weight of root, rl - root length.

Genotype	Conc. mM	Trait (%)						
		h	fm	dm	l	rfm	rdm	rl
PR-ST-3B	Control	100.0a	100.0b	100.0b	100.0a	100.0a	100.0a	100.0a
	4.2	35.9b	115.6a	129.3a	56.1b	20.1c	29.4bc	58.2bc
	4.4	27.9bc	67.5d	92.5c	50.8bc	27.6c	50.5b	45.0cd
	4.6	19.1c	81.3c	77.3e	41.2c	44.4b	15.6c	82.2ab
	4.8	20.2c	45.5e	85.7d	55.0b	14.9d	36.8bc	17.8d
	LSD _{0,01}	10.8	5.2	4.2	12.0	15.5	22.7	34.5
CMS ₁ -90A	Control	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
	4.2	26.1b	58.1b	74.8b	26.3b	26.3b	30.3a	53.8b
	4.4	13.6c	46.7bc	63.8bc	7.5bc	12.1b	55.9a	17.4b
	4.6	22.0bc	42.8c	61.0c	4.5c	30.7b	70.8a	39.9b
	4.8	19.0bc	56.6bc	75.2b	14.6bc	15.3b	60.7a	21.1b
	LSD _{0,01}	9.2	14.6	11.9	2.9	26.6	106.1	43.2
OD-3369A	Control	100.0a	100.0a	100.0a	100.0a	100.0	100.0b	100.0a
	4.2	59.7b	80.2b	91.4a	77.0ab	61.9c	93.8b	48.4c
	4.4	39.2b	61.4c	70.8bc	61.3b	49.8d	72.6c	53.7c
	4.6	56.4b	69.1bc	75.4b	55.9bc	89.4b	126.0a	77.1b
	4.8	27.1	42.9d	59.6c	27.0c	12.9e	26.0d	23.4d
	LSD _{0,01}	39.6	18.4	13.2	33.1	9.3	12.6	12.7

CONCLUSION

The results obtained in our study were in accordance with the results of Hartman *et al.* (1988), Raducanu and Soare (1992), and Vasić *et al.* (1999) who found correlation between the tolerance to oxalic acid in the calli and protoplasts and the resistance of field grown plants. Of the tested parameters, plant height and root length were found to be the most reliable indicators of plant resistance/susceptibility to *Sclerotinia*. Based on the data obtained, oxalic acid concentrations of 4 mM, 3 mM and 2 mM were chosen for further work in which test would be done on a larger number of genotypes.

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**UTILIZACION DEL ACIDO OXALICO PARA LA
INVESTIGACION IN VITRO DE LA RESISTENCIA A
Sclerotinia EN LAS PLANTAS DE GIRASOL NO DANADAS**

RESUMEN

Tres líneas consanguíneas del girasol que son recíprocamente diferentes en el grado de resistencia a *Sclerotinia* eran cultivadas en el fondo nutritivo al cual eran añadidas diversas cantidades de ácido oxálico. Después de dos semanas del cultivo, eran medidas la altura de la planta, masa de la parte fresca y seca de superficie, el largo de la raíz y la masa de la raíz fresca y seca. Los datos obtenidos eran analizados por medio de los testes ANOVA y LSD. Entre los parámetros investigados, la altura de la planta y el largo de la raíz eran los indicadores más ciertos de la resistencia/sensibilidad a *Sclerotinia*. A base de los datos obtenidos, las concentraciones de ácido oxálico de 4 mM, 3 mM y 2 mM fueron seleccionadas para el trabajo ulterior durante el cual será investigado el número considerable de los genotipos del girasol.

UTILISATION DE L'ACIDE OXALIQUE DANS L'EXAMEN IN VITRO DE LA RÉSISTANCE ENVERS LA *Sclerotinia* SUR DES PLANTES DE TOURNESOL INTACTES

RÉSUMÉ

Trois lignes inbred différentes par leur résistance à la *Sclerotinia* ont été cultivées dans un milieu nutritif auquel ont été ajoutées différentes concentrations d'acide oxalique. Après deux semaines de culture, la hauteur de la plante, la masse des parties aériennes fraîche et sèche, la longueur de la première paire de feuilles, la longueur de la racine et la masse de racine fraîche et sèche ont été évaluées. Les données obtenues ont été analysées au moyen de tests ANOVA et LSD. Parmi les paramètres examinés, ce sont la hauteur de la plante et la longueur de la racine qui sont apparues comme les indicateurs les plus sûrs de la résistance ou de la sensibilité à la *Sclerotinia*. À partir des données obtenues, les concentrations d'acide oxalique de 4 mM, 3 mM et 2 mM ont été choisies pour des recherches ultérieures au cours desquelles on pourra tester un plus grand nombre de génotypes.

