AGRICULTURAL ACADEMY, SOFIA

DOBRUDZHA AGRICULTURAL INSTITUTE
General Toshevo

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STUDIES ON THE AGGRESSIVE AND GENETIC VARIABILITY OF *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY IN BULGARIA

PH. D. THESIS

ABSTRACT OF PH. D. DISSERTATION

PROFESSIONAL FIELD: 6.2 PLANT PROTECTION

ACADEMIC SUBJECT „PLANT PROTECTION“

Research supervisor:
Prof. Dr. Ivan Dimitrov Kiryakov

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STUDIES ON THE AGGRESSIVE AND GENETIC VARIABBILITY РАЗНООБРАЗИЕ OF *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY IN BULGARIA

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for awarding the educational and scientific degree of Ph.D.

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This PH.D. thesis consists of 144 pages, including nine major chapters and two appendixes. Twenty-two tables and 40 figures are presented on the results from the experimental research work. The information provided is supported by 229 literary sources, two of which in the Cyrillic alphabet.

The experimental research work was carried out at Dobrudzha Agricultural Institute – General Toshevo during 2020 – 2024.

The Ph.D. thesis was discussed and its defense was appointed at a session of the Extended Scientific Council of the Cereal and Leguminous Crops Breeding Department of Dobrudzha Agricultural institute – General Toshevo (Protocol No ….).

The public defense of the Ph.D. thesis will take place on …. at…. at a session of the Scientific Jury in accordance with Order No ….. of the Chairman of Agricultural Academy - Sofia

Research supervisor:
Prof. Dr. Ivan Dimitrov Kiryakov
Reviewrs:
1.
2.
Expert opinions:
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3.

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1. INTRODUCTION

Sclerotinia sclerotiorum (Lib.) De Bary is a phytopathogenic fungus belonging to the family *Sclerotiniaceae* of the class *Leotiomycetes*, division Ascomycota, whose host range exceeds 400 plant species, predominantly dicotyledons. The fungus forms black sclerotia on the attacked organs, the size and shape of which vary, depending on the host and the tissues in or on which they are formed. The attack on the hosts can be carried out directly by germinating the sclerotia (mycelogenic development) or by ascospores formed in apothecia developed on the sclerotia (carpogenic development). The economic damage caused by the pathogen is related to the plant species and the genotypes used and can lead to a 100% loss of yield. Under the conditions of Bulgaria, the development of *S. sclerotiorum* is of predominantly mycelogenic nature.

The bibliographic review of *Sclerotinia sclerotiorum* shows that during the period 1972-2024, research in Bulgaria related to the pathogen was mainly focused on studying the resistance of germplasm, developing methods for evaluating genetic material, morphological and cultural features, the influence of abiotic factors and applied agricultural practices on the development and distribution of the fungus, as well as control measures related to the application of biological preparations and plant protection products. No literature sources related to studies on the aggressiveness and genetic diversity of the pathogen in our country have been identified, which would support breeding programs for resistance, as well as the development of appropriate agricultural practices to manage it.

2. PURPOSE AND OBJECTIVES OF THE RESEARCH

This study aims to investigate the genetic and aggressive diversity in populations of *Sclerotinia sclerotiorum* (Lib.) de Bary in Bulgaria, with a view of increasing the efficiency of the breeding process and developing an adequate strategy for control of the pathogen.

To achieve the above goal, the following main tasks have been set:

- Study on sunflower, oilseed rape and bean production areas to determine the distribution of *S. sclerotiorum* in Bulgaria;
- Determining the morphological and cultural characteristics of monosclerotiorum isolates of *S. sclerotiorum*;

- Study on the genetic diversity in *S. sclerotiorum* populations based on mycelial compatibility between isolates;
- Determining the aggressive diversity in *S. sclerotiorum* populations in the country;
- Study on the physiological resistance of bean samples under field and controlled conditions;
- Study on the resistance of wild specimens of the genus *Helianthus* and hybrid combinations from interspecific hybridization to the stem form of *S. sclerotiorum*.

3. MATERIALS AND METHODS

3.1. Collection and storage of plant samples.

During 2019-2021, surveys of sunflower, oilseed rape and common bean crops were carried out in Northern and Southern Bulgaria. Plants with symptoms of basal forms of the disease were collected from each production field (location). Sclerotia were separated from the attacked plants, placed in natrons bags and stored at 4°C until their processing. The collected samples were numbered with an alphanumeric code including the host, year of collection, location and number of the respective plant.

3.2. Isolation and storage of the obtained isolates.

PDA (HIMEDIA) medium was used for pathogen isolation. For this purpose, sclerotia were washed under running water for 2-4 hours, then surface sterilized with 0.6% NaClO for 3 minutes, followed by a double wash with sterile distilled water. The sterilized sclerotia were dried in a laminar flow aspirator and then placed on the culture medium and poured into 90 mm diameter Petri dishes. The dishes were incubated in a thermostat at 21±1ºC, in the dark. When the diameter of the colony growth reached 4-5 cm from the periphery, a disk (5 mm) was cut and placed on a new nutrient medium. The isolates were incubated under the same conditions until sclerotia formed. The formed sclerotia were dried in a laminar flow hood, then placed in glass ampoules with capacity of (5 ml) and stored in a refrigerator at 4°C until use.

3.3. Determining morphological and cultural characteristics of mono sclerotic isolates.

The study included 118 isolates from 17 sites in Bulgaria. For this purpose, the sclerotia from the respective mono sclerotic culture were superficially disinfected with 70% ethyl alcohol for 1 min, washed twice with

sterile water and after drying placed on PDA medium, and poured into 90 mm Petri dishes. After three days of incubation at 21°C, in the dark, a 5 mm agar disk was cut from the periphery of the colonies and placed on a new nutrient medium. Three dishes were used for each isolate. The dishes were incubated at 21°C, in the dark. The diametric growth of the colonies was recorded after 24 and 48 hours of incubation. The occurence of sclerotia and the colour of the colonies were recorded from 4 to 8 days. The number of sclerotia, their distribution and their weight in the dish were recorded 31 days after the start of incubation.

3.4. Determining mycelial compatibility between isolates.

3.4.1. Influence of culture medium and incubation period on the visualization of a compatible/incompatible reaction.

The study included 19 mono sclerotia isolates. The compatibility of the isolates was determined according to the method described by Schafer and Kohn (2006). Three nutrient media were used in the study: PDA, PDA+50 µl/L red dye (PDAC+50) and PDA+80 µl/L (PDAC+80). The presence of a compatible/incompatible reaction between the isolates was recorded after 5 to 7 days. The following phenotypic manifestations were recorded: MC (mycelial compatibility) – a fusion of the colonies; G (groove) – a clearly expressed dividing line on the upper side of the plates and the absence of a red line on the lower side of the plates; SG/L – a weakly expressed groove and a red line; SG/SL – a weakly expressed line and a red line; G/L – groove and a red line; G/SL – groove and a weakly expressed line. The absence of a groove at the contact boundary between the isolates was considered a compatible reaction.

3.4.2. Formation of mycelial compatible groups (MCGs).

The study included 154 mono sclerotia isolates from samples collected in 2019-2021 from 17 locations in Northern and Southern Bulgaria. The compatibility and self-compatibility of the isolates were established according to the method of Schafer and Kohn (2006) using the medium (PDAC+80). The designation of the local groups is based on their origin, as follows: NLNMCG , where N – location number, L – the region of the location, NMCG – sequence number of the compatible group established in the location.

To determine the genetic diversity between individual locations and regions, complementary tests were conducted with 94 mono sclerotia isolates included in the already formed local MCG groups. The designation of the additional groups is as follows: NLNMCG+ and so on.

3.5. Determining the aggressiveness of the isolates on the common bean variety GTB Blyan and the sunflower hybrid Deveda.

The study included two hosts – common bean variety GTB Blyan, sunflower hybrid Deveda, and 102 mono sclerotia isolates. The hosts involved in the study were sown in 45x30x10 cm pots, containing soil-peat mixture (1:1), and were grown at temperature 21-24/16-18ºC, respectively day/night. The infection of the plants was carried out by the Straw method (Petzoldt and Dickson, 1996), with the bean plants being inoculated at growth stage beginning of formation of the 2nd triple leaf, and the sunflower plants – at stage appearance of the 3rd pair of leaves (Fig. 1). For this purpose, the main stem of the plant was cut at a distance of 2 cm from the node of the primordial (not true) leaves of the bean plants and the first pair of leaves of the sunflower plants. A one-sided closed plastic straw (6 x 25 mm) was placed on the cut, containing an agar disc with mycelium cut from the periphery of a 3-day culture on PDA of the tested isolate. Five plants were inoculated with each isolate. After inoculation, the plants were incubated at temperature of 20-21/16-18ºC, day/night, respectively. The results were read 5 and 7 days after inoculation for beans and 3 and 5 days for sunflowers, and the length of the attacked tissue from the site of inoculum placement was measured in mm.

