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Mycelial Compatibility Groupings and Pathogenic Diversity of *Sclerotinia sclerotiorum* (Lib.) de Bary Populations on Canola in Golestan Province of Iran

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ABSTRACT

Genetic structure and pathogenic diversity of *Sclerotinia sclerotiorum*, the causal agent of canola white stem rot, were assessed through Mycalial Compatibility Groupings (MCGs), a comparison and comparing of isolate virulence. Fifty-seven isolates from three different regions in Golestan Province were selected for mycelial compatibility and as well for virulence tests. Within the 57 tested isolates, 35 MCGs were identified, 42.86% of which being constituted of single isolate specimens, were all collected from Ali Abad region. The observed MCGs differed within the three regions. From among the 35 MCGs, 25.71%, 28.57% and 45.72% belonged to Kalaleh, Hashem Abad and Ali Abad, respectively. In Kalaleh, nine MCGs were identified all of which fell into two isolates. Ten MCGs were identified within the Hashem Abad region, eight of which represented two isolates and the remaining were constituted from three isolates. Sixteen MCGs were detected in Ali Abad for which, except one MCG which was constituted of two isolates, the rest belonged to one isolate. Moreover, no MCG was identified as common among these regions. Shannon diversity index (H_o) of MCGs for the whole regions found to be was 0.86 (H_{tot}). Partition of total diversity (Htot) showed that 95.45% corresponded to a variation in diversity within *S. sclerotiorum* populations. Variation in isolate virulence was tested using a petiole inoculation technique under greenhouse conditions. Isolate virulence varied within the three regions. Moreover, in most cases the differences in virulence of isolates within MCGs were significant. The data indicated that populations of *S. sclerotiorum* obtained from the studied regions were composed of a heterogeneous mix of MCGs, therefore the population structure of this pathogen as well as variations in virulence of isolates must be considered in disease management systems in these regions.

Keywords: Mycelial compatibility groups, *Sclerotinia sclerotiorum*, Shannon index.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is a cosmopolitan necrotrophic fungal pathogen with a wide host range, including over 400 different plant species (Boland and Hall, 1994; Purdy, 1979). This ascomycete fungus significantly reduces the yields and quality of some important crops including canola (oilseed rape, *Brassica napus* L.) (Kirkegaard *et al*., 2006), sunflower (Gulya *et al*., 1997) and soybean (Danielson *et al*., 2004). This pathogen is the causal agent of sclerotinial stem rot in canola leading to serious losses in yield due to lodging and premature shattering of seedpods (Gugel and Morrall, 1986). Sclerotinial stem rot of canola is one of the most injurious diseases of this crop in Golestan Province, the canola cultivating center, of Iran.

Intraspecific variation in virulence (Marciano *et al*., 1983; Morral *et al*., 1972) and in such morphological characteristics as pigmentation of the mycelium, ascus, ascospore, sclerotial size and production

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(Boland and Smith, 1991; LeToumeau, 1979; Price *et al*., 1975a; Purdy, 1955) have been reported in *S. sclerotiorum*. To identify intraspecific variation of *S. sclerotiorum*, two presupposed unrelated criteria of mycelial compatibility groups (MCGs), and DNA fingerprinting have been employed (Kohn *et al*., 1990; 1991).

Mycelial compatibility is the capability of two fungal strains of hyphae to anastomose and form one integrated colony (Kohn *et al*., 1990). It has been suggested that MCGs represent genetically dissimilar individuals and each MCG is a particular genotype (Kohn *et al*., 1990). Infrequently outbreeding in *S. sclerotiorum*, immigration of strains from other sites (Glass and Kuldau, 1992), genetic exchange, meiotic recombination (Carbone *et al*., 1999), mitotic recombination, transitory selection, selective neutrality, and diversifying selection (Kohli *et al*., 1992) are possible sources of the MCG diversity.

MCGs' based grouping has been applied to *S. sclerotiorum* strains isolated from a variety of hosts in different countries (Atallah *et al.*, 2004; Kull *et al.*, 2004; Sexton *et al.*, 2006; Mert-Turk *et al.*, 2007; Mavarez *et al*., 2007).

Several studies on evaluating soybean resistance to *S. sclerotiorum* showed that results of resistance evaluations from greenhouse and field experiments were of low correlation and also, results from these studies across field locations are mixed (Kim *et al*., 1999; Nelson *et al*., 1991; Wegulo *et al*., 1998). These may be due in part to disregard for differences in MCG frequency among fields and variation in virulence of *S. sclerotiorum* isolates (Kull *et al*., 2004).

The extent of MCG diversity and variability in isolate and MCG pathogenicity related to *S. sclerotiorum* populations infesting canola fields in Iran has not been reported. Thus, the objectives of this study were (1) to identify MCGs among a collection of *S. sclerotiorum* isolates from various fields at different geographic locations from North Iran, (2) to appraise a measure of MCG diversity and (3) to determine virulence of isolates and their correlation with the identified MCGs.

MATERIALS AND METHODS

S. sclerotiorum Isolates

Three hundred naturally infected plants of canola were randomly collected from Hashem Abad, Kalaleh, and Ali Abad in Gorgan Province in 2006. Ten fields were sampled in each location. One hundred and seventy-three isolates were obtained. Sixty nine isolates were gathered from Hashem Abad included region, 67 from Kalaleh, isolates and 37 isolates from Ali Abad. Out of these, 57 isolates were selected to cover all locations (2-3 isolates randomly from each field (Table 1)). Isolates were obtained from sclerotia of infected plants. Sclerotia were surface sterilized by being soaked in 50% ethanol plus 30% sodium hypochlorite for 1 minute. Sclerotia were air dried on sterile filter paper for 10 minutes and placed on Potato Dextrose Agar (PDA; Merk), which was prepared of 40 g of PDA in 1 liter of distilled water. Plates were incubated in the dark at room temperature (20-22˚C). Hyphal tip culture was carried out on water agar (2% WA; Merk), which was prepared of 20 g of agar in 1 liter of distilled water. All the mycelial cultures were maintained on PDA slants and stored at 4˚C for use throughout the study.

