Results regarding the influence of *in vitro* stress induced by the *Phomopsis helianthi* filtrate on some physiological indices and on sunflower oil quantity and quality

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ABSTRACT

During 1997-2005 at ARDI Fundulea, many experiments for *in vitro* testing and selection of some Romanian sunflower genotypes with tolerance to *Phomopsis helianthi* have been performed. Fourteen out of the 30 tested genotypes were selected for their good response to the *in vitro* culture. Following the treatment applied on MS culture medium supplemented with 150ml/l filtrate, and, on the basis of the results obtained regarding the leaf index, chlorophyll content, 1000-kernel weight, seed oil percentage and its composition, genotypes with increased resistance to this pathogen have been selected. The determinations were performed by the Minolta Chlorophyll meter (SPAD units) for chlorophyll contents, nuclear magnetic resonance for oil content, and gas-chromatography for fatty acid composition of the seed oil.

Key words: chlorophyll content – *in vitro* culture – *in vitro* testing and selection – *Phomopsis helianthi*.

INTRODUCTION

Phomopsis helianthi (Diaporthe helianthi), causal agent of stem canker, is one of the most important pathogens of sunflower in Europe. It can cause significant losses in yield (10±50%) and in oil content (10±15%) when the environmental conditions are favorable for disease development. Stem canker was noticed for the first time in Yugoslavia in 1980 and in Romania in 1981 (Vrânceanu et al., 1992; Vrânceanu, 2000). In 1994, the inocula of *Phomopsis* were present in all the areas where sunflower is grown (Vear et al., 1997).

Using *in vitro* screening, the goals of this study were to contribute to the knowledge regarding the influence of stress induced by *Phomopsis helianthi* filtrate on some Romanian inbred lines and to the identification of inbred lines with a high level of tolerance to the pathogen (Raducanu et al., 1997a, 1997b; Raducanu, 1998; Hagima and Raducanu, 1998; Raducanu et al., 2002; Raducanu and Moraru, 2003; Raducanu et al., 2005).

MATERIALS AND METHODS

For *in vitro* testing to *Phomopsis helianthi* pathogen, a total of 14 Romanian inbred lines were used. As explants, immature embryos collected 10 days after pollination were inoculated on an MS medium, supplemented with 150ml/l *Phomopsis helianthi* filtrate and incubated for 21 days at 27ºC, 12/12 light/dark. After this period, phenotypically normal plants were transplanted into pots with a mixture of heavy soil and sand in 1:1 proportion and they were grown under controlled conditions until maturity.

On these plants, under different stages of vegetation, the following data were recorded: leaf index, chlorophyll content, TKW (thousand kernel weight), seed oil content and its composition.

The determinations were performed by the Minolta chlorophyll meter (SPAD units) for chlorophyll content, nuclear magnetic resonance (NMR) for oil content, and gas-chromatography (Shimadzu-GC-14B) for seed oil fatty acid composition.

The fatty acids were analyzed according to the conventional method (Schulte and Weber, 1989). The transesterification of triglycerides to fatty acid methyl esters was performed with trimethylsulfoniumhydroxid (TMSH). A capillary column (25 MX 0.32 MM ID) of 25m length on a Shimadzu gas chromatograph with flame ionization detector (FID) was used. Injector and detector were kept at 270 and 280 ºC, respectively. The carrier gas was nitrogen, with a flow rate of 20 ml/min. To calculate the total area of the peaks, an electronic integrator was used. The area of each fatty acid peak was expressed as a percentage of the total area.

The leaf index was calculated by the following formula:

L x l x 0.66 (L=length; l=width; 0.66=correction coefficient).

RESULTS

The ANOVA analyses and effects of *Phomopsis helianthi* filtrate on leaf index, chlorophyll content, 1000-kernel weight, seed oil percentage and its composition are presented in Tables 1 to 9.