Figure 1. STRAW-test in common beans and sunflowers to determine the aggressiveness of isolates

3.6. Study on the resistance of common bean accessions to *Sclerotinia sclerotiorum.*

3.6.1. Field studies.

The study was conducted under field conditions in 2021 and 2022. In 2021, 94 common bean cultivars were tested, grouped according to their habit type. The specimens were sown in two 3 m rows with interspacing of 30 cm.

They were inoculated using the STRAW method at the beginning of budding stage. For this purpose, the stem of the plant was cut at a distance of 3 cm from the last leaf node. A one-sided closed plastic straw containing an agar disk with mycelium of isolates S19.19.5/1 or R19.17.1 was placed on the cut. The results were reported 14 days after inoculation according to a 9-fegree scale (Fig. 2). The classification of the specimens according to their reaction was as follows: Highly resistant (VR) – 1.0; Resistant (R) - 1.1- 3.0; Moderately resistant (MR) - 3.1-5.0; Susceptible (S) - 5.1-7.0; Highly susceptible (VS) - above 7.0. In 2022, 21 samples were tested, showing varying degrees of physiological resistance. The sowing scheme, isolates, infection and counting method were the same as in 2021.

Figure 2. Nine-degree scale for assessing bean response to *Sclerotinia sclerotiorum* (yellow arrow – 1st; red – 2nd; blue – 3rd node)

3.6.2. Comparative testing of two methods for determining resistance of common bean to *Sclerotinia sclerotiorum* under controlled conditions.

The study was conducted under greenhouse conditions and included 21 bean samples. The efficacy of the two methods for determining the resistance of the bean samples involved in the study was investigated.

STRAW test. Infection was carried out at stage fully developed 1st triple leaf. The central stem of the plant was cut 3 cm from the node of the primordial leaves, after which a one-sided closed plastic straw containing an agar disk with mycelium of isolates R19171, S19323, S191951 or SS1914 was placed on the cut. Six plants were infected from each sample, and plants on which a straw with a clean agar disk was placed were used as controls. After inoculation, the plants were placed at 22-24ºC/16-18ºC day/night temperature. The reaction of the samples was recorded 7 days after inoculation on a 9-degree scale (Kiryakov and Genchev, 2002). Based on their score, the samples were grouped as follows: Highly resistant (VR) – 1.0; Resistant (R) - 1.1- 3.0; Moderately resistant (MR) – 3.1-5.0; Susceptible (S) – 5.1-7.0; Highly susceptible (VS) – above 7.0.

Oxalic test. Plant organs (stem and leaves) cut off before inoculation by the STRAW test were placed in vessels with 50 ml of oxalic acid solution (20 mM adjusted to pH=4.0 with 1 N NaOH). Plants immersed in sterile water adjusted to pH=4.0 with 1 N HCl were used as controls. Five plants were used from each sample. The plants were placed at temperature 16-18°C. The results were recorded 19h (5h day and 14h night) after placing the plants in the solutions according to the following scale: 1 – no symptoms; 3 – loss of turgor at the periphery of single leaves; 5 – loss of turgor at the periphery of the three leaves of the triple leaf; 7 – loss of turgor over ½ of the leaf petiole, whitening of the leaf petiole and stem; 9 – complete wilting of the leaves. Based on their score, the samples were grouped as follows: Highly resistant (VR) – 1.0; Resistant (R) - 1.1- 3.0; Moderately resistant (MR) - 3.1-5.0; Sensitive (S) - 5.1-7.0; Highly sensitive (VS) - above 7.0 (Kiryakov and Genchev, 2002).

3.7. Resistance of wild accessions and interspecific hybrid combinations of sunflower to the stem form of *Sclerotinia sclerotiorum*.

The studies were conducted in 2019 and 2020. The 2019 study included 15 hybrid combinations of interspecific hybridization with line 712A (cultivated *H. annuus*) and 10 wild *H. annuus* specimens, used as parental components. The materials were sown manually in 2 m rows with interspacing of 0.7 m and intra-row spacing of 0.30 m. Infection was carried out at budding-flowering growth stages using the STRAW method (Christov et al.,

2004). For this purpose, the petiole of two leaves from the middle floors for each genotype was cut at distance 2 cm from the leaf node. A one-sided closed plastic straw was inserted into the cut with an agar disk containing mycelium of isolate Ss1814 or Ss1941, cultivated on PDA medium. Four plants from each genotype were inoculated with each isolate. Plants, on which a straw containing a disc of pure PDA was placed, were used as a control. The results were recorded 14 days after inoculation on a 9-degree scale (Fig. 3): Based on their average score, the genotypes were grouped as follows: 1- Highly resistant (VR); 1.1-3.0 – Resistant (R); 3.1-5.0 Moderately resistant (MR); 5.1-7.0 Susceptible (S); above 7.0 – Highly susceptible (VS) (Christov et al., 2004).

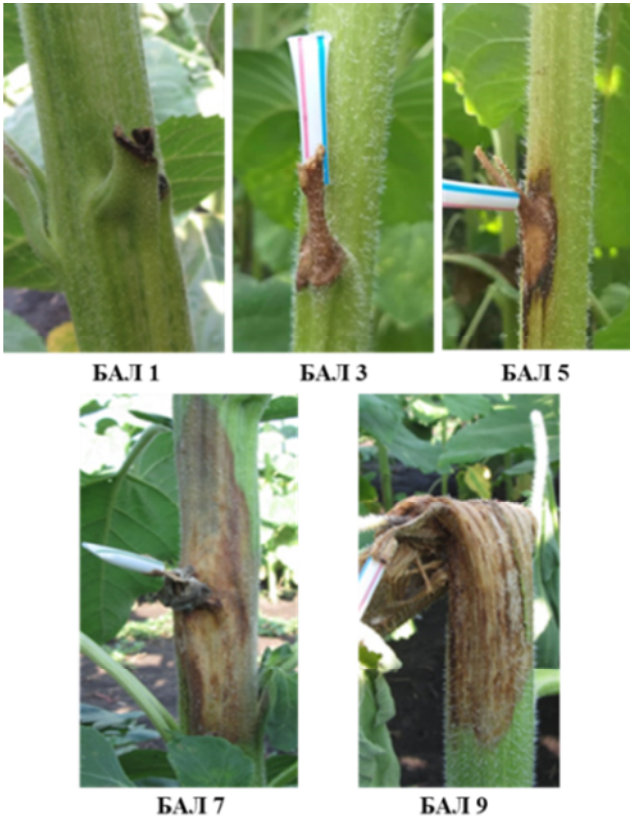

Figure 3. Nine-degree scale for assessing sunflower response to the stem form of *Sclerotinia sclerotiorum*

The 2020 study included 29 wild annual accessions of *Helianthus* ssp. from the collection of the Bulgarian Institute of Plant Protection - General Toshevo, grown, inoculated and recorded in 2019.

3.8. Statistical analysis of results

The genetic diversity in each location was determined by the Shannon index - H0=h0/ln k, where h0= -∑(pi ln pi), with pi being the frequency of the i-th MCG (frequency is defined as the ratio between the number of isolates belonging to the i-th MCG and the number of isolates in the sample), and k being the sample size (Aban et al., 2018). The clonal index (Ki) was calculated for each population as Ki=1 − [(number of MCGs)/(total number of isolates)] (Aban et al., 2018, Yan et al., 2022).

The aggressiveness of the isolates was determined based on the valuesof the area under the development curve (AUDPC):

$$AUDCP = \sum_{i=1}^{N-1} \frac{(Y_i + Y_{i+1})}{2} (t_{i+1} - t_i),$$

where yi – spot size at the beginning of the reporting period, yi+1 – spot size at the end of the reporting period, ti – reporting period (Simko and Piepho, 2012).

Univariate and multivariate analysis of variance, correlation analysis, and cluster analysis were performed using IBM SPSS (Statistics 19) software. The coefficient of variation was calculated using Microsoft Excel 2013.

4. RESULTS AND DISCUSSION

4.1. Areas surveyed and isolates obtained

Out of the 64 plant samples processed during the period 2019-2021 - sunflower, bean and oilsed rape collected from 17 locations in Bulgaria, 168 isolates were obtained (*Table 1*). The degree of attack over locations varied from 1% to 70%. The highest degree of attack of the basal form was observed in 2021 at location S21.1 (Karnobat) - 70%. In the remaining locations, attack only on single plants was established.

