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STUDIES ABOUT THE INFLUENCE *OF SCLEROTINIA SCLEROTIORUM* FILTRATES ON SOME QUANTITATIVE AND QUALITATIVE TRAITS IN ROMANIAN SUNFLOWER GENOTYPES *IN VITRO* AND *IN VIVO* TESTED

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Sclerotinia sclerotiorum Lib. de Bary was identified as a pathogen in *Helianthus annuus* L. by Funckel in 1861 and is nowadays considered a most important disease. Up to now no sunflower genotype with to *Sclerotinia* resistance has been found, because this pathogen has various infection ways.

In this paper we studied the effect of *Sclerotinia sclerotiorum* filtrates on some quantitative (oil percentage, weight of 1000 seeds) and qualitative (spectrum of helianthinin) traits on one side and evaluation of sunflower genotypes variability for resistance at this pathogen, on the other side.

Nine sunflower hybrids and eight inbred lines *in vitro* and *in vivo* with *Sclerotinia sclerotiorum* filtrates were tested.

The *in vitro* infection was done by supplemented the MS medium with 150 ml *Sclerotinia sclerotiorum* filtrates. Immature embryos, 10 day old, were inoculated and incubated for two weeks at 21 ^{0} C, at 12 hours daily photo period.

After this period, the normal plants were transferred in a mixture soil/sand, 1/1 and were growth until technical maturity in green house.

For *in vivo* testing the same genotypes were sown in the field and at button stage were infected by injected with 10 ml/plant *Sclerotinia sclerotiorum* filtrates.

The results obtained relieved that the weight of 1000 seeds was negatively influenced by *in vivo* treatments (lower was 11.2 g at LC-1050; 65 g at HA-300 genotypes as compared with control). The oil content (%) was significant lower *in vivo* treatment for all studied genotypes, except LC-1050 and LC-1020 inbred lines.

Regarding helianthinin spectrum the image obtained were specified for each genotype and treatment applied.Information concerning genetic variability of sunflower hybrids for *Sclerotinia sclerotiorum* resistance, would assist sunflower breeding in the development of resistance genotypes.

Key words: *in vitro* infection; *Sclerotinia sclerotiorum*, resistance; helianthinin spectrum.

INTRODUCTION

White rot produced by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most damaging sunflower diseases in the main cropping areas. This pathogen produces great damage by its attack on all parts of the plant (root -rot, stalk, head).

Screening the reaction of sunflower genotypes to this pathogen is a problem not yet solved by the application of artificial field testing methods. In 1990, Godoy et. al. confirmed the determining role of oxalic acid in *Sclerotinia sclerotiorum* disease. In pointing out of the role of oxalic acid in this disease, they relied both on its discovery in the infected tissues of the host plant and on the correlation of the various symptoms of the disease after infection with oxalic acid or inoculation with *Sclerotinia sclerotiorum* which contains oxalic acid. Rãducanu et. al. (1995) in their study, *Sclerotinia sclerotiorum* filtrates obtained from infected sunflower roots, stalks, heads, oxalic acid, a mixture of sclerotia filtrates isolated from root-rot, stalk and head, for *in vitro* testing used.

Results regarding that the lowest virulence was observed in the case of using oxalic acid and the highest virulence was recorded in the case of the one isolated from the root-rot and also when the mixture was used.

In this study the investigations were carried:

-to study the effects of *Sclerotinia sclerotiorum* filtrates on weight of 1000 seeds, oil content (%) and helianthinin spectrum

- to identify tolerance / resistance sunflower genotypes in goal its use in breeding programs for resistance to *Sclerotinia sclerotiorum*

- the specific helianthinin spectrum of investigated genotypes and modifications induced by *Sclerotinia sclerotiorum* toxins.