Mycelial Compatibility Grouping

Isolates were manipulated in all possible combinations on PDA, amended with 175 µl L^{-1} of McCormick's red food coloring (McCormick Crop., Dallas, Texas) according to a previously planned procedure (Schafer and Kohn, 2006). All pairings were scored after incubation in the dark and at room temperature (20-22˚C) for 7 and 14 days with each pairing performed twice.

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Table 1. Investigated *S. sclerotiorum* isolates grouped THROUGH MCG, isolate code, field number and location.

MCG	Isolate code	Field number	Location
1	H1.4	1	Hashem Abad, Golestan*
1	H1.8	1	Hashem Abad, Golestan
1	H1.9	1	Hashem Abad, Golestan
2	H2.2	2	Hashem Abad, Golestan
2	H2.3	2	Hashem Abad, Golestan
3	H2.5	2	Hashem Abad, Golestan
3	H3.3	3	Hashem Abad, Golestan
4	H3.5	3	Hashem Abad, Golestan
4	H3.6	3	Hashem Abad, Golestan
5	H3.8	3	Hashem Abad, Golestan
5	H4.4	4	Hashem Abad, Golestan
6	H4.6	4	Hashem Abad, Golestan
6	H4.9	4	Hashem Abad, Golestan
7	H5.6	5	Hashem Abad, Golestan
7	H5.7	5	Hashem Abad, Golestan
8	H6.7	6	Hashem Abad, Golestan
8	H7.10	7	Hashem Abad, Golestan
9	H8.5	8	Hashem Abad, Golestan
9	H8.9	8	Hashem Abad, Golestan
10	H8.10	8	Hashem Abad, Golestan
10	H9.3	9	Hashem Abad, Golestan
10	H9.4	9	Hashem Abad, Golestan
11	A1.4	1	Ali Abad, Golestan
12	A1.6	1	Ali Abad, Golestan
13	A2.1	2	Ali Abad, Golestan
14	A2.6	2	Ali Abad, Golestan
15	A3.2	3	Ali Abad, Golestan
16	A3.8	3	Ali Abad, Golestan
17	A4.3	4	Ali Abad, Golestan
18	A4.9	4	Ali Abad, Golestan
19	A5.1	5	Ali Abad, Golestan
20	A5.8	5	Ali Abad, Golestan
21	A7.5	7	Ali Abad, Golestan
22	A7.7	7	Ali Abad, Golestan
23	A8.6	8	Ali Abad, Golestan
24	A8.9	8	Ali Abad, Golestan
25	A9.5	9	Ali Abad, Golestan
26	A9.8	9	Ali Abad, Golestan
26	A10.3	10	Ali Abad, Golestan
27	K1.4	1	Kalaleh, Golestan
27	K1.11	1	Kalaleh, Golestan
28	K2.5	2	Kalaleh, Golestan
29	K2.6	2	Kalaleh, Golestan
29	K2.7	2	Kalaleh, Golestan
28	K2.9	2	Kalaleh, Golestan
30	K3.8	3	Kalaleh, Golestan
30	K3.9	3	Kalaleh, Golestan
31	K4.7	4	Kalaleh, Golestan
32	K5.6	5	Kalaleh, Golestan
32	K5.8	5	Kalaleh, Golestan
31	K6.6	6	Kalaleh, Golestan
33	K8.6	8	Kalaleh, Golestan
33	K9.1	9	Kalaleh, Golestan
34	K9.2	9	Kalaleh, Golestan
34	K9.9	9	Kalaleh, Golestan
35	K10.1	10	Kalaleh, Golestan
35	K10.10	10	Kalaleh, Golestan

Pairings were scored as mycelially compatible when no reaction line was observed within the interaction zone between paired isolates. Pairings were scored as mycelially incompatible when a thin to wide band of uniform, aerial mycelium or thin red line on the colony surface, reverse, or both was observed between adjacent paired isolates (Kohn *et al*., 1990).

To facilitate MCG determination of all isolates, a subset of 10 isolates from each region was selected and paired in all possible combinations and then representatives of each MCG were paired with all the remaining untested isolates. Finally, the representatives of each MCG were paired with those of each of the other MCGs. In the meantime, self-self pairings were performed as well.

Assessment and Comparison of MCG Diversity

The Shannon index, h_o, was calculated for each population as follows:

$h_o = -\Sigma(p_i \ln p_i)$

where p_i was the frequency of ith MCG. Frequency was defined as the ratio between the number of isolates belonging to the ith MCG and the number of isolates in the sample. To correct for differences in sample sizes among populations, MCG diversity values were normalized by the maximum diversity in each population, so that

$H_o = h_o/\ln k$

where k was the sample size. Total MCG diversity (H_{tot}) was partitioned into within, and among population components (Goodwin *et al*., 1992).