Table 1. Origin of plant samples and isolates during the period 2019-2021

Location code	Origin	District	Number of samples	Attack rate (%)	Crops	Number of isolates
S19.3*	Dropla	Dobrich	3	≤3	Sunflower	9
S19.5	Tsarichino	Dobrich	3	≤3	Sunflower	9
S19.7	Kabile	Yambol	3	≤3	Sunflower	8
R19.8	Razgrad	Razgrad	1	≤1	Rapeseed	3
S19.9	Radko Dimitrievo	Shumen	1	≤1	Sunflower	1
S19.10	Rish1	Shumen	2	≤1	Sunflower	6
S19.11	Rish2	Shumen	1	≤1	Sunflower	3
S19.12	Vulchin	Burgas	5	5	Sunflower	15
R19.13	Straldzha	Yambol	5	5	Canola	10
S19.15	Lovech	Lovech	2	≤1	Sunflower	4
S19.16	Selanovtsi	Vratsa	5	5	Sunflower	15
R19.17	Slivo Pole1	Ruse	2	≤1	Rapeseed	6
S19.18	Senokos	Dobrich	1	≤1	Sunflower	1
S19.19	General Toshevo 1	Dobrich	5	5	Sunflower	13
S19.20	General Toshevo 2	Dobrich	2	≤1	Sunflower	3
S20.1	Poroyno	Silistra	6	40	Sunflower	18
S20.2	Sitovo	Silistra	2	≤1	Sunflower	6
S20.4	Slivo Pole2	Ruse	4	≤3	Sunflower	12
S21.1	Karnobatт	Burgas	9	70	Sunflower	24
SS1.4	General Toshevo 3	Dobrich	1	≤1	Common bean	1
SS4.1	General Toshevo 4	Dobrich	1	≤1	Sunflower	1
Total number			**64**			**168**

* The first digit in the code is the year the samples were collected, and the second is the location number

4.2. Morphological and species characteristics of the isolates.

4.2.1. Colony morphology.

After cultivating 118 isolates of S. sclerotiorum on PDA medium, 3 types of colony growth were observed - scattered, smooth and fluffy (Fig. 4). The highest percentage was characterized by isolates with a smooth type of colonies - 78%. A fluffy type of mycelium was observed in 13%, and scattered - in 9%. In terms of colony colour, the studied isolates were divided into 3 groups – beige, brown and white. The brown colour of the colonies was observed in 7% of the isolates. The beige colour was recorded in 25% of the colonies, and the highest percentage was observed in the isolates with white colour of the colonies - 68%. Based on the arrangement of sclerotia in a Petri dish, the studied isolates were divided into 4 groups: The largest percentage of the tested isolates had sclerotia arranged along the edge of the dish - 48%, followed by isolates with sclerotia in the form of a peripheral ring - 31%, with

scattered sclerotia were 14%, and 7% of the isolates were with a sub-peripheral ring.

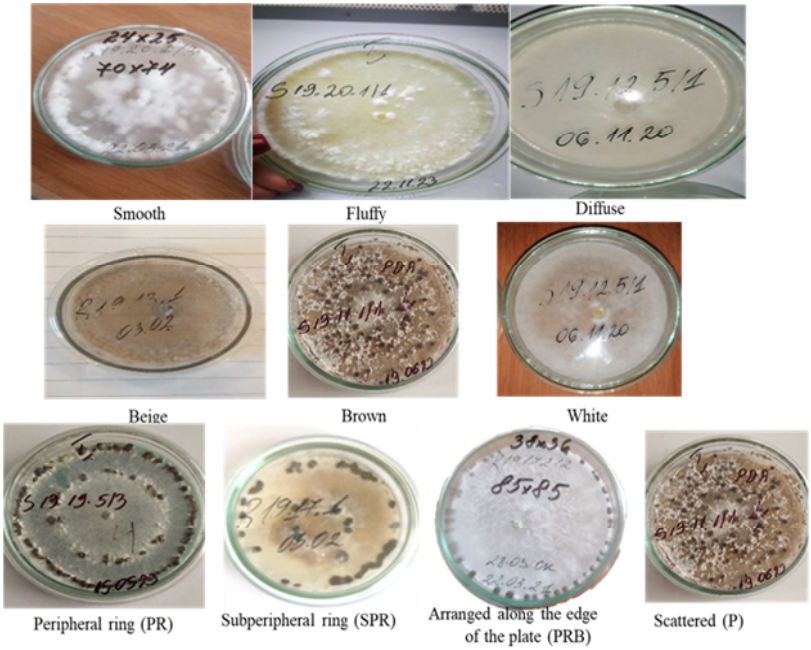

Figure 4. Morphological features of *S. sclerotiorum* isolates

4.2.2 Diametrical growth and development rate

The diametrical growth of the studied isolates, after 48 h of incubation on PDA medium, ranged from 29.50 for location S19.3 to 90.00 mm, with a mean value of 82.00 mm, the differences between these values being significant ($LSD_{0.05}$=1.52) (*Table 2*). In seven locations, the maximum diametrical growth of the isolates reached 90 mm. Significant differences were found between the minimum and maximum values, both within and between locations. The colony growth rate ranged from 0.61 to 1.88 mm/h. In eight locations - S19.3; S19.5; S19.10; S19.12; S19.16; S19.19; S20.1; S21.1, a maximum growth rate of 1.88 mm/h was recorded (Table 2). A functional relationship (r=1, P<0.001) was established between diametrical growth and growth rate.

Table 2. Diametrical growth (mm) and growth rate of 118 isolates of *S. sclerotiorum* over locations, after 48 h incubation at 21ºC on PDA medium

Location code	Number of isolates	Diametrical growth (mm)				Growth rate (mm/h)			
		MIN	MAX	AVR	VC%	MIN	MAX	AVR	CV%
S19.3.	7	***29,50***	**90,00**	72,29	28,29	***0,61***	**1,88**	1,51	28,29
S19.5.	9	**82,75**	**90,00**	87,75	2,83	1,72	**1,88**	1,83	2,83
R19.8.	1	69,25	69,25	69,25	-	1,44	1,44	1,44	-
S19.9.	1	64,50	64,50	64,50	-	1,34	1,34	1,34	-
S19.10.	5	79,50	**90,00**	87,15	5,25	1,66	**1,88**	1,82	5,25
S19.11.	2	46,25	***51,50***	***48,88***	7,60	0,96	***1,07***	1,02	7,6
S19.12.	13	51,25	**90,00**	77,62	18,4	1,07	**1,88**	1,62	18,4
R19.13.	7	77,00	87,50	84,18	4,39	1,60	1,82	1,75	4,39
S19.15.	2	77,75	87,25	82,50	8,14	1,62	1,82	1,72	8,14
S19.16.	14	72,00	**90,00**	85,63	1,05	1,50	**1,88**	1,78	6,53
S19.17.	4	87,50	89,25	**88,13**	0,88	**1,82**	1,86	1,84	0,88
S19.19.	11	65,00	**90,00**	83,07	8,41	1,35	**1,88**	1,73	8,41
S19.20.	5	**82,25**	88,50	85,00	3,31	1,71	1,84	1,77	3,31
S20.1.	11	57,5	**90,00**	80,34	11,64	1,20	**1,88**	1,67	11,64
S21.1.	24	70,25	**90,00**	84,85	7,90	1,46	**1,88**	1,77	7,9
SS1.4	1	63,75	63,80	63,75	-	1,33	1,33	1,33	-
SS4.1	1	76,25	76,30	76,25	-	1,59	1,59	1,59	-
Average	*118*	*67,78*	*82,23*	*77,71*		*1,41*	*1,71*	*1,62*	

$LSD_{0.05}$=3.00 for min and max of diametral growth;
$LSD_{0.05}$=0.63 for min and max speed

4.2.3. Number and weight of sclerotia in a dish.

The number of sclerotia in a Petri dish varied from 18 to 114, with the differences between the minimum and maximum values of the isolates, both in individual locations and between locations, being significant. The minimum value at location S19.20 (General Toshevo 2) was 18 pcs. sclerotia in a dish, with positive significant differences observed compared to all studied isolates. The maximum value for sclerotia was observed at location S20.1 (Poroyno – 114 pcs.) followed by S19.5 (Tsarichino – 110.5 pcs.), with no significant differences established, but the differences compared to the remaining 15 locations were significant. The weight of sclerotia in a Petri dish varied from 0.042 g (General Toshevo 2) to 0.89 g (Gen. Toshevo 1). A significant correlation was found between the number and weight of sclerotia in a Petri dish ($r=0.546$, $P<0.001$), but the correlation was low with regard to diametrical growth and growth rate ($r=0.207$, $P>0.05$).