MATERIALS AND METHODS

A total of eight sunflower inbred lines (LC-1004; HA-300; LC-1029; LC-1020; LC-1003; LC-996; LC-1010 and LC-1050) and nine sunflower hybrids (LC-1010 x LC-1050; LC1-004 x LC-996; LC-1010 x LC-996; HA-300 x LC-1004; LC-1020 x LC-1029; LC-1004 x HA-300; HA-300 x LC-1003; LC-1020 x LC-1050; LC-1020 x LC-996),for *Sclerotinia sclerotiorum* resistance, *in vitro* and *in vivo* were evaluated. All these genotypes provided by the sunflower breeding department of RICIC Fundulea.

For *in vitro* testing, immature embryos, 10 days old, on MS medium, supplemented with *Sclerotinia sclerotiorum* filtrates (V_1 = control, V_2 = MS + 150 ml / l medium, *Sclerotinia* filtrates isolated from head, V_3 = MS + 150 ml / l medium, *Sclerotinia* filtrates isolated from head T_{29} and V_4= MS + 150 ml / l medium, *Sclerotinia* filtrates isolated from root), were inoculated and incubated for two weeks at 21^0C and 12/12 light / dark.

After this period, phenotypic normal plants were transplanted in pots with a mixture of a heavy soil and sand in 1/1 proportion and were growth until technical maturity in controlled conditions.

For *in vivo* testing the same genotypes were sown in the field and at the flowering button stage, with the same *Sclerotinia sclerotiorum* filtrates (like *in vitro*) by injection with 10 ml filtrates / plant, were infected.

The following data were recorded: weight of 1000 seeds, oil content (%) and helianthinin spectrum; for the quantitative traits, ANOVA and correlation between *in vitro* and *in vivo* results, were used.

For separation of helianthinin, a modified method after I.N. Anisimova at. al. 1991 was used.