Virulence Assessment

Virulence of isolates was determined using a petiole inoculation technique (Zhao *et al*., 2004). Canola cv. OKpi, was used in all virulence tests. Canola seeds were planted in a soil, peat, and perlite mixture

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(1:1:1) into 14 cm diameter pots. Four uniform plants were kept per pot and grown in a greenhouse at 21±2˚C. Plants were 7 to 8 weeks of age at inoculation time. To inoculate plants, the open end of a blue tip was punched into the margin of a 4-day-old colony to cut an 8-mm plug out of PDA culture of each isolate. Petioles of the third fully expanded leaves were severed at 2.5 cm away from the main stem, using a scalpel blade. The petiole was pushed through the agar plug within the pipette tip. As for control, agar plug with no fungus was employed. Inoculated plants were hand misted with water, and maintained under nylon chambers in air conditioned greenhouse at 20±2˚C for four days. Experimental design for all the isolate virulence tests was a randomized, complete block one of three replications, and four plants per replication, with the experiment being conducted twice.

Four separate scoring systems were employed to assess the virulence of each isolate. Stem Lesion Length (SLL; cm) was measured 7, 10, and 14 days after inoculation (Figure 1). A lesion phenotype (LP) index was devised to classify phenotypes for all the inoculated plants. The LP index consisted of a 0-5 scale and was assessed on the final day of SLL measurements. Lesion phenotypes were categorized as follows: 0= No symptom, 1= Water-soaked lesion on petiole; 2= Small lesion at junction of petiole and at stem, no water-soaking, and no wilt; 3= Small water-soaked lesion and no wilting; 4= Expanded, sunken water-soaked lesion and no wilt; 5= Expanded, sunken, water-soaked lesion resulting in irreversible wilt of foliage (Zhao *et al*., 2004). Furthermore, Girdling Percent (GP) was found out 14 days after inoculation. A 0-4 scale was assigned to GP as follows: 0= No lesion; 1= 25% of stem surrounded by lesion; 2= 50% of stem by lesion affected; 3= 75% of stem affected by lesion; 4= The whole stem surrounded by lesion. The Area Under Disease Progress Curve (AUDPC) found out as follows:

$$AUDPC=[[(Y_1+Y_2)/2](t_2-t_1)]+[[(Y_2+Y_3)/2](t_3-t_2)]$$

where Y_1, Y_2 and Y_3 were lesion length 7, 10 and 14 days past inoculation, while t_1, t_2, and t_3 represented days of scoring.

Data Analysis

To determine if isolates varied in virulence for the three sets; SSL, LP, girdling and AUDPC data along with the combined data of these four indexes were analyzed using Analysis of Variance (ANOVA) and employing MSTATC software. Duncan's Multiple Range Test (DMRT) was employed to determine the significant (P= 0.01) differences among mean values.

RESULTS

MCG Diversity and Distribution

Among the 57 *S. sclerotiorum* isolates, 35 MCGs were identified (Table 1). All the isolates were compatible within themselves.

Figure 1. The symptoms of Sclerotinia stem rot on canola as by using petiole inoculation technique in greenhouse.

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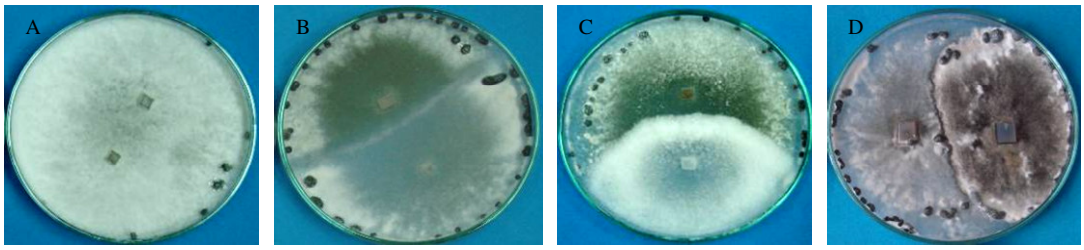

Figure 2. Four representative 4 types of mycelial compatibility of *Sclerotinia sclerotiorum*: (A) Represents complete compatibility, two paired isolates have merged to form one colony (level 0); (B) Partial compatibility, a distinct thin band of mycelia observed in the interaction zone (level 1); (C) Intermediate incompatibility, reaction line visible as abundant, white patches of aerial mycelium in the reaction zone on the colony surface (level 2), (D) completely incompatible, a red reaction line observed between interfering paired isolates (level 3).

Pairs that were 100% compatible (level 0) merged to form one colony with no distinct zone of incompatibility visible where the mycelia came into contact (Figure 2-A). Three levels of incompatibility were distinguished. Level 1 incompatibility (not completely compatible, partial compatibility): when a sharp distinct thin band of mycelia was observed within the interaction zone (Figure 2-B); Level 2 incompatibility (not completely incompatible): reaction line was visible as abundant tufts, white patches of aerial mycelia in the reaction zone on the colony surface (Figure 2-C); and Level 3 incompatibility (100% incompatible): when a red reaction line observed between the interfering paired isolates (Figure 2-D). Interestingly, most of the non-sclerotium forming isolates (H3.8, H4.4, H8.10, H9.3, H9.4) in pairings with all other isolates produced a uniform wide band of aerial mycelia on the colony surface.