Table 3. Number and weight of sclerotia in a Petri dish (g) for 118 isolates of *S.sclerotiorum* after 30 days of incubation at 21ºC on PDA medium

Location code	Number of isolates	Number of sclerotia per dish				Weight of sclerotia in a dish (g)			
		MIN	MAX	AVR	CV%	MIN	MAX	AVR	CV%
S19.3	7	39,0	71,5	56,29	19,05	0,15	0,69	0,36	51,42
S19.5	9	46,5	110,5	70,5	28,30	0,194	0,55	0,36	36,28
R19.8	1	36,0	**36,0**	***36,0***	-	0,211	0,21	0,21	-
S19.9	1	48,0	48,0	48,0	-	0,269	0,27	0,27	-
S19.10	5	46,5	66,5	58,3	12,90	0,205	0,45	0,33	26,37
S19.11	2	56,0	58,0	57,0	2,48	0,155	0,26	0,21	34,84
S19.12	13	39,5	57,5	51,19	10,02	0,078	0,46	0,33	32,14
R19.13	7	40,0	63,5	51,57	15,67	0,214	0,48	0,34	28,87
S19.15	2	39,5	49,5	44,5	15,89	0,164	0,17	0,17	2,08
S19.16	14	37,5	71,0	53,79	19,75	0,139	0,49	0,29	35,89
S19.17	4	49,5	58,0	53,25	7,84	0,32	0,44	0,4	13,46
S19.19	11	39,0	71,5	54,05	19,00	0,184	**0,89**	0,37	50,81
S19.20	5	***18,0***	54,5	40,9	33,76	***0,042***	0,58	0,29	67,03
S20.1	11	29,5	**114,0**	58,55	41,43	***0,078***	0,56	0,28	45,84
S21.1	24	45,0	79,0	59,5	16,98	0,167	0,66	0,35	33,99
SS1.4	1	**80,5**	80,5	**80,5**	-	0,382	0,38	0,38	-
SS4.1	1	57,5	57,5	57,5	-	0,522	0,52	0,52	-
Average	118	*43,97*	*67,47*	*54,79*		*0,20*	*0,47*	*0,32*	

$LSD_{0.05}$=10.67 for min and max of sclerotia number;
$LSD_{0.05}$=0.14 for min and a max of weight in a dish

The results show that despite the significant differences between the pathogen populations at the separate locations, their cultural characteristics were not related to the origin of the isolates.

The cluster analysis performed did not show a correlation between the diametrical growth, the number of sclerotia and their weight with the geographical origin and the host from which they were isolated. Isolates from the same origin fell into different clusters (*Fig. 5*).

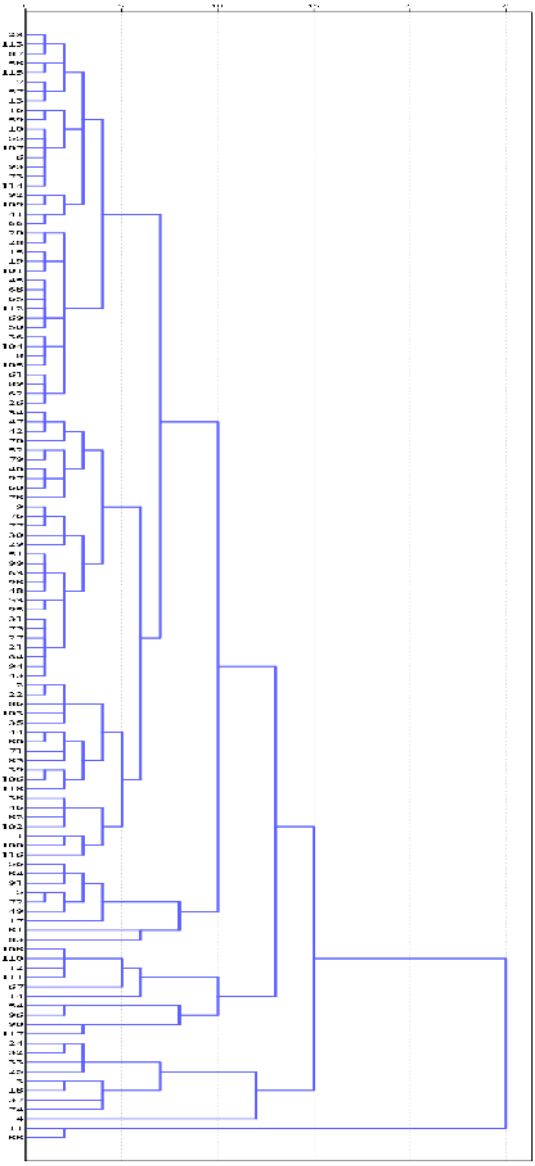

Figure 5. Dendrogram representing the Euclidean distance between 118 isolates based on their cultural characteristics (diameter growth, development rate, number of sclerotia and weight of sclerotia per dish)

4.3. Genetic diversity in *Sclerotinia sclerotiorum* populations.

4.3.1 Influence of culture medium and incubation period on the visualization of a compatible/incompatible reaction.

Developing the methodology for determining mycelial compatibility/incompatibility between 19 isolates of *S. sclerotiorum*, it was found that the PDAC+ dye medium gave better visualization. The test results showed that the dye used did not affect the development of the isolates. Figure 6 shows a compatible and an incompatible reaction between isolates of the fungus on PDA and PDAC+50. At this concentration of the dye, the dividing line (furrow) between the incompatible isolates was much more clearly defined than that in the medium without dye. Increasing the amount of dye in the medium to 80 µl/L allowed for a clearer delineation of the dividing line, as well as a well-defined red line on the underside of the plates (*Fig. 6*). The optimal period for reading was between 4 and 7 days of incubation. The results of the study showed that after 5 days of incubation of the isolates, the dividing line was much clearer, but no sclerotia formation was observed in the colonies. On the seventh day, the dividing line was thinner and formed sclerotia were observed in the colonies, which were located on both sides of the dividing line. In parallel, on the seventh day, a more distinct red line was observed on the underside of the plates.

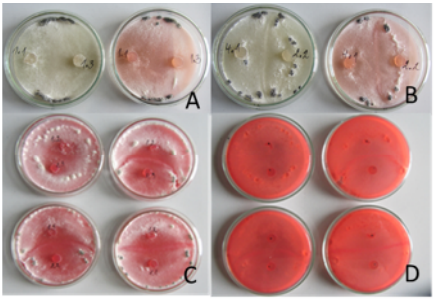

Figure 6. Phenotypic expression in complementary compatibility tests
A - compatible reaction on PDA and PDAC+50 media; B - incompatible reaction on PDA and PDAC+50; C - Compatible and incompatible reaction on PDAC+80, upper side; D - Compatible and incompatible reaction on PDAC+80, lower side

4.3.2. Mycelially compatible groups at separate locations

The studied 154 mono sclerotia isolates were self-compatible, which allowed for their further inclusion in the investigation. In total, the isolates from the individual locations formed 108 local MCGs, the Shannon index (Htot) for the entire population amounting to 0.895 (*Table 4, Fig. 7*). Sixty-

five or 42.2% of the studied isolates formed an independent group, with this percentage varying from 25 to 100% at the separate locations. Thirty of the groups were made up of 2 isolates (19.5%), 8 of 3 (5.2%), and one with 5 (0.5%) and 6 (0.5%) isolates, respectively. In 3.9% of the isolates, the participation of the same isolate in more than one MCG was observed. Five per cent of isolates obtained from single plants were assigned to different MCGs, indicating parallel infection of plants with more than one genotype. The clonal index for all populations was 0.299.

Table 4. Local mycelial compatibility groups (MCG) of *S. sclerotiorum* at 17 locations from 9 regions in Bulgaria

District	Origin	Crop	Number of isolates	Number of MCGs	MCG code	H_o*	K_i**
Dobrich	Dropla	Sunflower	8	7	1D1-1D7	0,875	0,125
	Tsarichino	Sunflower	8	6	2D1-2D6	0,833	0,250
	Dobrich	Sunflower	13	8	3D1-3D8	0,746	0,385
	General Toshevo	Sunflower	6	5	4D1-4D5	0,871	0,167
Silistra	Poroyno	Sunflower	17	11	1S1-1S11	0,828	0,353
	Sitovo	Sunflower	6	3	2S1-2S3	0,564	0,500
Ruse	Slivo Pole1	Rapeseed	6	4	1RU1-1RU4	0,693	0,333
	Slivo Pole2	Sunflower	12	7	2RU1-2RU7	0,787	0,417
Shumen	Rish 1	Sunflower	6	4	1SH1-1SH4	0,742	0,333
	Rish 2	Sunflower	3	3	2SH1-2SH3	1,000	0,000
Burgas	Vulchin	Sunflower	11	8	1B1-1B8	0,822	0,273
	Karnobat	Sunflower	20	15	2B1-2B15	0,894	0,250
Yambol	Straldzha	Sunflower	9	8	1Y1-1Y8	0,929	0,111
	Kabile	Sunflower	8	5	1R1-1R5	0,718	0,375
Lovech	Lovech	Sunflower	4	4	1L1-1L4	1,000	0,000
Vratsa	Selanovtsi	Sunflower	14	7	1V1-1V7	0,634	0,500
Razgrad	Razgrad	Rapeseed	3	3	2R1-2R3	1,000	0,000
Total			**154**	**108**		**0,895**	**0,299**