Table 1. ANOVA of the leaf area of some Romanian sunflower genotypes after *Phomopsis helianthi* treatment

Source of variation	SS	DF	MS	F value
Genotypes (A)	34198.51	13	2630.65	60.049***
A error	1139.02	26	43.808	-
Treatment (B)	2584.12	1	2584.12	67.034***
A x B	4020.47	13	309.267	8.022***
B error	1079.37	28	38.549	

Table 2. The effects of *Phomopsis helianthi* filtrate on leaf area in some Romanian sunflower genotypes

NO.	**Genotypes**	Average leaf area (cm^2 / genotype)			
		Control		treatment	
		Average	Difference from average[1]	Average	Difference from control[2]
1	LC 4001	17.366	-16.125^^	22.800	-10.691
2	LC 4002	12.933	-20.558^^^	162.000	-17.291**
3	LC 4005	27.566	-5.925	25.466	-8.025
4	LC 4006	41.800	8.308	26.000	-6.891
5	LC 4007	23.866	9.625	14.100	19.391***
6	LC 4010	62.166	28.675^^^	44.000	10.508
7	LC 4011	36.166	2.675	34.833	1.341
8	LC 4016	14.600	-18.891^^	15.166	-18.325**
9	LC 4018	37.433	3.914	16.100	-17.391**
10	LC 4019	55.633	22.141^^^	31.833	-1.658
11	LC 4020	110.666	77.175^^^	62.566	29.075***
12	LC 4022	16.266	-17.225^^	11.533	-21.958***
13	LC 4024	68.300	34.802^^^	49.866	16.375***
14	LC 4025	21.766	-11.725^	20.166	-13.325*
	Average	39.037		35.216	

[1]^, ^^, ^^^ Significantly different from average for P<0.05; P<0.01; P<0.001, respectively.
[2]*, **, *** Significantly different from control for P<0.05; P<0.01; P<0.001, respectively.

Table 3. ANOVA of the chlorophyll content in some Romanian sunflower genotypes after *Phomopsis helianthi* filtrate treatment

Source of variation	SS	DF	MS	F value
Genotypes (A)	3418.58	13	262.968	19.575***
A error	349.271	26	13.433	-
Treatment (B)	460.615	1	460.615	43.124***
A x B	593.004	13	45.615	4.271***
B error	299.075	28	10.681	

Table 4. The effects of *Phomopsis helianthi* filtrate on chlorophyll content in some Romanian sunflower genotypes

NO.	**Genotypes**	Average chlorophyll content (SPAD/units)			
		Control		Treatment	
		Average	Difference from average[1]	Average	Difference from control[2]
1	LC 4001	24.500	-5.508	28.500	-1.508
2	LC 4002	22.433	-7.575	17.600	-12.408**
3	LC 4005	26.633	-3.375^^	20.766	-9.241**
4	LC 4006	30.233	0.225	23.866	-6.141**
5	LC 4007	30.400	0.391^^^	25.400	-4.608
6	LC 4010	35.800	5.791	36.000	-6.141**
7	LC 4011	35.800	5.791	39.866	9.858**
8	LC 4016	38.800	8.917^^	31.933	4.925
9	LC 4018	42.733	12.725	31.566	1.558
10	LC 4019	42.866	12.725	36.800	6.792**
11	LC 4020	42.733	12.725	31.266	-1.258
12	LC 4022	26.166	-3.841^^^	24.266	-5.741
13	LC 4024	38.500	8.492^^^	20.800	-9.408
14	LC 4025	22.866	-7.142^	18.900	-11.108***
	Average	32.890		27.680	

[1]^, ^^, ^^^ Significantly different from average for P<0.05; P<0.01; P<0.001, respectively.
[2]*, **, *** Significantly different from control for P<0.05; P<0.01; P<0.001, respectively.

Table 5. ANOVA of the TKW in some Romanian sunflower genotypes after *Phomopsis helianthi* filtrate treatment.

Source of variation	SS	DF	MS	F value
Genotypes (A)	819.046	13	63.003	21.632***
A error	75.726	26	2.912	-
Treatment (B)	150.934	1	150.934	117.678***
A x B	77.645	13	5.972	4.657***
B error	35.912	28	12.826	

Table 6. The effects of *Phomopsis helianthi* filtrates on TKW in some Romanian sunflower genotypes

