RESEARCHES ON RESISTANCE SOME SUNFLOWER HYBRIDS TO THE ARTIFICIAL INFECTION WITH *SCLEROTINIA SCLEROTIORUM*

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Abstract

Among the main diseases that cause significant production (quantitative and qualitative), decreases in sunflower, the white rot (white rot, white mould) is frequently met. It is also know as wet rot (cottony soft rot, watery soft rot), and withering (wilt), cancer (canker), etc. (Mordue and Holliday, 1976). Disease known to be spread rapidly, it can be found in several regions of the country, particularly in Moldavia. The fungus attacks different organs of the plant: roots, stems, leaves, capitulum (Castano *et al*., 1987). *Sclerotinia sclerotiorum* (Lib.) De Bary is a parasite attacks over 400 plant species of 75 botanical families, of which many species are crops with particular importance in agriculture (sunflower, rapeseed, soybean). This disease produces losses of yield due to its virulence in certain environmental conditions and due to the impossibility of compliance a rotation by most farmers.
In this study, we have tested the resistance of some sunflower hybrids (*Helianthus annuus* L.) to the pathogen *Sclerotinia sclerotiorum*. The research material is represented by 12 commercial sunflower hybrids. For the artificial inoculation, we have used the method provided by Rashid (1997). For the artificial infection, we have used 2 isolates the fungus, provided from Romania and Germany. There have been noticed differences in virulence of the pathogen isolates used and also differences in the response of the studied genotypes. Sunflower genotypes responded differently to artificial inoculation with two isolates of *Sclerotinia* depending on the environmental conditions of the year 2011, at the Ezăreni teaching resort from Iasi. Thus, the Iasi isolate, behaved in a more aggressive way, compared to the Giessen isolate, which presented lower virulence.

Key words: *Sclerotinia sclerotiorum*, genetic diversity, artificial infection

Sunflower (*Heliantus annuus*) is as plant oil of great economic and industrial importance. The area cultivated sunflower in Romania occupies the third place, after maize and wheat. Due oil contents (33-56%) in seeds its quality the sunflower is one the main sources of vegetable fats used in human nutrition and the most important source of oil for Romania (Bîlteanu Gh., 1991).

Sclerotinia sclerotiorum (Lib. of Bary) was first described in 1837 by Libert as the *Peziza sclerotiorum* and identified by Fuckel in 1861 [Purdy], as the fungus that causes most of the damage, up to 100%, in many crops (Sackston, 1992). Mycelium and ascospores tests proved to be efficient for the detection of differences between the sunflower genotypes with resistance to *Sclerotinia sclerotiorum* and also between the attack mode on the capitulum (Tourveieille and Vear 1984, Vear and Guillaumin, 1977).

MATERIAL AND METHOD

As research material, were used 12 commercial sunflower hybrids from the company Pioneer Hi-Breed International "(table 1).

Table 1

Name and provenience of the hybrids (Consent given by: Piooner Romania)

Crt. No	Hybrid	Female genitor line	Male genitor line
1	PR63A86	T0001LF	09849LM
2	PR64G46	N0626LF	B0524LM
3	PR64A15	E0012LF	F009LM
4	PR63A90	PHA320	PHA319
5	PR64E83	U9605LF	B0349LM
6	PR64E71	U9605LF	B0524LM
7	PR64A71	U9605LF	U01P6LM
8	PR64A83	U9605LF	U9612LM
9	PR64F50	T0511LF	PHA232
10	PR64J80	T9933LF	F0143LM
11	PR63A62	T0001LF	T0162LM
12	PR64A89	U9605LF	F0143LM

Field experiments were carried at the "Ezăreni" farm, using the method of randomized blocks with three repetitions.