Among the 57 tested isolates, 35 MCGs were identified, of which 42.86% were unique, defined as one MCG that constituted single isolate specimens, all collected from Ali Abad location. The observed MCGs differed within the three regions (Figure 3). From among the 35 MCGs, 25.71%, 28.57% and 45.72% belonged to Kalaleh, Hashem Abad and Ali Abad, respectively (Figure 4). In Kalale, nine MCGs were identified all of which consisted of two isolates. Ten MCGs were identified within the Hashem Abad population; eight of which were composed of two isolates and the remaining two each consisted of three isolates. Sixteen MCGs were observed in Ali Abad, except one MCG which was constituted of two isolates

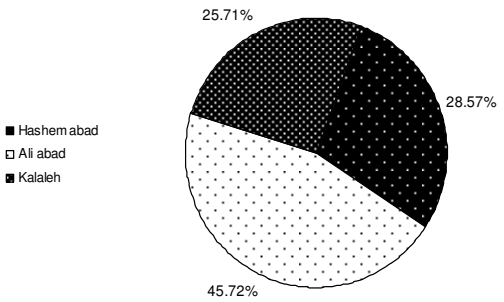

Figure 2. Percentage of total MCGs within the three different regions of Golestan Province.

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Table 2. Mycelial compatibility groupings (MCGs) and MCG diversity (H_o) of *S. sclerotiorum* populations from different locations.

Location	MCG	Sample size	H_o [a]	H_{pop} [b]/ H_{tot}	$(H_{tot}-H_{pop})/H_{tot}$
Hashem Abad	1, 2, 3, 4, 5, 6, 7, 8, 9, 10	22	0.74		
Ali Abad	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26	17	0.97		
Kalaleh	27, 28, 29, 30, 31, 32, 33, 34, 35	18	0.76		
Total		57	0.86	0.95	0.45

H_o= Normalized MCG diversity; H_{pop}= The average H_0; H_{pop}/H_{tot}= The proportion of the total MCG diversity ascribed to variations between individuals within a population, $(H_{tot}-H_{pop})/H_{tot=}$ The proportion of total MCG diversity ascribed to difference among populations.

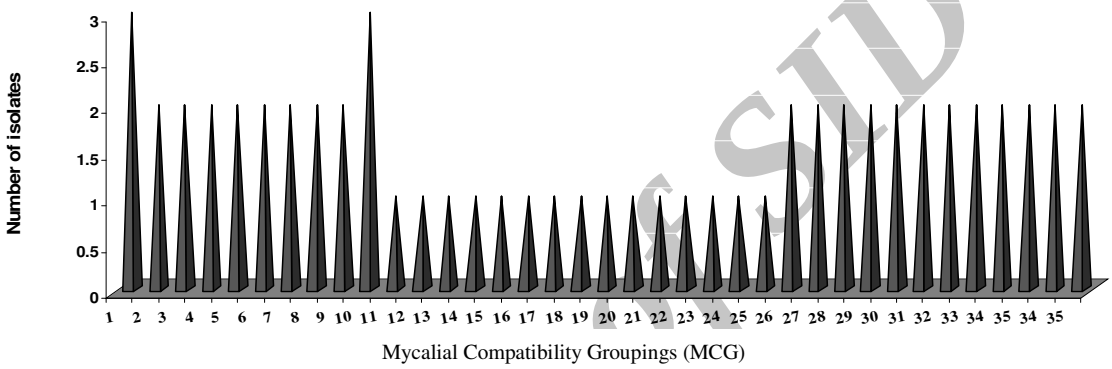

Figure 3. Histogram representing the number of isolates that belong to each MCG.

the others were each composed of one isolate. Moreover, no common MCG was identified among the regions.

Shannon diversity index (H_o) of MCGs for the three regions (Table 2) was found out 0.86 (H_{tot}), ranging from 0.74 for Hashem Abad to 0.97 for Ali Abad and with a mean of 0.82 (H_{pop}). Overall, diversity was higher in the populations from Ali Abad than in those from Kalaleh and Hashem Abad. Partition of total diversity (Htot) showed that 95.45% corresponded to a variation of diversity within *S. sclerotiorum* populations, while only 4.55% of diversity was responsible for variability among those populations.

Isolate Virulence within the Three Regions

Analysis of variance (ANOVA) showed that there were significant differences among virulence of isolates (P= 0.01) in all the four scorings along with the combined data (Table 3). Among four different scoring, AUDPC, girdling, and AUDPC+girdling (combined data of AUDPC and girdling) proved the most suitable AUDPC had the lowest Coefficient of Variation (CV) of all four scoring systems under controlled conditions (Table 3). Girdling is considered as an important disease index, because it causes death of plants. In this respect, isolates of Hashem Abad had mean AUDPC values ranging from 2.05 to 7.35. Furthermore, in this region mean girdling ranged from 0 to 0.56. Ali Abad had mean AUDPC values and mean girdlings ranging from 3.82 to 7.61 and from 0 to 0.46, respectively. Kalaleh carried mean AUDPC values ranging from 4.33 to 8.58 and mean girdling ranging from 0 to 0.58 (Table 4). The least aggressive isolate (H9.4) came from Hashem Abad and responsible for a mean AUDPC value of 2.05 and mean girdling value of 0. This

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Table3. Variance analysis for disease severity of the studied *S. sclerotiorum* isolates.

source	Degrees of Freedom	Mean Square	F value
SSL data			
Block	2	0.057	0.743
Isolate	57	0.172	2.247*
Error	114	0.077	
Total	173		
LP data			
Block	2	0.027	1.322
Isolate	57	0.074	3.601*
Error	114	0.021	
Total	173		
Girdling data			
Block	2	0.035	1.115
Isolate	57	0.071	2.278*
Error	114	0.031	
Total	173		
AUDPC data			
Block	2	1.432	0.729
Isolate	57	5.733	2.922*
Error	114	1.962	
Total	173		
AUDPC+Girdling data			
Block	2	0.462	0.765
Isolate	57	1.675	2.773*
Error	114	0.604	
Total	173		

* Significant at the 0.01 probability level.

isolate having slow growth rate produced no sclerotia on PDA medium. The most aggressive isolate (K1.11) came from Kalaleh and bore the highest mean AUDPC as well as mean girdling values.