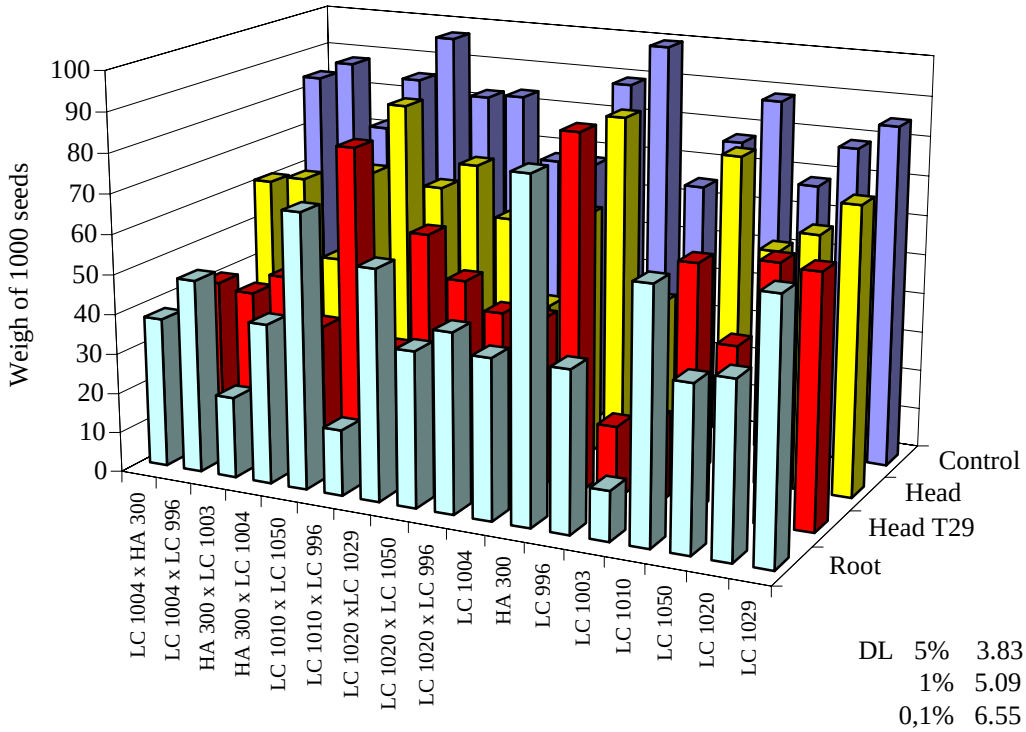
RESULTS AND DISCUSSIONS

ANOVA for weight of 1000 seeds at *in vitro* tested, on media supplemented with different *Sclerotinia sclerotiorum* filtrates (head, head T29, root) have shown a significant interactions between genotype and inoculum type, F factor is registering highly significant value both the genotype and treatment and genotype x treatment interaction.

The best tolerance was found at LC-1029 and HA-300 inbred lines and at the genotypes where these lines are present. At these genotypes the weight of 1000 seeds decreased the lowest after *in vitro* treatment but it was significant.

With these excepts, all the tested genotypes showed a sensitive reaction at the *Sclerotinia sclerotiorum* filtrates and the strongest virulence was observed at the root isolated (Fig. 1).

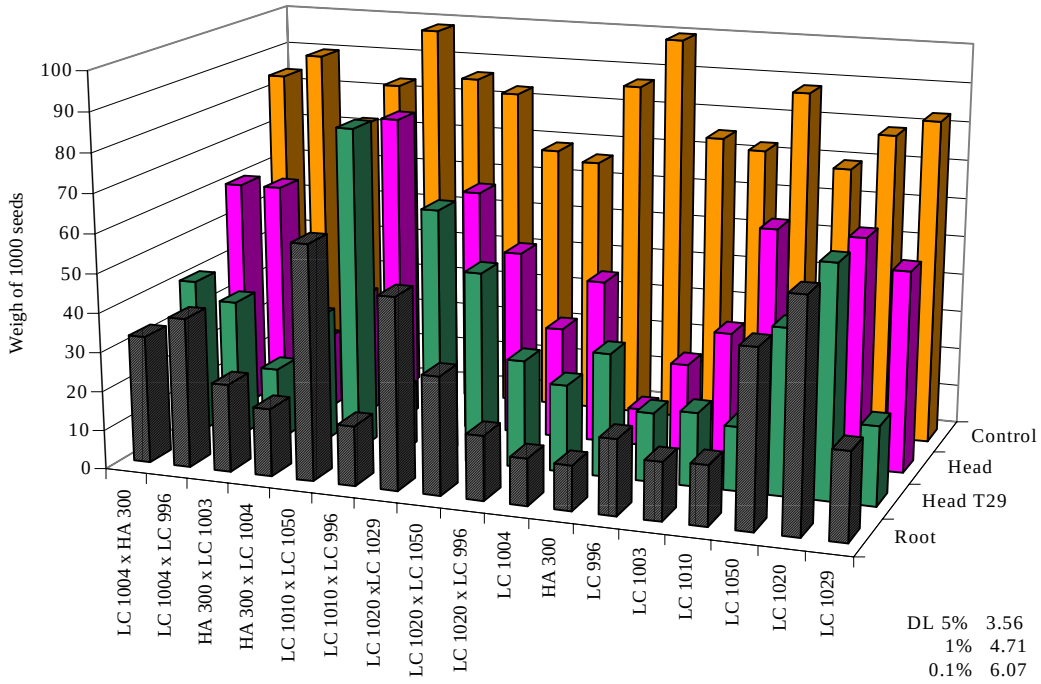
Fig. 1 Influence of *Sclerotinia sclerotiorum* filtrates on weight 1000 seeds at *in vitro* testing



Source of variation	SS	DF	MS	F value
A factor	24258.67	16	1516.16	328.05**
A error	147.89	32	4.62	-
B factor	9523.91	3	3174.63	565.94**
A x B	9191.42	48	191.48	34.1365**
B error	572.16	102	5.6	-

In the case of the treatments *in vivo*, weight of 1000 seeds was drastic diminished at all the genotypes except LC-1020, LC-1050, LC-1010 x LC-1050 genotypes, at the V_3, comparatively with the control. This parameter varied between 80 g (LC-1010 x LC-1050) at V_1 and 11.437 g(HA-300) at V_3 (Fig.2).

