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Effect of *Sclerotinia sclerotiorum* on Sunflower Seeds Quality

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Abstract: The investigation was carried out at Dobrudzha Agricultural Institute – General Toshevo, Bulgaria, and encompassed the period 2009–2010. The experiment involved four double haploid fertility restorer lines (DH-R-2, DH-R-7, DH-R-116 and DH-R-128), which have shown in our previous studies various degree of resistance to *Sclerotinia sclerotiorum* de Bary under artificial infection conditions. Ten plants from each investigated line were inoculated by the Straw-method at stage 5-6th pair of leaves. The plants were self-pollinated and the seeds obtained from them, as well as the seeds from the check plants (not infected), were analyzed for the traits 1000 seed weight, % of kernel, oil in kernel and protein content in kernel. Variations in the quality characteristics of the sunflower seeds were found in all investigated lines, the degree of quality "deterioration" having different expression according to the tolerance of the line to the pathogen. Lines DH-R-2 and DH-R-7 formed seeds with lower seed weight and percent of kernel. These seeds had lower oil and protein content. The established variations in the quality of the seeds between lines DH-R-116 and DH-R-128 and the check variants were not statistically significant.

Keywords: sunflower, Sclerotinia sclerotiorum, 1000 seed weight, percent of kernel, oil and protein content in kernel

Introduction

Many diseases caused by fungi, bacteria and viruses, are capable of deteriorating the quality and quantity of sunflower seed production. One of the most important fungal pathogen is *Sclerotinia sclerotiorum* (Lib.) de Bary which is distributed in almost all production regions. *Sclerotinia* wilt (root rot), middle stalk rot, and head rot are recognized in sunflower fields. *Sclerotinia* disease can cause serious yield losses of sunflower. The losses may be up to 100 % under

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suitable conditions (20 °C and 70 % air humidity) in fields where the fungus is spread (Ivanov *et al*., 1989; Ivanov, 1998; Mairevi and Gulya, 1992; Rashid, 1993; Leite, 2014).

The most effective way to reduce damages from sclerotinia attack is developing of forms with genetic resistance which is a priority in a number of research projects and investigations (Ronicke *et al*., 2004; Ronicke *et al*., 2005). To date, sunflower genotypes with different levels of resistance to *Sclerotinia sclerotiorum* have been identified, but no fully resistant genotypes are available (Davar *et al*., 2012). Resistance is the ability of an organism to exclude or overcome, completely or in some degree, the effect of a pathogen or other damaging factor (Agrios, 1988).

The establishment of parental lines of hybrids with some degree of resistance to the pathogen is a prerequisite for the creation of tolerant hybrids and respectively for the reduction of the losses in yield and quality of sunflower seeds caused by sclerotinia. In this relation it is interesting to consider the question to what extent the quality characteristics of the sunflower seeds produced by plants of R lines with some degree of resistance to sclerotinia maintain their values under artificial infection with the pathogen.

The aim of this investigation was to clarify the influence of *Sclerotinia sclerotiorum* mid-stalk rot on some quality characteristics of sunflower seeds by artificial infection of the plants.

Materials and methods

The field experiments were carried out at Dobrudzha Agricultural Institute – General Toshevo, Bulgaria. The investigation was performed with four doubled haploid R-lines developed by the method of gamma-induced parthenogenesis. The lines were of different origin: lines DH-R-116 and DH-R-128 were produced from experimental hybrid combination in which one of the parental lines originated from interspecific cross between *H.annuus* and *H. argophyllus*; lines DH-R-2 and DH-R-7 were obtained from *H. annuus* L. hybrids. The lines differed by their response to artificial inoculation with *Sclerotinia sclerotiorum*, line DH-R-128 demonstrating considerable tolerance to the pathogen, while line DH-R-7 showed high susceptibility to sclerotinia (Drumeva *et al*., 2008). The other two lines had response within the range outlined by DH-R-128 and DH-R-7.