The dendrogram obtained from the means of girdling showed different levels of virulence among isolates. Five groups of virulences were obtained among all the isolates through UPGMA (Figure 5). Group IV consisted of two isolates (highly aggressive). Group III, composed of 4 isolates was of high virulence. Groups V and I exhibited intermediate virulence while group II low virulence. All of non sclerotia producing isolates were exhibited to fall in group II.

Isolate Virulence within MCGs

In most cases the differences in virulence of isolates within the MCGs were significant (P= 0.01). For example, isolates K1.11 and K1.4 from MCG 27 had a range of mean AUDPC and mean girdling range of from 8.58 to 5.12 and 0.58 to 0.02, respectively. But in few cases it happened that isolates, virulence within MCGs did not differ (P= 0.01). For instance, isolates K2.5 and K2.9, belonging to MCG 28, exhibited no differences (P= 0.01) in mean AUDPC values and in mean values of girdling.

DISCUSSION

A distribution of mycelial compatibility grouping in *S. sclerotiorum* isolates from

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Table 4. Comparison of mean AUDPC, girdling, and AUDPC+girdling values using Duncan's Multiple Range Test *(*P= 0.01).

Isolate code	AUDPC mean	Girdling mean	AUDPC+Girdling mean
H1.4	6.42 ABCD*	0.33 ABCD	3.38 ABCDE
H1.8	5.24 ABCDE	0.04 CD	2.64 ABCDEF
H1.9	6.31 ABCD	0.33 ABCD	3.32 ABCDE
H2.2	5.7 ABCDE	0.14 ABCD	2.93 ABCDEF
H2.3	7.35 ABC	0.56 AB	3.96 ABC
H2.5	3.87 BCDE	0.00 D	1.94 BCDEF
H3.3	4.17 BCDE	0.08 CD	2.13 BCDEF
H3.5	4.79 BCDE	0.29 ABCD	2.54 ABCDEF
H3.6	3.16 DEF	0.00 D	1.58 EFG
H3.8	3.82 CDE	0.00 D	1.91 CDEF
H4.4	3.60 CDE	0.00 D	1.80 DEFG
H4.6	6.35 ABCD	0.39 ABCD	3.37 ABCDE
H4.9	6.36 ABCD	0.25 ABCD	3.30 ABCDE
H5.6	5.28 ABCDE	0.27 ABCD	2.78 ABCDEF
H5.7	5.34 ABCDE	0.02 D	2.68 ABCDEF
H6.7	4.89 ABCDE	0.10 BCD	2.50 BCDEF
H7.10	5.55 ABCDE	0.25 ABCD	2.90 ABCDEF
H8.5	6.42 ABCD	0.23 ABCD	3.32 ABCDE
H8.9	5.67 ABCDE	0.31 ABCD	2.99 ABCDEF
H8.10	3.72 CDE	0.00 D	1.86 CDEF
H9.3	3.17 DEF	0.00 D	1.59 EFG
H9.4	2.05 EF	0.00 D	1.02 FG
A1.4	6.68 ABCD	0.17 ABCD	3.42 ABCDE
A1.6	4.48 BCDE	0.19 ABCD	2.33 BCDEF
A2.1	4.56 BCDE	0.19 ABCD	2.38 BCDEF
A2.6	6.00 ABCD	0.10 BCD	3.05 ABCDEF
A3.2	5.08 ABCDE	0.00 D	2.54 ABCDEF
A3.8	4.59 BCDE	0.14 ABCD	2.37 BCDEF
A4.3	5.73 ABCDE	0.12 ABCD	2.93 ABCDEF
A4.9	5.59 ABCDE	0.27 ABCD	2.93 ABCDEF
A5.1	5.79 ABCD	0.33 ABCD	3.06 ABCDEF
A5.8	7.61 AB	0.46 ABCD	4.03 AB
A7.5	6.16 ABCD	0.17 ABCD	3.16 ABCDE
A7.7	3.82 CDE	0.83 CD	1.95 BCDEF
A8.6	6.43 ABCD	0.21 ABCD	3.32 ABCDE
A8.9	5.84 ABCD	0.12 ABCD	2.98 ABCDEF
A9.5	5.46 ABCDE	0.00 D	2.73 ABCDEF
A9.8	7.26 ABC	0.42 ABCD	3.84 ABCD
A10.3	5.85 ABCD	0.27 ABCD	3.06 ABCDEF
K1.4	5.12 ABCDE	0.02 D	2.57 ABCDEF
K1.11	8.58 A	0.58 A	4.58 A
K2.5	5.22 ABCDE	0.00 D	2.61 ABCDEF
K2.6	6.12 ABCD	0.19 ABCD	3.15 ABCD
K2.7	5.71 ABCDE	0.62 CD	2.89 ABCDEF
K2.9	5.30 ABCDE	0.00 CD	2.65 ABCDEF
K3.8	5.97 ABCD	0.12 ABCD	3.05 ABCDEF
K3.9	5.30 ABCDE	0.00 D	2.65 ABCDEF
K4.7	6.53 ABCD	0.29 ABCD	3.41 ABCDE
K5.6	4.57 BCDE	0.19 ABCD	2.38 BCDEF
K5.8	5.70 ABCDE	0.83 CD	2.89 ABCDEF
K6.6	4.87 ABCDE	0.21 ABCD	2.54 ABCDEF
K8.6	5.66 ABCDE	0.17 ABCD	2.91 ABCDEF
K9.1	6.21 ABCD	0.29 ABCD	3.25 ABCDE
K9.2	6.30 ABCD	0.19 ABCD	3.25 ABCDEF
K9.9	5.56 ABCDE	0.00 D	2.78 ABCDEF
K10.1	7.16 ABC	0.50 ABCD	3.83 ABCD
K10.10	4.33 BCDE	0.62 CD	2.19 BCDEF
control	0.00 F	0.00 D	0.00 G

* Letters beside the numbers in the last three columns show statistical groups.