Fig. 2 Influence of *Sclerotinia sclerotiorum* filtrates on weight 1000 seeds at *in vivo* testing

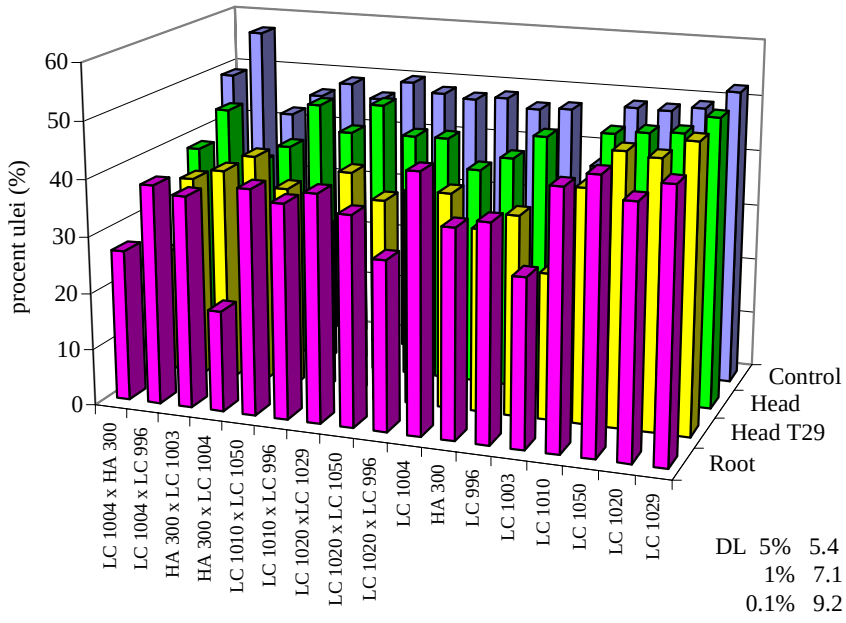


Source of variation	SS	DF	MS	F value
A factor	2829.73	16	1768.17	481.56**
A error	117.49	32	3.67	-
B factor	47682.16	3	15894.08	3298.18**
A x B	18697.67	48	389.53	80.83**
B error	491.54	102	4.81	-

Concerning at the oil content parameter, *in vitro* testing have shown that this was affected by decreased from 57.273% (LC -1004 x LC-996) at control, until 38.850% at V_3.

The lowest decreased was registered at LC-1020 x LC-1029 and LC-1050, from 50.07% (control), at 40.03 (V_3); 48.275% (control) at 47.261% (V_3), respectively (Fig.3).