The design of the experiment was uniform in the both years of the study. 30 plants of each of the investigated R lines were grown in plots of 7.35 m2. Randomised ten plants at stage 5-6th pair of leaves from each variant were

inoculated under field conditions by the Straw-method (Encheva and Kiryakov, 2002), successfully applied from the authors for artificial inoculation of sunflower plants with *Phomopsis helianthi*. The artificial infections with *Sclerotinia sclerotiorum* of all plants was done the same day. Plants were inoculated at stage 5-6th pair of leaves. A petiole of the fourth pair of leaves from each plant was cut, so that 1.5–2 cm of it where left to the stem. A plastic straw (30 × 6 mm) with one end closed was inserted in the place of incision (Figure 1). The straw contained agar disc from the periphery of a 3 days old culture of isolate Ss-1 on nutrition medium PDA at 22 ± 1 °C. The reaction of the plant was rated three times every 7 days according to a 6-degree scale (Drumeva *et al.*, 2008).

Figure 1: Artificial infection with sclerotinia and different degrees of damage on sunflower.

In parallel with this, 5 plants from each line were left uninfected for check variants. All plants, the inoculated ones and the checks, were deliberately self-pollinated. Seeds from all infected and uninfected plants were manually

collected at maturity stage, separately per plant. The harvested seeds of all infected plants in frame of each of the investigated lines were mixed and two mean samples each of 50 seeds were taken. The same procedure was followed prior to sampling the seeds of uninfected plants of each line. The following traits were analyzed: 1000 seed weight, % of kernel, % of oil in kernel and protein content in defatted kernel (%).

Thousand seed weight and the value of kernel were determined by analyzing two samples, each of 50 seeds. Oil percent was determined by the method of Rushkovsky (1957), and protein content – by Kjeldahl's method.

To assess the effect of Sclerotinia mid-stalk rot on the quality of the sunflower seeds depending on the genotype of the investigated lines and the year conditions, three-factor dispersion analysis was applied. The three sources of variation – year conditions (factor A), genotype of the investigated lines (factor B) and the influence of the pathogen on each of the studied quality indices of sunflower seeds (factor C) were analyzed by using the software SPSS 16.0.

The analysis was performed according to this model:

$$y_{ijk} = \mu + (\alpha)_i + (\beta)_j + (\gamma)_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + e_{ijk}$$

Using t-criterion, the statistical differences in the variations of the quality indices of the sunflower seeds in comparison to the check variants were determined, as well as the significance of the obtained results with regard to the investigated indices. Data were processed with the help of Biostat Version 5.1 (Penchev, 1998).

Results and discussion

Seed formation is a process influenced by multiple factors, including meteorological conditions, the response of the studied genotypes to inoculation with the pathogen and to the specific environment during the investigation. At seed formation of sunflower, the values of the mean monthly air temperatures are very important, especially during the period from flowering to seed maturity (July-August). Most favorable during this period is considered a mean monthly temperature within the range 22–25 ^{o}C (Pustovoit, 1975).

The temperature during April, when sunflower planting is usually done, was close to the norm in 2009, while in 2010 it was higher (Figure 2). The rest of the months were warmer than the norm in both years of investigation. Concerning vegetation rainfalls (April-September), in 2009 the sum of rainfalls was close to the climatic sum. In 2010 it was higher with 21.6 %. The precipitation in 2010

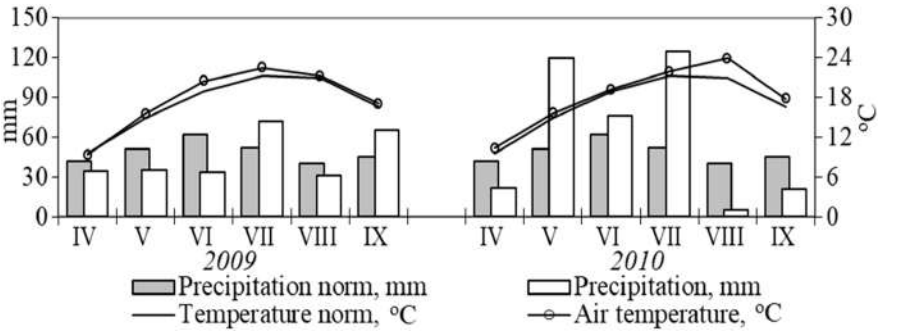


Figure 2: Monthly precipitation sums and air temperatures from April to September during 2009–2010.

significantly exceeded the precipitation in 2009, especially during May-June providing the necessary moisture in the critical stages of sunflower budding and flowering.