*Shannon index: Ho - normalized MCG diversity; Hpop - average for Ho (0.825); the ratio of total MCG diversity resulting from variation among individuals in the population (Hpop/tot) = 0.922; the ratio of total MCG diversity resulting from observed differences among populations (Htot − Hpop/Htot) = 0.778.
**Ki - clonal index

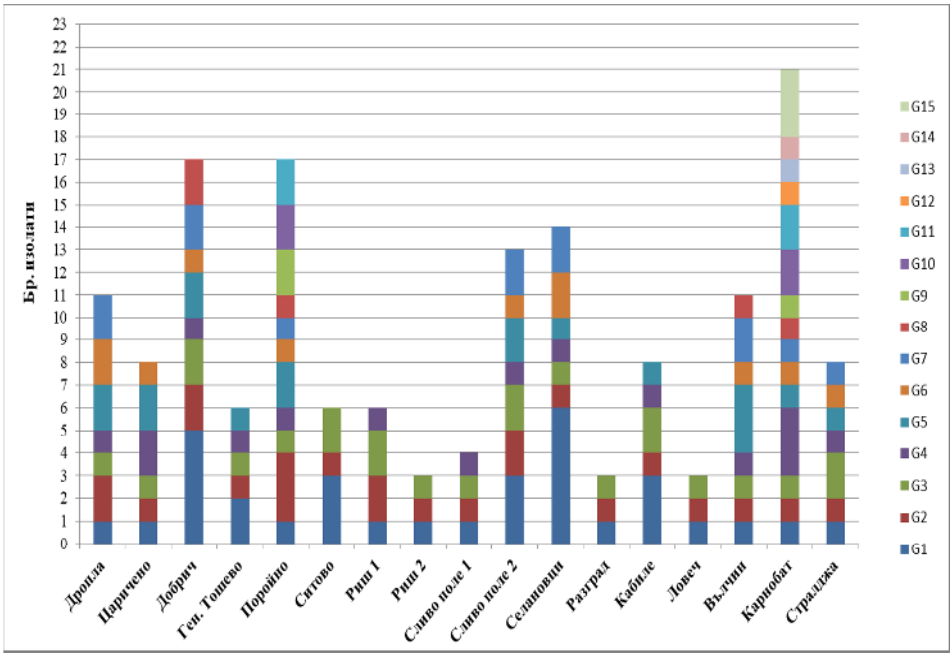

Figure 7. Number of isolates in the identified MCGs (G1 to G15 reflects the number of identified MCGs at the respective location)

The locations are as follows: Dropla, Tsarichino, Dobrich, Poroyno, Sitovo, Rish 1, Rish 2, Slivo pole 1, Slivo pole 2, Selanovtsi, Razgrad, Kabile, Lovech, Valchin, Karnobat, Straldzha

4.3.3. Compatibility of isolates from different locations.

When studying the compatibility between 94 isolates from different locations participating in the formed local groups, six complementary tests were conducted, in which 10 interlocal compatible groups were formed (Table 5). In four of the tests - Kabile-Razgrad, Rish 1-Rish 2, Slivo Pole 1-Slivo Pole 2, Dobrich-Slivo Pole 1-Vulchin-Stralja-Selanovtsi - no compatibility was established between the isolates from the different locations. In the fifth test, 8 generalized groups were formed, one of which (1D5x2D4x1SH3x1Y8) included six isolates from four locations (Dropla – Tsarichino - Rish 1 - Straldzha), located in three regions of Bulgaria. In the sixth test including 20 isolates from five locations, two compatible groups were formed - 1D3x4D2x1B2 and 2D5x1V7 (Table 5). The combined group 1D3x4D2x1B2 included three isolates from Dropla, Dobrich and Valchin, and 2D5x1V7 from Tsarichino and Selanovtsi.

Table 5. Compatibility between isolates of *S. sclerotiorum* from different locations

Добрич			Русе		Шумен		Бургас	Ямбол		Враца	Разград	
Дропла	Цариченo	Добрич	Сливо поле-1	Сливо поле-2	Риш-1	Риш-2	Вълчин	Страджа	Кабиле	Селановци	Разград	Общ. брой изолати
-	-	-	-	-	-	-	-	-	NO$^{8/8}$	-	NO$^{3/3}$	11
-	-	-	-	-	NO$^{5/5}$	NO$^{3/3}$	-	-	-	-	-	8
-	-	-	NO$^{6/6*}$	NO$^{8/8}$	-	-	-	-	-	-	-	14
-	-	NO$^{2/2}$	NO$^{2/2}$	-	-	-	NO$^{4/4}$	NO$^{4/4}$	-	NO$^{8/8}$	-	20
1D5$^{2/6}$	2D4$^{2/6}$	-	-	-	-	-	-	-	-	-	-	21
1D5$^{2/6}$	2D4$^{1/6}$	-	-	-	1SH3$^{2/2}$	-	-	1Y8$^{1/1}$	-	-	-	
1D5$^{2/6}$	-	-	-	-	-	-	1B5$^{1/6}$	-	-	-	-	
1D6$^{1/6}$	2D5$^{1/6}$	-	-	-	-	-	-	-	-	-	-	
1D2$^{1/6}$	-	-	-	-	-	-	1B5$^{3/6}$	-	-	-	-	
-	2D4$^{2/6}$	-	-	-	-	-	1B5$^{2/6}$	-	-	-	-	
1D5$^{1/6}$	2D5$^{1/6}$	-	-	-	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	1B5$^{1/6}$	1Y8$^{1/1}$	-	-	-	
1D3$^{1/3}$	-	4D2$^{1/2}$	-	-	-	-	1B2$^{1/4}$	-	-	-	-	20
-	2D5$^{1/3}$	-	-	-	-	-	-	-	-	1V7$^{1/4}$	-	

*Number of isolates included in the pooled MCG/total number of isolates from the location included in the test. The locations are as follows: Dropla, Tsarichino, Dobrich, Slivo pole 1, Slivo pole 2, Rish 1, Rish 2, Valchin, Straldzha, Selanovtsi, Kabile, Karnobat, Razgrad

4.4. Aggressiveness of isolates.

4.4.1. Aggressiveness of the isolates towards the common bean variety GTB Blyan.

The area under the development curve (AUDPC) for the 102 isolates of variety GTB Blyan varied from 114.4 to 455.4, the differences between them being significant at LSD0.05 (44.47) (*Table 6*). The minimum value at the studied locations was the lowest at location S19.5 (Tsarichino), and the highest at S19.17 (Slivo Pole 1). The maximum values at the locations varied from 179.8 for R19.8 (Razgrad) to 455.40 for S19.16 (Selanovtsi). Significant differences were observed at 13 locations, compared to the lowest value of AUDPC (114.40), and such differences were not found between locations S19.3, S19.5, S19.10 and S19.12. Compared to the highest value of the parameter (455.40), no differences were found at seven of the locations. These results indicate that the aggressiveness of the isolates towards GTB Blyan is not related to the geographical distribution of the isolates. The coefficient of variation in the locations with more than one isolate varied from 7.32 to

44.12%, implying that the isolates from one location vary in their aggressiveness towards bean.

Table 6. The aggressiveness of 102 isolates of *S. sclerotiorum* to variety GTB Blyan, by origin

Code	Origin	District	Number of isolates	Serial number of isolate	MIN	MAX	AVR	CV%
S19.3	Dropla	Dobrich	9	1-9	138,40	395,00	209,58	44,12
S19.5	Tsarichino	Dobrich	9	10-18	114,40	393,80	260,04	42,13
R19.8	Razgrad	Razgrad	1	19	179,80	179,80	179,80	-
S19.10	Rish 1	Shumen	5	20-24	157,00	362,60	262,32	30,27
S19.11	Rish 2	Shumen	1	25	357,20	357,20	357,20	-
S19.12	Vulchin	Burgas	10	26-35	141,60	417,80	272,04	38,25
R19.13	Straldzha	Yambol	6	36-41	200,40	438,80	326,13	25,92
S19.15	Lovech	Lovech	3	42-44	270,60	349,00	334,00	12,66
S19.16	Selanovtsi	Vratsa	11	45-55	240,60	455,40	384,07	15,55
S19.17	Slivo Pole1	Ruse	3	56-58	385,80	437,60	415,07	6,40
S19.18	Senokos	Dobrich	1	59	299,00	299,00	299,00	-
S19.19	General Toshevo 1	Dobrich	9	60-68	165,60	438,60	357,92	22,84
S19.20	General Toshevo 2	Dobrich	4	69-72	362,20	436,20	406,40	8,13
S20.1	Poroyno	Silistra	6	73-78	236,00	370,60	299,80	19,48
S20.2	Sitovo	Silistra	4	79-82	288,80	376,60	331,90	12,37
S20.4	Slivo Pole2	Ruse	2	83-84	196,00	392,20	294,10	47,17
S21.1	Karnobat	Burgas	18	85-102	301,40	412,80	371,29	7,32
Avereg			*102*		*237,34*	*383,12*		
LSD$_{0,05}$					***44,47***			