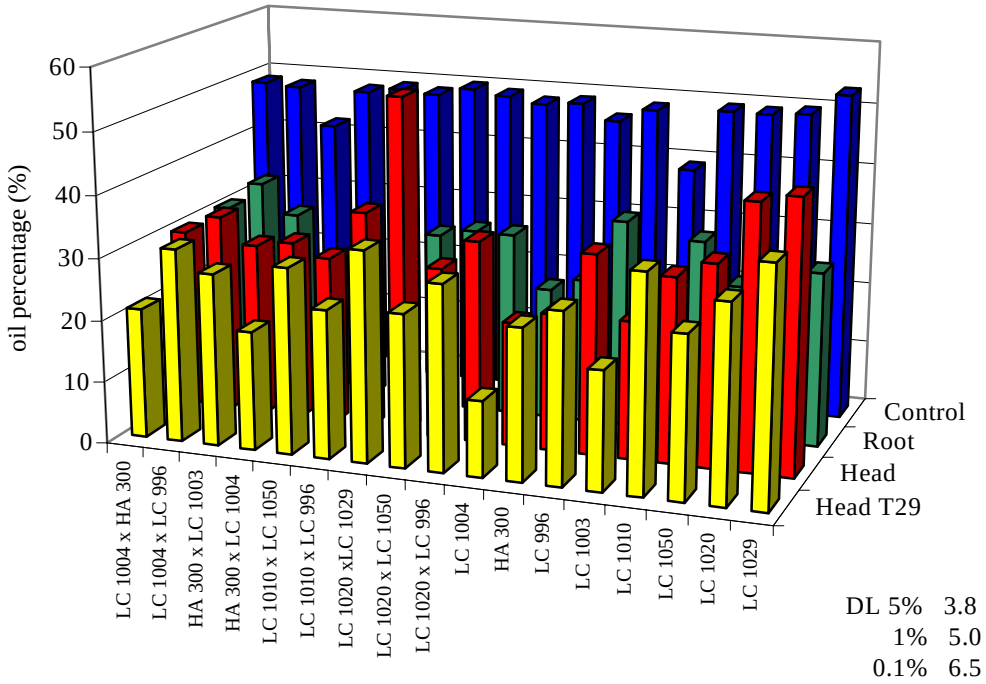
Fig. 3 Influence of *Sclerotinia sclerotiorum* filtrates on oil content at *in vitro* testing



Source of variation	SS	DF	MS	F value
A factor	2981.07	16	186.31	12.65***
A error	471.05	32	14.72	-
B factor	1773.9	3	591.3	53.43***
A x B	2106.63	48	43.88	3.96**
B error	1128.67	102	11.06	

Oil content was diminished much more in the case the *in vivo* treatments, but LC -1029, LC-1010 and LC-1010 x LC-1029 showed an high degree of tolerance for all type of filtrates used. Content oil varied between 54.5% (LC-1020 x LC-1029) at control and 20.5% (LC-1010 x LC-996) at V_3 (Fig.4).

Fig. 4 Influence of *Sclerotinia sclerotiorum* filtrates on oil content at *in vivo* testing



Source of variation	SS	DF	MS	F value
A factor	3348.84	16	209.3	50.06***
A error	133.73	32	4.18	-
B factor	8649.92	3	2883.3	519.72***
A x B	2111.82	48	43.99	7.93**
B error	565.87	102	5.54	-

Between the *in vitro* and *in vivo* results obtained after treatments applied for the weight 1000 seeds and oil content, a significantly correlation was found (Fig.5).