NO.	**Genotypes**	Average TKW(g)			
		Control		Treatment	
		Average	Difference from average[1]	Average	Difference from control[2]
1	LC 4001	13.766	-0.408	13.533	-0.640
2	LC 4002	18.733	4.599^^^	18.366	4.192**
3	LC 4005	20.966	6.792^^^	18.900	4.726***
4	LC 4006	21.223	7.059^^^	15.600	1.426
5	LC 4007	12.300	-1.874	10.366	-3.807**
6	LC 4010	16.800	2.626^	10.600	-3.574**
7	LC 4011	17.566	3.392^^	15.466	1.292
8	LC 4016	14.666	0.492	13.033	-1.106
9	LC 4018	14.000	-0.174	10.933	-3.240**
10	LC 4019	14.033	-0.140^^^	10.466	-3.707
11	LC 4020	18.933	4.759^^^	12.933	-1.240
12	LC 4022	12.700	-1.474	11.100	-3.074**
13	LC 4024	11.700	-2.474^	9.400	-4.744***
14	LC 4025	9.700	-4.474^^^	8.966	-5.207***
	Average	14.459		12.833	

[1]^, ^^, ^^^ Significant different from average for P<0.05; P<0.01; P<0.001, respectively.
[2]*, **, *** Significantly different from control for P<0.05; P<0.01; P<0.001, respectively.

Table 7. ANOVA of the oil content in some Romanian sunflower genotypes after *Phomopsis helianthi* filtrate treatment

Source of variation	SS	DF	MS	F value
Genotypes (A)	1302. 19	13	100.169	48.209***
A error	54.022	26	20. 778	-
Treatment (B)	516.031	1	512.031	160.344***
A x B	195.958	13	15.073	4.684***
B error	90.111	28	3.218	

Table 8. The effects of *Phomopsis helianthi* filtrate on the oil content in some Romanian sunflower genotypes

NO.	**Genotypes**	Average oil content (%)			
		Control		Treatment	
		Average	Difference from average[1]	Average	Difference from control[2]
1	LC 4001	29.333	- 4.972^^^	27.200	- 7.104***
2	LC 4002	37.700	3.395^	27.600	6.671***
3	LC 4005	39.266	4.961^^^	33.633	0.671
4	LC 4006	39.200	4.895^^^	36.333	2.028
5	LC 4007	27.200	- 7.104^^^	29.033	- 5.271***
6	LC 4010	41.900	7.595^^^	36.000	1.695
7	LC 4011	29.933	- 4.371^^^	27.366	-6.938***
8	LC 4016	36.233	1.928	28.433	-5.871***
9	LC 4018	38.033	3.728^^^	33.333	1.005
10	LC 4019	41.100	7.795^^^	38.500	4.195*
11	LC 4020	43.866	9.561^^^	35.400	1.095
12	LC 4022	38.866	4.561^^^	30.000	- 4.305*
13	LC 4024	33.700	- 0.538	30.133	-4.171*
14	LC 4025	37.566	3.261^	34.033	-2.272
	Average	36.706		31.928	

[1]^, ^^, ^^^ Significantly different from average for P<0.05; P<0.01; P<0.001, respectively.
[2]*, **, *** Significantly different from control for P<0.05; P<0.01; P<0.001, respectively.

Table 9. Retention time for fatty acids from the standard solution

No. peak	Retention time (min.)	Fatty acid (formula)	The fatty acid
1	12.55	C 16:0	Palmitic acid
	17.48	C 18:0	Stearic acid
2	17.48	C 18:1	Oleic acid
3	20.36	C 18:2	Linoleic acid
4	22.65	C 18:3	Linolenic acid

DISCUSSION

The results obtained by gas chromatography underlined the fact that of the five fatty acids from sunflower oil, oleic acid decreases after treatment in all genotypes, excepting the LC 4010 line. At the same time, the linoleic acid percentage increases after treatment in nine out of the tested lines. We positively noticed the fact that the linolenic acid, which reduces oil stability, was detected only in three genotypes but in very small quantities. ANOVA for the leaf index emphasized a very different behavior of the tested lines, with significant positive or negative differences between genotypes, depending on both tolerance degree to disease and response to the *in vitro* culture. Eight genotypes in which the leaf area was not diminished by the treatment as compared with the control have been identified.

As regards the chlorophyll content, it was ascertained that for all tested genotypes, at the treatment variant, the average/variant was diminished with 5.2 SPAD units vs. the control.

In variants treated with the filtrate, TKW was drastically diminished in seven out of the 14 genotypes. The oleic acid content showed a higher decrease in comparison with the control in all lines excepting the LC 4010 line.

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