4.4.2. Aggressiveness of isolates towards sunflower hybrid Deveda.

The area under the development curve (AUDPC) for the 102 isolates in hybrid Deveda varied from 104.0 to 521.8, with the differences between them being significant at LSD0.05 (62.05) (Table 7). The minimum value at the studied locations is lowest at location S19.3 (Dropla) and highest at S19.18 (Senokos). The maximum values at the locations vary from 259.2 for R19.8 (Razgrad) to 521.8 for S19.19 (General Toshevo 1). No significant differences were found in the minimum value of AUDPC (104.0) between locations S19.3 and S19.12. No differences were observed at four of the studied locations concerning the maximum value of the parameter (521.8). These results confirm the lack of relationship between geographic origin and the aggressiveness of isolates towards hybrid Deveda. The coefficient of variation

at locations with more than one isolate varied from 4.47 to 30.93%, indicating that differences in the aggressiveness of isolates were observed at some locations.

Table 7. Aggressiveness of 102 isolates of *S. sclerotiorum* towards hybrid Deveda, by origin

Code	Origin	District	Number of isolates	Serial number of isolate	MIN	MAX	AVR	CV%
S19.3	Dropla	Dobrich	9	1-9	104,00	352,00	259,73	30,93
S19.5	Tsarichino	Dobrich	9	10-18	247,00	514,00	331,91	24,74
R19.8	Razgrad	Razgrad	1	19	259,20	259,20	259,20	-
S19.10	Rish 1	Shumen	5	20-24	232,00	395,20	298,40	21,25
S19.11	Rish 2	Shumen	1	25	278,20	278,20	278,20	-
S19.12	Vulchin	Burgas	10	26-35	159,80	502,00	299,42	33,95
R19.13	Straldzha	Yambol	6	36-41	239,80	377,80	296,67	17,19
S19.15	Lovech	Lovech	3	42-44	255,80	300,20	282,87	8,39
S19.16	Selanovtsi	Vratsa	11	45-55	292,00	414,00	358,27	11,84
S19.17	Slivo Pole1	Ruse	3	56-58	323,40	487,20	386,40	22,83
S19.18	Senokos	Dobrich	1	59	335,00	335,00	335,00	-
S19.19	General Toshevo 1	Dobrich	9	60-68	230,00	521,80	405,04	29,32
S19.20	General Toshevo 2	Dobrich	4	69-72	331,00	363,20	345,65	4,74
S20.1	Poroyno	Silistra	6	73-78	221,00	296,20	259,87	11,52
S20.2	Sitovo	Silistra	4	79-82	233,00	332,40	300,95	15,45
S20.4	Slivo Pole2	Ruse	2	83-84	255,60	353,60	304,60	22,75
S21.1	Karnobat	Burgas	18	85-102	231,40	388,40	301,46	17,72
Avereg			*102*		*248,72*	*380,61*	*311,98*	
LSD$_{0.05}$					***62,05***			

4.4.3. Aggressiveness of the isolates towards both hosts

The analysis of variance for the AUDPC values to the two hosts revealed a significant influence of the factor *isolate* ($F=28.094$; $P<0.001$) and the combined effect of the *isolate x culture* factors ($F=10.514$; $P<0.001$), while the independent influence of the factor *culture* ($F=2.513$; $P>0.05$) was of low significance.

The average maximum value of AUDPC for the two hosts varied from 121.2 to 475.4, with significant differences at LSD0.05. In 35 isolates, higher aggressiveness toward variety GTB Blyan was observed, the differences being significant (Fig. 8). Thirty-one isolates were more aggressive toward hybrid Deveda. In the remaining isolates, no significant differences were observed in terms of their aggressiveness toward the two hosts.

The correlation analysis performed showed a moderate, positive correlation between the aggressiveness of the isolates towards both hosts and the rate of colony growth (r=0.311, P=0.05). A weak, but insignificant correlation was found between MCGs and the mean values of AUPDC, averaged for both hosts (r=0.140, P>0.05). The performed cluster analyses grouped the studied isolates from different locations into common subgroups, which was an indicator that their aggressiveness was not related to their geographical location and the host from which they were isolated.

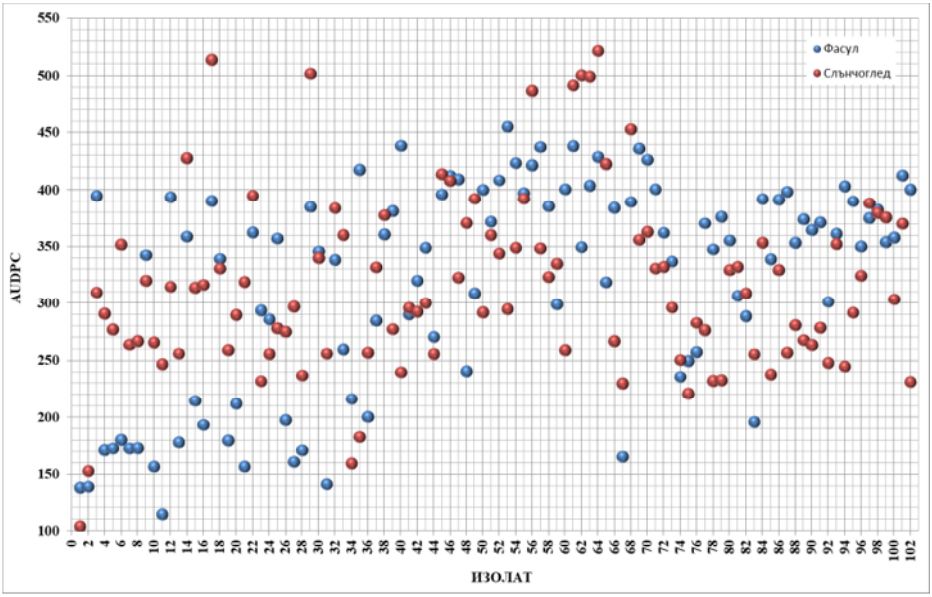

Figure 8. AUDPC for 102 *S. sclerotiorum* isolates, averaged across both hosts ($LSD_{0.05}$=53.99)

The serial number of the exams in the respective locations is indicated in Tables 6 and 7.

4.5. Physiological resistance of common bean accession to *Sclerotinia sclerotiorum*.

4.5.1. Field studies

The results of the 2021 field analysis for physiological resistance of 89 common bean accessions show that 30.33% of the samples reacted with a sensitive to highly sensitive response to isolate S19.19.5/1, and 34.83% to isolate R19.17.1. Resistant to medium resistant in determinant varieties (type I) was shown by 83.9% of the samples (Fig. 9). In the non-determinate

samples, the distribution of resistant and medium resistant materials was as follows: type II – 66.7%; type III – 38.5%; type IV – 81.8%. The higher percentage of resistance in the determinant samples was probably due to the stronger lignification in the leaf nodes.

Figure 9. Distribution of 89 common bean accessions depending on their reaction to two isolates of *Sclerotinia sclerotiorum* in 2021

* Growth habit; R – resistant; MR – moderately resistant; S – sensitive; VS – highly sensitive

The analysis of variance that was carried out to determine the influence of the conditions during the year on the reaction of 21 common bean genotypes inoculated with two isolates of the pathogen in 2021 and 2022, showed a significant influence of the factors *Year* ($F=7.430$, $P<0.01$), *Genotype* ($F=5.047$, $P<0.001$), as well as the interaction between *Year x Genotype* ($F=3.598$, $P<0.001$) and *Isolate x Year* ($F=2.014$, $P<0.01$). On average, no varieties with a resistant reaction to the two isolates were

identified for the two years of study (Table 8). Samples Astor, A 195, Izabell, Rodopeya and IIRR 7585 exhibited average resistance.