The artificial infection was made on 252 plants, using two isolates of the pathogen, one

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from Romania (Iasi), and one from Germany (Giessen) when the capitulums were mature. Measurements and observations were carried out, a week after the infection, by measuring the lesions from the capitulum. There have been measured the maximum size if the lesion and the capitulum diameter. Two weeks after the infection, there were given notes from 1-8 depending on the size of the lesion. 1 – lesion less than 1 cm lesion; 2 - lesion between 1 - 4 cm; 3 - lesions between 4.1 - 8 cm; 4 - when 25% of the capitulum was infected; 5 - when 50 % of the capitulum was infected; 6 when 75% from the capitulum was infected; 7 - 100% of the capitulumwas infected; 8 – the capitulum has been destroyed and even fellen down.

1. Fungus isolation

The sclerotia used to obtain the mycelium were disinfected with a solution of sodium hypochlorite (NaOCl) 1% and ethyl alcohol (C2H6O) 70% for 4 minutes, then were washed three times with distilled water. They were transferred to Petri plates with PDA medium (potato-dextrose-agar). It was used a sclerotia for every Petri plate. The PDA medium is obtained from 200 g potatoes, 20 g dextrose, 20 g of agar and 1000 ml of distilled water. The plates with medium and sclerotia were incubated in dark conditions and a temperature of 18^{o}C for 7 days.

After cultivation on PDA medium, the *Sclerotinia sclerotiorum* isolates presented different virulence.

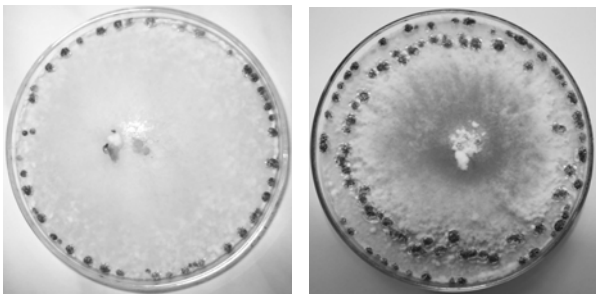

Figura 1 **Petri plates with mycelium and sclerotia of *Sclerotinia sclerotiorum* (original)**

2. Artificial inoculation with *Sclerotinia sclerotiorum*

Artificial inoculations with *Sclerotinia sclerotiorum* was carried out using the Rashid method (1997). The sclerotia were cultivated in Petri plates on PDA medium at 25°C for 1 - 2 weeks. A piece of mycelium was cut and transferred to a glass tube containing 30 g of autoclaved millet (*Panicum miliaceum* L.) and tap water (40 ml). The grains were covered with mycelium a 1 - 2 weeks at 25°C.

For the artificial infection, a small slice was cut from the capitulum, with a scalpel (fig. 2). 1 - 2 grains covered with mycelium were placed into each incision and then taped.

Figura 2 **Incision made on the sunflower capitulum (original)**

RESULTS AND DISCUSSIONS

The *Sclerotinia sclerotiorum* infection is highly dependent on environmental conditions. Due to high humidity conditions during the artificial infection, the presence of the disease was evident, the attack being observed 7 days after the inoculation, due to the appearance of brown spots on the capitulum, that spread in the every next week.

Using the dates resulted after the measurements, there was calculated the average for each variant and the average for each repetition, with ANOVA test. The plants that didn't show symptoms were removed from the statistical calculation. There were noticed significant differences in the virulence of the two isolates. The Iasi isolate showed a higher virulence, in the environmental conditions of the Ezareni farm, compared to Giessen isolate.