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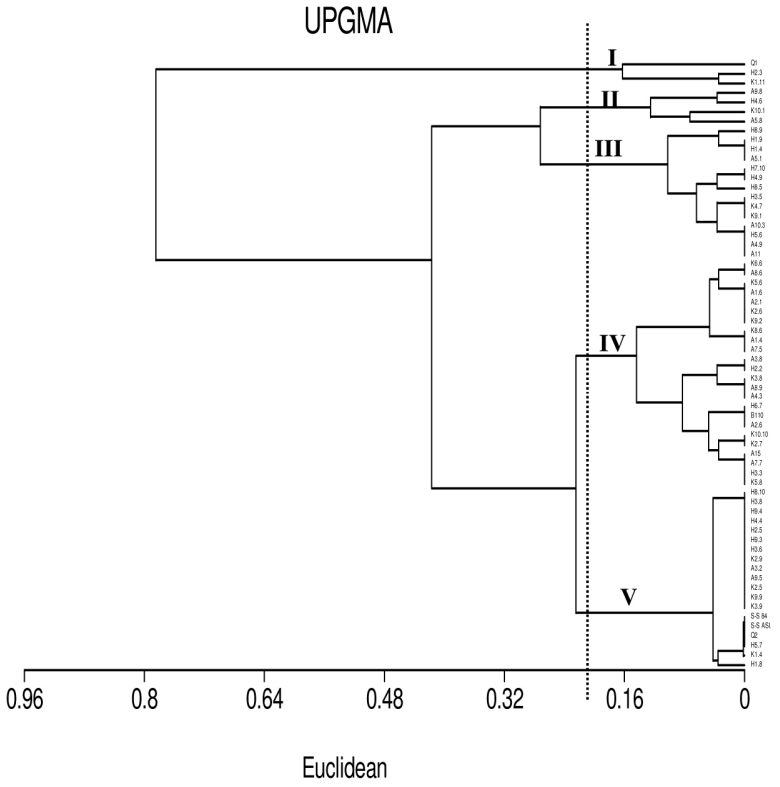

Figure 5. Clustering of 64 isolates of *S. sclerotiorum* as based on mean girdling values.

Iran was for the first time reported in this study. Populations of *S. sclerotiorum* from the studied locations formed a heterogeneous mix of MCGs. This is in agreement with those obtained from canola and soybean crops in Canada (Kohli *et al*., 1992; 1995; Kohn *et al*., 1991; Hambleton *et al*., 2002), cabbage and soybean in USA (Cubeta *et al*., 1997; Kull *et al*., 2004), giant buttercup and cauliflower in New Zealand (Carpenter *et al*., 1999) as well as sunflower in China (Li *et al*., 2008). In the current study, each MCG being composed of 1-3 isolates; would be indicative of a much greater sexual reproduction than in other parts of the world (Cubeta *et al*., 1997; Kohli and Kohn, 1998; Kohli *et al*., 1992). Furthermore, the results indicated a high level of MCG diversity. Genetic exchange, meiotic recombination (Carbone *et al*., 1999), mitotic recombination, transitory selection, selective neutrality, diversifying selection (Kohli *et al*., 1992), infrequent outbreeding in *S. sclerotiorum*, immigration of strains from other sites (Glass and Kuldau, 1992), could account for the possibilities of MCG diversity. Stem rot of canola is caused by airborne ascospores, the outcomes of a sexual event (homothallic and perhaps outcrossed), proposing that such events related to sexual reproduction as meiotic recombination are the important sources of MCG diversity of *S. sclerotiorum* in canola.

In some mycelial interactions there existed partial compatibilities (level 1) that might be mediated as by the sharing of some, (but not all) compatibility loci or alleles in recombination. This could be attributed to either the reshuffling of mycelial compatibility loci or alleles in recombination (Cubeta *et al*., 1997).

The data indicated that each canola field included one or two MCGs of low frequencies. This may be the result insufficient number of isolates that selected

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for determining MCG in each field (2-3 isolates each field) and so it would be necessary to take more isolates to be tested from each field.

Moreover, no common MCGs were found within the three studied regions. This may show little or no movement of propagules or indicate selection for particular MCG genotypes affected by environmental conditions and competition (Kull *et al*., 2004). Furthermore, lack of common MCGs among these regions may be due to small sample size. To detect common MCGs, additional sampling from these regions is required. The data indicated that all the unique MCGs were collected from Ali Abad. Localization of unique MCGs was observed in winter canola in Harrison, Ontario (Kohn *et al*., 1991). The existence of unique MCGs in a sampling area suggests that new MCGs and so new genotypes are evolving. The emergence of new genotypes could indicate that MCGs could be adapted to specific field microclimates. Also, evolving of new genotypes may be associated with increased cropping of canola in such areas (Hambleton *et al*., 2002), leading to movement of *S. sclerotiorum* onto this crop from several other host plants, or due to ascogenous system in sexual reproduction, indicating a sexual population in contrast to the clonal population structure as indicated by other studies (Kohli *et al*., 1992; Cubeta *et al*., 1997).