Таблица 8. Response of 21 bean samples to two isolates of *S. sclerotiorum* in 2021 and 2022

№	Accession	Growth habit	S19.19.5/1			R19.17.1			Genotype average
			2021	2022	Ср.	2021	2022	AVR.	
			Score	Score		Score	Score		
1	Astor	IIIb	4,0	6,0	5,0	6,3	3,3	4,8	4,9
2	Eleksir	IIIb	6,0	4,3	5,1	5,8	5,8	5,8	5,4
3	Vulkan	IIIb	3,8	6,5	5,1	4,0	6,5	5,3	5,2
4	A195	Ia	3,8	3,0	3,4	4,3	3,0	3,6	3,5
5	Dobr. 7	IIIb	4,5	5,8	5,1	5,8	9,0	7,4	6,3
6	Dobr. ran	IIIb	6,3	4,8	5,5	6,5	9,0	7,8	6,6
7	Pukliv 2	IIb	8,3	7,5	7,9	8,5	7,3	7,9	7,9
8	Abritus	IIa	2,8	8,8	5,8	8,5	3,3	5,9	5,8
9	Ludogorie	IIa	3,3	8,0	5,6	6,3	3,5	4,9	5,3
10	Skitiya	IIa	8,5	5,5	7,0	8,0	7,3	7,6	7,3
11	Miziya	Ia	4,8	7,8	6,3	6,0	6,8	6,4	6,3
12	Helis	Ia	4,0	8,3	6,1	6,3	6,0	6,1	6,1
13	Izabel	Ia	3,0	3,8	3,4	3,3	4,8	4,0	3,7
14	Radoil	IVa	7,3	4,8	6,0	6,8	5,3	6,0	6,0
15	Blyan	IIa	6,8	5,3	6,0	5,5	4,8	5,1	5,6
16	Ustrem	IIa	4,5	5,3	4,9	5,5	5,5	5,5	5,2
17	Trakiya	Ia	6,0	7,3	6,6	7,5	3,3	5,4	6,0
18	Rodopeya	IVa	3,3	4,8	4,0	3,5	3,8	3,6	3,8
19	Pirina	IVa	8,5	5,0	6,8	5,0	6,3	5,6	6,2
20	Vezhen	IIa	5,0	6,5	5,8	4,8	5,0	4,9	5,3
21	IIRR 7585	Ia	3,8	6,5	5,1	2,5	3,3	2,9	4,0
Average for the isolate			5,2	6,0	5,5	5,7	5,4	5,5	

$LSD_{0.05}$: Genotype – 1.41; Isolate x Genotype – 1.99; Year x Isolate x Genotype – 2.82

4.5.2. Comparative testing of bean samples' resistance to Sclerotinia sclerotiorum by applying direct and indirect methods

The analysis of variance conducted for the reaction of 21 common bean accessions to four isolates of *S. sclerotiorum* when applying the STRAW test in greenhouse conditions showed high significance of the independent and combined influence of the studied factors.

Table 9. Resistance of 21 bean samples to *S. sclerotiorum* using direct and indirect methods of analysis

№	Accession	Oxalate test	STRAW				
			SS19.1.4	R 19.17.1	S19.19.5/1	S19.3.2/3	Average
1	Astor	5.6	2.8	4.4	4.8	5.2	4.3
2	Eleksir	7.6	4.0	4.2	4.8	3.4	4.1
3	Vulkan	7.8	4.0	5.6	3.6	4.8	4.5
4	A195	4.8	3.8	3.2	4.0	4.6	3.9
5	Dobr. 7	9.0	4.6	5.2	4.6	4.4	4.7
6	Dobr. ran	8.2	4.2	3.8	4.8	4.4	4.3
7	Pukliv 2	8.2	4.2	6.0	5.6	5.8	5.4
8	Abritus	8.0	4.0	7.6	6.4	7.0	6.3
9	Ludogorie	4.4	5.2	6.8	5.6	6.4	6.0
10	Skitiya	3.6	2.4	4.4	7.4	5.4	4.9
11	Miziya	7.0	5.0	6.2	4.6	4.8	5.2
12	Helis	5.8	6.2	5.8	4.8	5.0	5.5
13	Izabel	5.0	3.6	3.8	3.4	4.2	3.8
14	Radoil	7.0	3.8	4.4	5.0	3.2	4.1
15	Blyan	6.6	4.2	4.6	4.0	5.2	4.5
16	Ustrem	7.0	5.0	5.0	4.0	5.0	4.8
17	Trakiya	7.8	4.2	6.2	5.4	4.4	5.1
18	Rodopeya	3.6	3.6	4.8	4.2	4.4	4.3
19	Pirina	4.0	2.8	4.2	4.0	5.8	4.2
20	Vezhen	8.4	4.2	6.0	2.8	5.8	4.7
21	IIRR 7585	8.6	4.0	5.2	3.4	3.6	4.1
Average			*4.1*	*5.1*	*4.6*	*4.9*	

$LSD_{0.05}$: 2.04 for Oxal test; 0.82 for Genotype; 0.36 for Isolate; 0.36 for Genotype x Isolate.

Significant differences were observed, both between the studied samples and in terms of their reaction to individual isolates (Table 9). The analysis conducted under controlled conditions confirmed the average resistance of samples Astor, A 195, Izabell, Rodopeya and IIRR 7585 established in the field analysis. A weak positive correlation (r=0.120, sig=0.606) was established between the oxalic test and the STRAW test. A weak positive correlation was also established between the oxalic test and the

reaction of the samples to isolates SS19.1.4 (r=0.182, sig=0.640) and R19.17.1 (r=0.071, sig=0.649). A weak negative correlation was found between the oxalic acid test and the reaction of the samples to isolates S19.3.2/3 (r= -0.112, sig=0.256) and S19.19.5/1 (r= -0.058, sig=0.558). The obtained results allow recommending the application of the oxalic acid test in the initial generations of the breeding materials, after a preliminary comparative analysis of the parental forms by both methods.

44.6. Study on sunflower resistance to the stem form of *Sclerotinia sclerotiorum*

Of the 10 one-year-old wild specimens of *H. annuus* included in the field analysis in 2019, E-129, E-115, E-110 and E-154 showed moderate resistance to the two isolates, the differences in the score between the isolates not being significant (*Table 10*).

Table 10. Reaction of 15 hybrid sunflower combinations and 10 annual wild specimens of *H. annuus* to two isolates of *S. sclerotiorum*

Code №	Acession/ Hubid	SS19.4.1	SS19.1.4	Avr.	Code №	Acession/ Hubid	SS19.4.1	SS19.1.4	Avr.
H1	712 A x E-155-2	7,8	3,0	5,4	H14	712 A x E-155-3	7,3	3,0	5,2
H2	712 A x E-116	7,8	4,0	5,9	H15	712 A x E-117	8,0	6,5	7,3
H3	712 A x E-154-2	5,8	4,5	5,2	A3	E-110*	3,8	3,0	3,4
H4	712 A x E-115	5,5	4,0	4,8	A5	E-115*	4,3	3,8	4,1
H5	712 A x E-125-2	6,0	5,3	5,7	A8	E-116*	3,8	5,8	4,8
H6	712 A x E-110-2	3,5	3,8	3,7	A10	E-117*	5,5	4,0	4,8
H7	712 A x E-129	4,8	2,8	3,8	A17	E-120*	6,0	4,0	5,0
H8	712 A x E-155-1	7,5	4,5	6,0	A22	E-125*	6,3	6,3	6,3
H9	712 A x E-120	6,3	8,0	7,2	A23	E-127*	7,0	8,0	7,5
H10	712 A x E-125-1	8,0	6,0	7,0	A33	E-129*	3,3	3,5	3,4
H11	712 A x E-127	8,0	8,5	8,3	A47	E-154*	3,8	3,0	3,4
H12	712 A x E-154-1	5,8	6,8	6,3	A49	E-155*	5,3	3,3	4,3
H13	712 A x E-110-1	4,0	4,3	4,2	*Average for the isolate*		*5,8*	*4,8*	

$LSD_{0.05}$ - 0.44 for isolate; 1.56 for genotype and 2.05 for isolate x genotype
**one-year-old wild specimens of H. annuus*

Of the 15 hybrid combinations studied, resulting from interspecific hybridization, average resistance to the two isolates of S. sclerotiorum was demonstrated by H4 =712A x E-115, H6=712A x E-110-2, H7=712A x E-129 and H13=712A x E-110-1 (Table 10). The resistance of these hybrids corresponded to the resistance of the wild samples used as resistance donors.

Of the 29 wild samples of Helianthus spp. studied in 2020, average resistance to the isolates included in the study was shown by nine, seven of which were from H. annuus and one each from H. petiolaris and H.p. ssp.runyonii (Table 11). In both years of the experiment, isolate SS19.4.1 showed higher aggressiveness to the studied genotypes compared to SS19.1.4.