It can be summarized that the climatic conditions during 2010 were favorable for realization of the sunflower's production potential; simultaneously the favorable temperatures and the higher humidity in that year were a prerequisite for development of the fungal pathogens, including *Sclerotinia sclerotiorum* (Lib.) de Bary.

The year conditions, the genotype of the lines and the effect of the pathogen, considered separately, influenced to the highest degree of statistical significance the quality indices of the sunflower seeds: 1000 kernel weight and percent of kernel, while the combined effect of these three factors was not significant (Table 1).

The probable reason for the lack of statistical significance of the mutual effect of the three factors is the good plasticity of the sunflower plant which manifests its adaptability according to the variations of the vegetation conditions over years. Highest relative influence on the trait 1000 seed weigh had the genotype of the investigated lines: averaged for two years, its relative effect according to the other factors was 49.06 %. The relative effect of the year conditions according to this trait, averaged for the two years, was 22.47 %, and the effect of the pathogen – 20. 48 %.

Concerning percent of kernel in seed, the highest relative effect was that of the pathogen – it was 43.7 % averaged for two years. This allowed the conclusion that the degree of the pathogen's harmfulness on the quality characteristics of the sunflower seeds was manifested primarily through this trait. Dorrell and Huang (1978) also reported for significant reduction of seed

Table 1: Results from the dispersion analysis on the effect of the year conditions, the genotype of the investigated lines and the pathogen (*Sclerotinia sclerotiorum*) on some quality characteristics of seeds.

Indices	Source of variation	df	F	Sig
1000 seed weight (g)	Factor A. Years	1	33.412	0.000
	Factor B. Lines	3	24.316	0.000
	Factor C. Pathogen	1	30.46	0.000
	A x B	3	0.797	0.505
	A x C	1	1.622	0.212
	B x C	3	2.577	0.071
	A x B x C	3	0.046	0.987
Kernel (%)	Factor A. Years	1	15.218	0.000
	Factor B. Lines	3	5.817	0.003
	Factor C. Pathogen	1	36.858	0.000
	A x B	3	0.766	0.522
	A x C	1	0.641	0.429
	B x C	3	4.117	0.014
	A x B x C	3	0.25	0.861
Percent of oil in kernel (%)	Factor A. Years	1	3.554	0.069
	Factor B. Lines	3	40.034	0.000
	Factor C. Pathogen	1	26.719	0.000
	A x B	3	0.583	0.630
	A x C	1	2.19	0.149
	B x C	3	6.436	0.002
	A x B x C	3	0.135	0.938
Protein content in defatted kernel (%)	Factor A. Years	1	1.249	0.272
	Factor B. Lines	3	20.976	0.000
	Factor C. Pathogen	1	4.185	0.049
	A x B	3	0.044	0.987
	A x C	1	0.545	0.466
	B x C	3	4.564	0.009
	A x B x C	3	0.113	0.952

df – degree of freedom. F – Fisher's criterion.

weight of infected by the pathogen plants, as a result of the wilting. The interaction of the genotype of the lines with the pathogen also had some effect on the percent of kernel in seed but it was not significant. The genotype of the lines and the effect of the pathogen were determining for the accumulation of oil in kernel. There was also some effect of the year although without statistical significance. A number of authors point out in their investigations that oil content in seed is determined by the genotype

and the agro ecological conditions, the effect of which is strongly expressed at stage seed filling (Stanoević *et al.*, 1992).

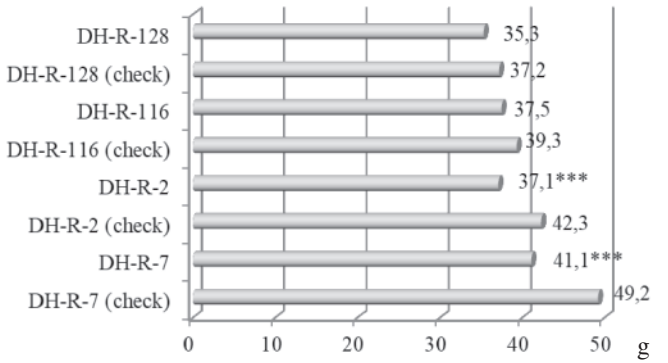
In this investigation the presence of an additional factor – the virulent influence of the pathogen, conditioned the highest effect of the genotype of the investigated lines for the formation of the above trait, and also the higher effect of the line-pathogen interaction. The relative effect on the formation of this trait, averaged for the two years of study, was 69.1% of the genotype and 11.09% of the genotype-pathogen interaction.