It was found that among the four different scorings, AUDPC, girdling, and AUDPC+girdling were the most suitable. Among four different scorings' AUDPC carried the lowest coefficient of variation (CV) of all the four scoring systems under controlled conditions. AUDPC score was assessed after 7, 10, and 14 days past inoculation. Thus, this approach would distinguish differences in disease progress caused by different isolates. From a pathological girding point of view girdling is an important index because it causes verse and death of plants. Highly virulent isolates exhibit a girdling lesion so severe that the stem portion above the lesion topples over. The girdling score was employed to classify plant phenotypes at a single point in time, late in the inoculation period. Thus, it may be more practical for virulence assessment of a large number of isolates, because it involves a single evaluation at the end of an experiment.

Different levels of virulence of *S. sclerotiorum* have been previously examined. A study report (Kull *et al*., 2004) through use of a limited-term, plug inoculation technique, showed that isolate aggressiveness varied in some studied locations. The author also showed that aggressiveness values for isolates within MCGs composed of members from multiple locations were highly significant, but isolate aggressiveness within locally observed MCGs, composed of members from a single location, did not differ. The present study revealed a considerable range in AUDPC values and as well in girdling values within MCGs constituted from individuals from a single location or even from a single field. These pathogenic variations in isolates and in MCGs do not seem to be related with their geographic origins. Variability in MCGs virulence within each field suggests that MCGs may conserve appropriate phenotypes or it more likely could indicate that there is no phenotype for this virulence (or aggressiveness). Moreover, it may indicate that MCG is not a suitable marker for virulence. Within the Hashem Abad fields, MCG 2 was the most aggressive, while MCG 10 the least. In Kalaleh, MCG 27 was the most aggressive, while MCG 35 the least aggressive. Among the unique MCGs, MCG 20, isolate A5.8, and MCG 22, isolate A7.7, resulted in the highest and lowest AUDPC values, respectively, within the 57 isolates compared in the virulence tests. Even isolates that had been single-ascospore cultures from the same sclerotium were totally different regarding their morphological, physiological and pathological characteristics (Morrall *et al*., 1972). Additionally, upon comparison, the isolates from different sources, notable variation in cultural morphology and

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virulence was found among protoplast-regenerated isolates of *S. sclerotiorum* (Boland and Smith, 1991).

Oxalic acid is an essential virulence factor of *S. sclerotiorum*, because mutants, which are deficient in oxalate biosynthesis are less pathogenic than wild-type fungi. (Godoy *et al*., 1990). Contrary to wild type fungus, oxalate-deficient *S. sclerotiorum*, which is incapable of producing oxalic acid during infection of petals (Godoy *et al*., 1990; Jamaux *et al*., 1995) produces no sclerotia *in vitro* and is less virulent (Rollins and Dickman, 2001). The results indicated isolates (H3.8, H4.4, H8.10, H9.3, H9.4) that produce no sclerotia and have low radial growth rate on PDA medium were the least aggressive. These isolates may produce less oxalic acid than the others but this claim should be put to testification.

Mycelial (vegetative) compatibility/inco mpatibility is a self-self/non-self recognition system controlled by multiple loci (Sexton and Howlet, 2004). The large number of MCGs that have been detected in field populations is consistent with the existence of several loci at which there are at least two alleles in the population (Kohli, *et al*., 1992). Thus, it should be considered that MCG or Vegetative Compatibility Group (VCG) system in fungi is not essentially clonal. Generally, in a sexually reproducing population, it is expected to have a high level of MCG diversity. There probably will be no correlation between a character such as virulence and MCG if pathogenicity is controlled by one or a few genes (Leslie, 1993).

The mixed results across field locations used for resistance screening trails (Kim *et al*., 1999) and the lack of correlation between field and greenhouse evaluations (Kim *et al*., 2000; Nelson *et al*., 1991; Wegulo *et al*., 1998) may be due to the differences in MCG frequency between fields and variation in virulence of *S. sclerotiorum* isolates (Kull *et al*., 2004). Therefore, to improve disease management efforts, it is more effective to select several isolates for evaluating resistance or developing disease resistance markers than evaluations conducted with one isolate.

In conclusion, with respect to Ali Abad designated high level of MCG diversity, majority of which were unique, with different levels of virulence, it could be found that the emergence of virulence and fungicide resistant isolates on different cultivars is possible in this region. Also, with respect to this result; it is suggested that evaluation of canola cultivars for resistance to Sclerotinia stem rot (in field environments) to be conducted in Ali Abad. Moreover, experiments of isolate-cultivar interactions could be done with isolates prevalent in this region.