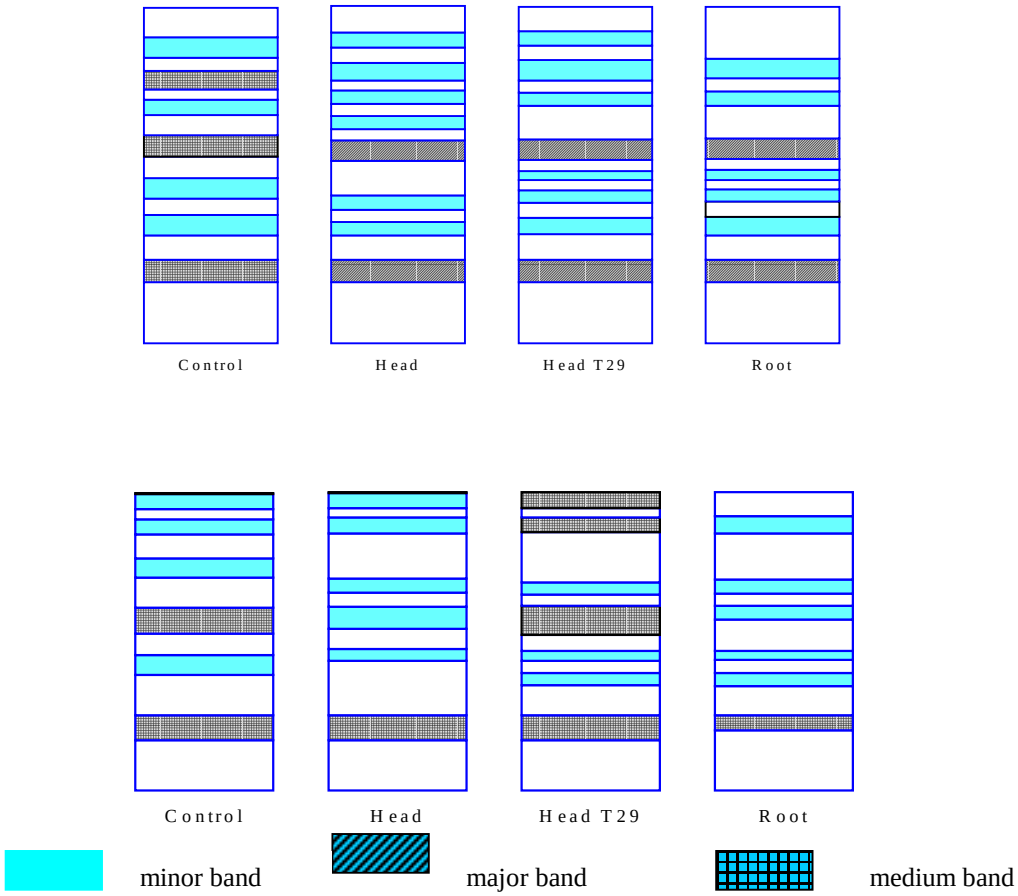
Fig.5 Correlation between the *in vitro* and *in vivo* reaction at treatments with *Sclerotinia sclerotiorum* filtrates

Trails	**weight 1000 seeds; %oil (head)**	**weight 1000 seeds; %oil (head T_{29})**	**weight 1000 seeds; %oil (root)**
weight 1000 seeds%oil (head)	**0.457*** **0.837*****		
weight 1000 seeds%oil (head T_{29})		**0.622**** **0.861*****	
weight 1000 seeds%oil (root)			**0.493*** **0.537***

Concerning the influence of toxins on helianthinin spectrum, our results indicated that this was specific for each genotype and treatment. For example, at LC-1050, toxins determined appearance of new two helianthinin bands with medium electrophoretic migration and reduced of number of bands with high molecular weight.

For LC-1020 genotype the helianthinin spectrum was influenced both the toxins and the genotype;so, two bands were clear revealed near the start and width of band with high molecular weight was less compared with the control (Fig.6).

Fig. 6. Electrophoretic spectrum of helianthinin at LC 1050 and LC 1020



CONCLUSIONS

The correlations between the results obtained at *in vitro* and *in vivo* testing permited the utilisation of the first method (beside by clasical method); its advantages are: practise economyc of time and space.

The culture filtrates used for testing had some effects on studied parameters: reduced of weight 1000 seede, oil content and modification of helianthinin spectrum, respectively.

The helianthinin electrophoretic spectrum was specific for each genotypes but it was influenced by the toxins types and also by infection method applied.

REFERENCES

ANISIMOVA, I.N., GAVRILJUK, I.P., KONAREV, V.G. 1991 - Identification of sunflower lines and varieties by helianthinin electrophoresis. Plant Varieties and Seeds, 4, 133-141.

GODOY, G., STEADMAN, J.R., DICKMAN, M.B., DAM, R.1990 - Use of mutants to demonstrate the role of oxalic acid in pathogenity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris.* Physiol. Mol. Pl. Path. 37: 179-191.

RÃDUCANU, F., SOARE, G., CRAICIU, D.S. 1995 - Screening the reaction of sunflower genotypes to *Sclerotinia sclerotiorum* Lib. de Bary by anther culture. RAR, pg.1-5.