With regard to the other main quality characteristics of sunflower seeds – protein content in defatted kernel, mainly the effect of the genotype of the lines was statistically significant, with relative value of 75.75%. The interaction between the line and the pathogen also had certain effect on the trait but without statistical significance. The relative effect of this interaction on protein content in seeds was 16.48%. The year conditions did not have a significant influence on the qualitative expression of this trait. This is confirmed also by the investigations of other authors who point out that protein content in kernel is a relatively inert trait not significantly affected by the year conditions, and that the genotype has a determining role for the higher protein in sunflower seeds (Dorrell and Huang, 1978; Marinković *et al.*, 2003).

Variation in the qualitative characteristics of the sunflower seeds, averaged for the period of study, was found in all investigated lines, the degree of quality "deterioration" having different expression in the individual lines. Lines DH-R-7 and DH-R-2 suffered the strongest effect being highly susceptible to the pathogen (Drumeva *et al.*, 2008). In the other two lines (DH-R-116 and DH-R-128), some variations in the values of the qualitative indices were also registered, but the established differences were minimal and within the range of the acceptable statistical error; the only exception was observed with regard to percent of kernel.

Highest variations were found with regard to the traits 1000 seed weight (Figure 3) and percent of kernel in seed (Figure 4). Similar results were reported by Dorrell and Huang (1978). In the susceptible genotypes (lines DH-R-7 and DH-R-2), the reduction of the quantitative variations of the above two indices was with the highest degree of statistical significance.

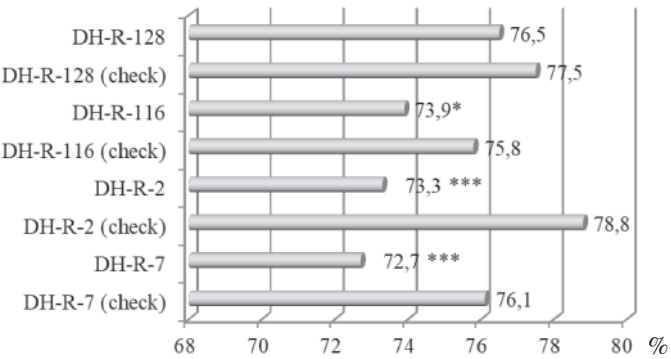
There was a significant variation ($P = 0.05$) in the percent of kernel in line DH-R-116 which demonstrated moderate resistance to the pathogen (Drumeva *et al.*, 2008). Since the relative effect of the pathogen was high with regard to this trait (43.7%), the observed variations can be interpreted unequivocally concerning the damages the pathogen caused on the quality of the sunflower seeds. Simultaneously, this index was directly related to the components of yield and can provide information on the pathogen's harmfulness not only on



GD 0.05 – 2.273 *GD 0.01 – 3.356* *GD 0.001 – 5.188*

*, **, *** – Significance of effects and variations at P levels 0.05, 0.01 and 0.001, respectively

Figure 3: Variations of the value of the trait 1000 seed weight in lines artificially inoculated with the pathogen (*Sclerotinia sclerotiorum*) averaged for the period of investigation.