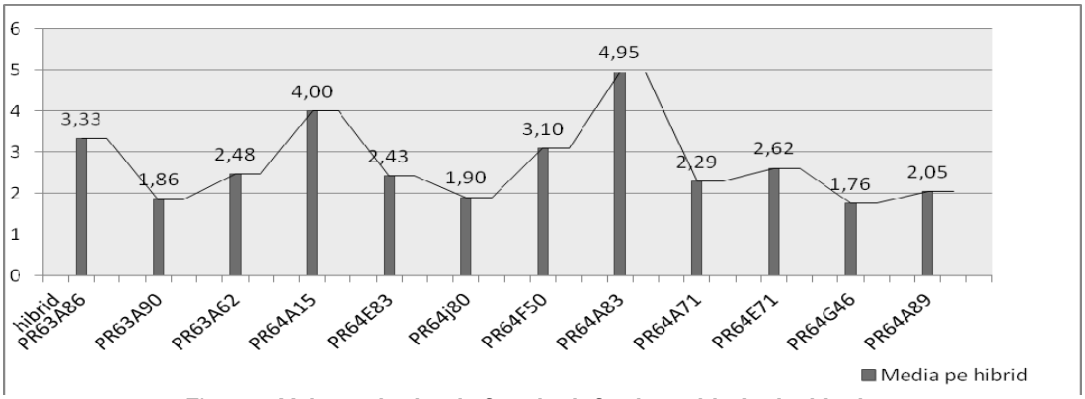

Figure 4 **Values obtained after the infection with the Iasi isolate**

As it can be observed in fig. 4, the lowest value of infection, after averaging the notes, 1.76, was recorded at the PR64G46 hybrid, which demonstrates that this genotype presented a higher resistance to *Sclerotinia sclerotiorum*, compared to the PR64A83 hybrid, which is very susceptible to the disease, with an average of 4.95.

After calculating the averages for the two isolates, it is obvious that the infection made with Giessen isolate recorded lower values, due to its lower virulence. The most resistant hybrid is PR64J80, with an average of 1.52, and the most susceptible is PR63A86, with an average of 3.81. The largest dimension measured had 14 cm, at the PR63A86 genotype, and the smallest spot of 0.21 cm was measured at PR64J80 genotype.

In figure 6 and 7 it can be observed the attack rate of the 12 sunflower genotypes. The attack frequency was calculated by dividing the number of plants or plant organs affected to the number of plant or organs observed. For the Iasi isolate, the highest attack frequency has been 34.27% for the R63A86 genotype, wich shows that the virulence of this isolates is higher, comparative to the one of the PR64E71 and PR64G46 genotypes, that had an attack frequency of 0.4%.