Table 11. Response of 29 annual wild accessions of *Helianthus* spp. to two isolates of *S. sclerotiorum*

Code.№	Accession	Species*	Isolate		Avr.
			SS19.1.4	SS19.4.1	
HA 1	E035-47p- Еал.д./3- H17	*H. annuus*	6,6	4,2	5,4
HA 2	E045-29E/2H17	*H. annuus*	3,8	3,4	**3,6**
HA 3	E093-EE/2H18	*H. annuus*	9,0	8,0	8,5
HA 4	E081-13pЕанг.H18	*H. annuus*	5,8	4,8	5,3
HA 5	E092-40Еант./2H18	*H. annuus*	3,8	3,6	**3,7**
HA 6	E118-8E/1H18	*H. annuus*	4,6	4,6	**4,6**
HA 7	E119-23E/2H18	*H. annuus*	4,2	3,8	**4,0**
HA 8	E121-45E/2H18	*H.. annuus*	5,6	3,0	**4,3**
HA 9	E127-44p-E13-H18	*H. annuus*	8,0	4,8	6,4
HA 10	E153-29E/1H19	*H. annuus*	3,8	3,8	**3,8**
HA 11	E152-47E/1H19	*H. annuus*	3,4	3,4	**3,4**
HA 12	E174-132E/1H19	*H. annuus*	6,6	4,6	5,6
HP 13	020Op18r	*H. petiolaris*	6,8	4,8	5,8
HP 14	021Op	*H. petiolaris*	4,2	4,0	**4,1**
HP 15	37	*H. petiolaris*	5,0	5,4	5,2
HP 16	105	*H. petiolaris*	6,8	7,6	7,2
HPP 17	142	*H. pe. ssp.petiolaris*	7,4	8,0	7,7
HD 18	50	*H. debilis*	6,0	5,0	5,5
HD 19	104	*H. debilis*	7,8	6,4	7,1
HDS 20	89	*H.d. ssp. silvestris*	5,2	5,2	5,2
HDC 21	137	*H.d. ssp.cucumerifolius*	6,4	6,8	6,6
HDT 22	141	*H.d. ssp. tardiflorus*	6,4	7,0	6,7
HPr 23	143	*H. praecox*	6,2	7,6	6,9
HPr 24	144	*H. praecox*	7,2	7,6	7,4
HPrH 25	27	*H.pr. ssp.hirtus*	6,4	6,2	6,3
HPrPr 26	28	*H.pr. ssp.praecox*	5,6	6,4	6,0
HPrH 27	148	*H.pr. ssp.hirtus*	6,2	5,2	5,7
HPrR 28	149	*H.pr. ssp.runyonii*	3,2	3,2	**3,2**
Hag 29	E130	*H. agrophyllus*	6,0	4,2	5,1
Average for the isolate			*5,8*	*5,3*	

**H.d. ssp – Helianthus debilis ssp; H.pr.ssp – Helianthus praecox ssp; H. pe. ssp-. H. petiolaris ssp.*

$LSD_{0.05}$ - 0.35 for isolate; 1.36 for genotype and 1.92 for isolate x genotype

5. MAIN CONCLUSIONS

Based on the conducted research and the analysis of the results obtained on the aggressiveness and genetic diversity of *Sclerotinia sclerotiorum* in Bulgaria, the following main conclusions can be drawn:

1. During the period 2019 – 2021, the attack on sunflower crops by the root and basal form of *Sclerotinia sclerotiorum* varied from single plants to 70%. In oilseed rape and bean crops, the attack varied from single plants to 5%.
2. According to their morphological features, the 118 studied isolates of *Sclerotinia sclerotiorum* were divided into three groups depending on the type of colonies, as well as the colour of the colonies, and into four groups according to the location of the sclerotia in the dish. The beginning of the formation of sclerotia was within 3 to 8 days. A functional relationship ($r=1$, $P<0.001$) was established between the diametral growth and the growth rate, as well as a significant positive relationship between the number of sclerotia and the weight of sclerotia in the dish. Significant differences were established in terms of the cultural characteristics of the isolates, both at individual locations and between them. The morphological and cultural features of the isolates were not related to their geographical origin and the host from which they were isolated.
3. The PDAC+80 medium was most suitable for visualizing the phenotypic expression when conducting complementary compatibility tests. 108 local MCGs were formed, the total Shannon index (Htot) for the studied populations being 0.895, and the clonal index being 0.299. A predominant clonal spread of the pathogen at the individual locations was proven. The 10 inter-location MCGs formed confirmed the presence of clonal spread of the pathogen between spatially distant locations.
4. Weak positive correlations were established between the aggressiveness of the isolates towards the common bean variety GTB Blyan and the sunflower hybrid Deveda with the diametral growth and rate of colony development, as well as between the aggressiveness towards the bean and the local MCGs. Cluster analyses group the studied isolates from different locations into common subgroups, regardless of their geographical origin and the host from which they were isolated.

5. Significant variation in the physiological resistance of 89 bean samples, grouped according to the habit type, was found. A reliable influence of the factors Conditions of the *Year* and *Genotype*, as well as the combined influence of the factors *Year x Genotype* and *Isolate x Genotype*, was proven. On average, varieties with a resistant reaction were not found during the two years of study. Samples Astor, A 195, Izabell, Rodopeya and IIRR 7585 exhibited moderate resistance under field conditions. A weak positive correlation (r=0.120, sig=0.606) was found between the oxalic test and the STRAW test, applied under controlled conditions. The direct and indirect methods confirmed the moderate resistance of the samples Astor, A 195, Izabell, Rodopeya and IIRR 7585, also established under field conditions.
6. Of the 15 hybrid combinations studied under field conditions, the result of interspecific hybridization, moderate resistance to *S. sclerotiorum* was demonstrated by 712 A x E-115, 712 A x E-110-2, 712 A x E-129 and 712 A x E-110-1. The resistance of these hybrids corresponded to the resistance of the wild *Helianthus annuus* accessions, used as paternal donors of resistance. Of the 29 wild annual *Helianthus* accessions studied, moderate resistance was shown by nine, seven of which were from *H. annuus* and one each from *H. petiolaris* and *H.praecox ssp. runyonii.*

6. PUBLICATIONS RELATED TO THE DISSERTATION

Kiryakov, I. & K. Zhecheva (2019). Mycelial compatibility and aggressiveness of Bulgarian *Sclerotinia sclerotiorum* isolates. *Field Crops Studies, 12 (3), 9-22 (BG).*

Zhecheva, K. & Kiryakov, I. (2021). Aggressiveness of *Sclerotinia sclerotiorum* isolates to *Phaseolus vulgaris* and *Helianthus annuus*. *Field Crops Studies, 14 (2-3-4), 9-16 (BG).*

Zhecheva, K., & Kiryakov, I. (2023). Comparative testing of the resistance of bean accessions to white mold (*Sclerotinia sclerotiorum*) by a direct and indirect method. *Bulgarian Journal of Crop Science, 60(5), 40-46 (BG).*

Zhecheva, K., Koleva, M., & Kiryakov, I. (2024). *Sclerotinia sclerotiorum* genetic diversity in Bulgaria. *Bulgarian Journal of Crop Science, 61(5) 97-104 (BG).*

7. SCIENTIFIC AND SCIENTIFIC-APPLIED CONTRIBUTIONS

1. This is the first study in our country related to the genetic diversity of *Sclerotinia sclerotiorum*, based on mycelial compatibility between isolates, including 156 isolates from 17 production fields in 11 regions of Northern and Southern Bulgaria.
2. For the first time in our country, the aggressiveness of 102 isolates of *Sclerotinia sclerotiorum* from 17 production crops of sunflower, rapeseed and mature beans has been studied. The results obtained provide an opportunity to increase the efficiency of selection programs for resistance in beans and sunflowers.
3. For the first time in our country, a large-scale study has been conducted related to the morphological and cultural characteristics of 118 isolates of *Sclerotinia sclerotiorum* originating from different geographical areas and hosts.
4. Under field conditions, the physiological resistance of 89 common bean accessions from the heart-shaped collection of the Dobrudzha Agricultural Institute to *Sclerotinia sclerotiorum* was monitored. The results obtained confirm the physiological resistance of some accessions indicated as donors of resistance by our and foreign authors.
5. The opinion of other authors regarding the possibilities of applying the Oxal test to determine the physiological resistance in common beans to *Sclerotinia sclerotiorum* has been confirmed. The results obtained show that the accuracy of the method can be increased after preliminary testing of the parental components by parallel application of the direct and indirect testing methods.
6. The physiological resistance to *Sclerotinia sclerotiorum* of the main varieties of dray common beans for the country has been confirmed. The information presented will assist agricultural producers in choosing a variety in areas with an established distribution of the pathogen.
7. The possibility of transferring resistance to the stem form of *Sclerotinia sclerotiorum* from the annual wild species *Helianthus annuus* to cultivated sunflower has been confirmed.
8. For the first time in Bulgaria, the resistance of annual wild species of *Helianthus* spp. to more than one isolate of *Sclerotinia sclerotiorum* has been studied. The results obtained allow for the inclusion of resistant specimens in sunflower breeding programs.