GD 0.05 – 1.923 *GD 0.01 – 2.840* *GD 0.001 – 4.390*

*, **, *** – Significance of effects and variations at P levels 0.05, 0.01 and 0.001, respectively

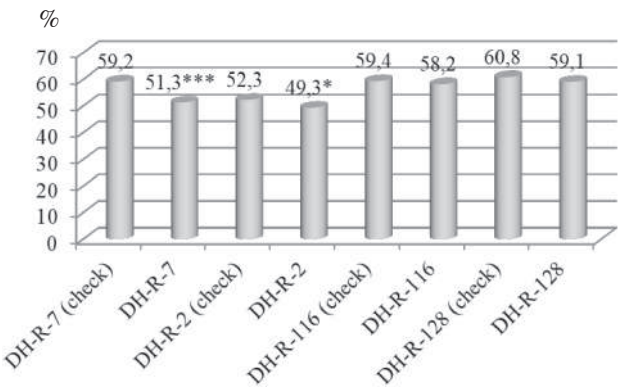
Figure 4: Variations in the value of the trait percent of kernel in lines artificially inoculated with the pathogen (*Sclerotinia sclerotiorum*) averaged for the period of investigation.

the qualitative indices but also on the production potential of the sunflower plant.

Investigating the effect of the *Sclerotinia scleriorum* on sunflower, Ivanov (1998) has also pointed out that the pathogen had greater influence on the elements of yield, a component of which are the traits 1000 seed weight and seed weight per head, than on the content of oil and protein in the kernel.

Similar conclusions were made also by Dorrell and Huang (1978). Other authors (Ivanov *et al*., 1989) have pointed out that the sunflower plants infected with *Sclerotinia sclerotiorum* form seeds with lower seed weight, with lower percent of kernel, and lower content of oil and protein.

In this investigation the dispersion analysis carried out proved the effect of the pathogen with regard to all investigated qualitative traits; the relative percent of this effect varied by traits but was highest on percent of kernel, which is one of the traits directly related to the elements of yield. At the same time, similar to Ivanov *et al*. (1989), we observed lower oil content in the kernels of lines which possessed higher susceptibility to the pathogen. Oil content in the kernel of line DH-R-7 was with 7.9 % lower than the check variant (P = 0.001), and in line DH-R-2 the oil content decreased with 3 % (P = 0.05) (Figure 5). In the other two lines the variations between the infected and the check variants were not significant.

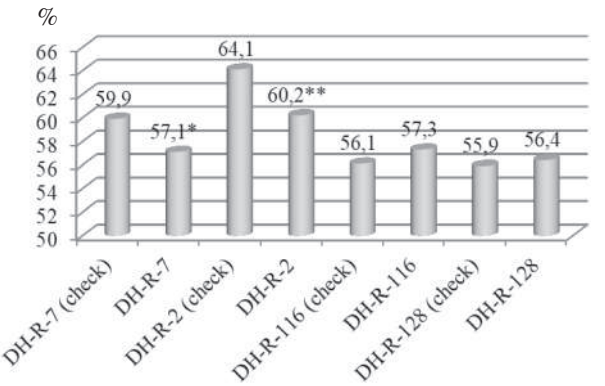


GD 0.05 – 2.534 *GD 0.01 – 3.742* *GD 0.001 – 5.784*

*, **, *** – Significance of effects and variations at P levels 0.05, 0.01 and 0.001, respectively

Figure 5: Variations in the percent of oil in kernel of lines artificially infected with the pathogen (*Sclerotinia sclerotiorum*), averaged for the period of investigation.

The protein content in the seeds of lines DH-R-H-R-2 also decreased, the differences according to the check variants being with lower degree of statistical significance (Figure 6). The protein content in kernel was lower with 2.7 % (P = 0.05) in DH-R-7, and with 3.9 % (P = 0.01) in DH-R-2. The variations between the check and the infected variants in the rest of the lines were not significant.



GD 0.05 – 2.284 *GD 0.01 – 3.373* *GD 0.001 – 5.213*

*, **, *** – Significance of effects and variations at P levels 0.05, 0.01 and 0.001, respectively

Figure 6: Variations in the percent of protein in defatted kernel of lines artificially infected with the pathogen (*Sclerotinia sclerotiorum*), averaged for the period of investigation.

Conclusions

The pathogen had significant effect on the investigated qualitative traits of the sunflower seeds – 1000 kernel weight, percent of kernel, oil in kernel and protein in kernel; this effect was relatively highest on the percent of kernel in seed.

The plants from the lines susceptible to the pathogen – DH-R-7 and DH-R-2 infected with sclerotinia formed seeds with lower weight, percent of kernel, oil and protein content. The established variations in the qualitative traits of the seeds of lines DH-R-116 and DH-R-128 and of the check variants were not significant.

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