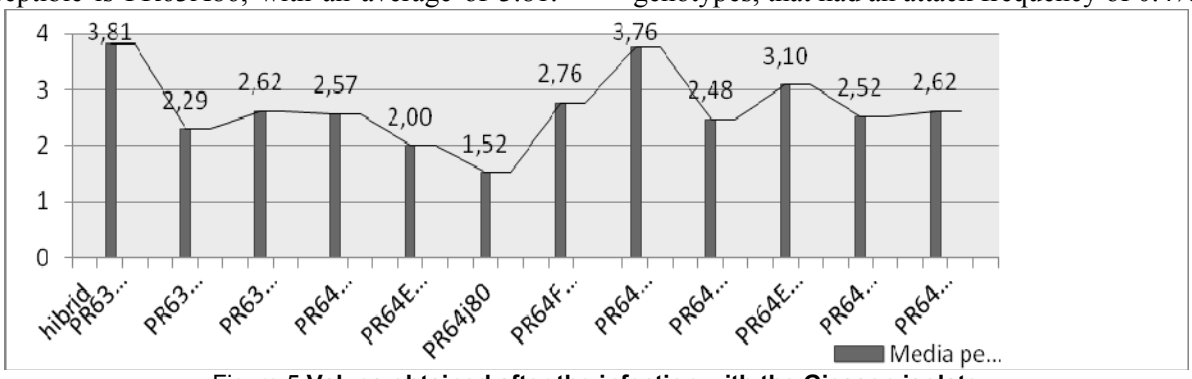

Figure 5 **Values obtained after the infection with the Giessen isolate**

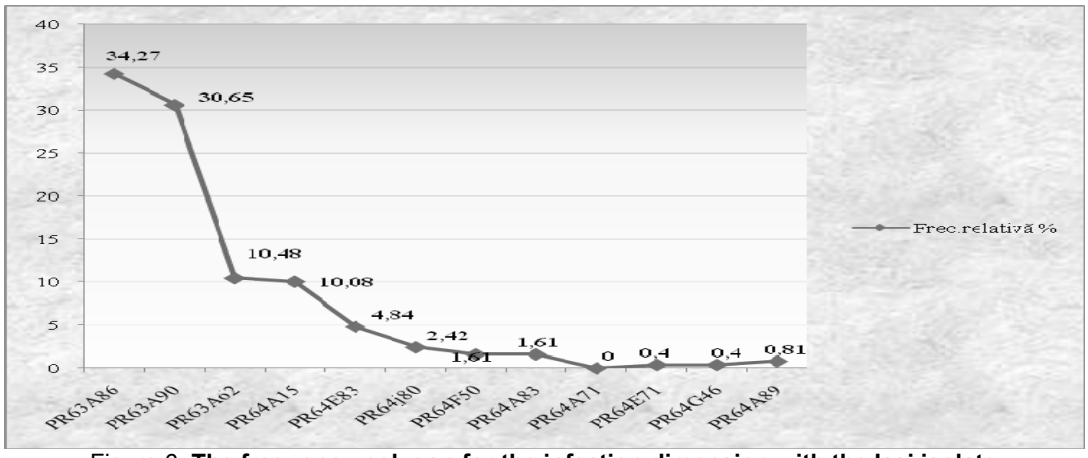

Figure 6 **The frequency polygon for the infection dimension with the Iasi isolate**

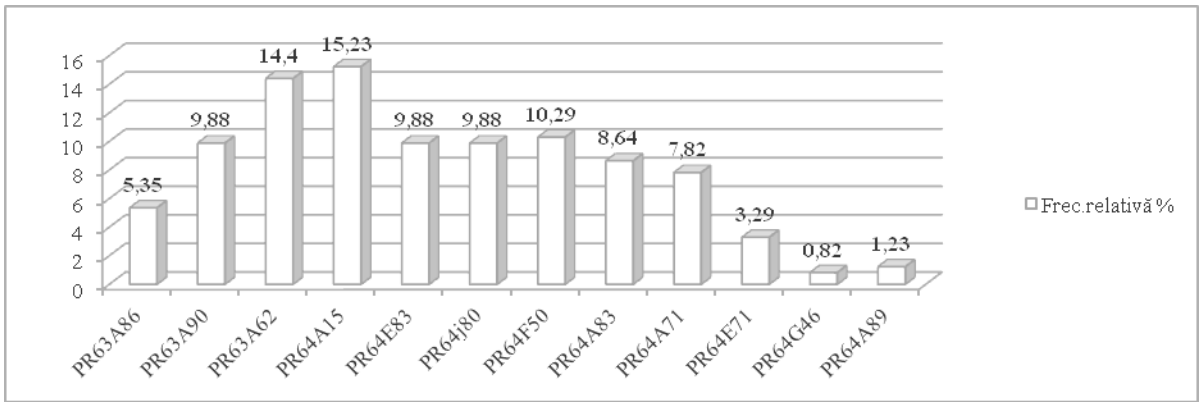

Figure 7 **The frequency histogram for the infection with the Giessen isolate**

For the Giessen isolate, the highest virulence was shown by the PR64 A15 genotype, with an attack frequency of 15.23% and the lowest was shown by the PR64G46 genotype, with an attack frequency of only 0.82%.

CONCLUSIONS

There were noticed significant differences between the virulence of the two isolates. Thus, due to the environmental conditions in the area, the Iasi isolate was more aggressive on the sunflower genotypes, compared to the Giessen isolate, which presented lower virulence, in the same environmental conditions.

Comparing the averages resulted from the notes given to the infection, the Iasi isolate presented higher virulence, with a maximum average of 4.95 and a minimum average of 1.76, while Giessen isolate had a maximum average 3.81 and a minimum one of 1.52. For the attack frequency, the Iasi isolate showed values ranging from 34.27% and 0.4% and Giessen isolate has values ranging from 15.23% and 0.82%.

The high percentage of infection of the genotypes studied in this paper and the very significant genotype variance indicates that this method can be successfully used in the selection of sunflower plants with resistance to *Sclerotinia sclerotiorum*. In future, there will be carried out trials, for obtaining genotypes with active resistance to the pathogen *Sclerotinia sclerotiorum*.

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