REFERENCES

1. Atallah, Z. K., Laget, B. Chen, X. and Johnson, D. A. 2004. High Genetic Diversity, Phenotypic Uniformity and Evidence of out Crossing in *Sclerotinia sclerotiorum* in the Columbia Basin of Washington State. *Phytopathol.*, **94**: 737-742.
2. Boland, G. J. and Hall, R. 1994. Index of Plant Hosts of *Sclerotinia sclerotiorum. Can. J. Plant Pathol.*, **16**: 93–100.
3. Boland, G. J. and Smith, E. A. 1991. Variation in Culture Morphology and Virulence among Protoplast Regenerated Isolates of *Sclerotinia sclerotiorum. Phytopathol.*, **81**: 766-770.
4. Carbone, I., Anderson, J. B. and Kohn, L. M. 1999. Patterns of Descent in Clonal Lineages and Their Multilocus Fingerprints are Resolved with Combined Gene Genealogia. *Evolution*, **53**: 11-21.
5. Carpenter, M. A., Frampton, C. and Stewart, A. 1999. Genetic Variation in New Zealand Populations of the Plant Pathogen *Sclerotinia sclerotiorum. N. Z. J. Crop Hortic. Sci.,* **27**: 13-21.
6. Cubeta, M. A., Codey, B. R. Kohli, Y. and Kohn, L. M. 1997. Clonality in *Sclerotinia sclerotiorum* on Infected Cabbage in Eastern North Carolina. *Phytopathol.*, **87**: 1000-1004.
7. Danielson, G. A., Nelson, B. D. and Helms, T. C. 2004. Effect of *Sclerotinia* Stem Rot

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on Yield of Soybean Inoculated at Different Growth Stages. *Plant Dis*., **88**: 297–300.
8. Durman, S. B., Menendez, A. B. and Godeas, A. M. 2005. Variation in Oxalic Acid Production and Mycelial Compatibility within Field Populations of *Sclerotinia sclerotiorum*. *Soil Biol. Biochem.*, **37**: 2180-2184.
9. Durman, S. B., Menendez, B. and Godes, A. M. 2003. Mycelial Compatibility Groups in Buenos Aires Field Populations of *Sclerotinia sclerotiorum*. *Aus. J. Bot.*, **51**:421-427.
10. Ford, E. J., Miler, R. V., Gray, H. and Sherwood, J. E. 1995. Heterokaryon Formation and Vegetative Compatibility in *Sclerotinia sclerotiorum*. *Mycol. Res.*, **99**: 241-247.
11. Glass, N. L. and Kuldau, G. A. 1992. Mating Type and Vegetative Incompatibility in Filamentous Ascomycetes. *Ann. Rev. Phytopathol.*, **30**: 201-224.
12. Godoy, G., Steadman, J. R., Dickman, M. B. and Dam, R. 1990. Use of Mutants to Demonstrate the Role of Oxalic Acid in Pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris*. *Physiol. Mol. Plant Pathol.*, **37**: 179–191.
13. Goodwin, S. B., Allard, R. W., Hardy, S. A. and Webster, R. K. 1992. Hierachical Structure of Pathogenic Variation among *Rhynchosporium secalis* Populations in Idaho and Oregan. *Can. J. Bot.*, **70**: 810-817.
14. Gugel, R. K. and Morrall, R. A. A. 1986. Inoculum-disease Relationships in Sclerotinia Stem Rot of Rapeseed in Saskatchewan. *Can. J. Plant Pathol.*, **8**: 89-96.
15. Gulya, T. M., Rashid, K. Y. and Masirevic, S. M., 1997. Sunflower Diseases. In: "*Sunflower Technology and Production*", (Ed.): Schneiter, A. A.. Agronomy, Madison, WI, PP. 263–379.
16. Hambleton, S., Walker, C. and Kohn, L. M. 2002. Clonal Lineages of *Sclerotinia sclerotiorum* Previously Known from Other Crops Predominate in 1999-2000 Samples from Ontario and Quebec Soybean. *Can. J. Plant Pathol.*, **24**: 309-315.
17. Jamaux, I., Gelie, B. and Lamarque, C. 1995. Early Stages of Infection of Rapeseed Petals and Leaves by *Sclerotinia sclerotiorum* Revealed by Scanning Electron Microscopy. *Plant Pathol.*, **44**: 22–30.
18. Kim, H. S., Hartman, G. L., Manandhar, J. B., Grief, G. L., Steadman, J. R. and Diers, B. W. 2000. Reaction of Soybean Cultivars to Sclerotinia Stem Rot in Field, Greenhouse, and Laboratory Evaluations. *Crop Sci.*, **39**: 64-68.
19. Kim, H. S., Sneller, C. H. and Diers, B. W. 1999. Evaluation of Soybean Cultivars for Resistance to Sclerotinia Stem Rot in Field Environments. *Crop Sci.*, **39**: 64-68.
20. Kirkegaard, J. A., Robertson, M. J., Hamblin, P. and Sprague, S. J. 2006. Effect of Blackleg and *Sclerotinia* Stem Rot on Canola Yield in the High Rainfall Zone of Southern New South Wales, Australia. *Aust. J. Agril. Res.*, **57**: 201–212.
21. Kohli Y., L.J. Brunner H. Yoell and L.M. Kohn, 1995. Clonal Dispersal and Spatial Mixing in Populations of the Plant Pathogenic Fungus, *Sclerotinia sclerotiorum*. *Molecular Ecology* 4, 69-77.
22. Kohli, Y. and Kohn, L. M. 1998. Random Association among Alleles in Clonal Populations of *Sclerotinia sclerotiorum*. *Fungal Genetic Biol.*, **23**: 139-149.
23. Kohli, Y., Monail, R. A. A., Anderson, J. B. and Kohn, L. M. 1992. Local and Trans-Canadian Clona1 Distribution of *Sclerotinia sclerotiorum* on Canola. *Phytopathol.*, **82**: 875-880.
24. Kohn, L. M., Carbone, I. and Anderson, J. B. 1990. Mycelial Interaction in *Sclerotinia sclerotiorum*. *Exp. Mycol.*, **14**: 255-267.
25. Kohn, L. M., Stasovski, E., Carbone, I., Royer, J. and Anderson, J. B. 1991. Mycelial Incompatibility and Molecular Markers Identify Genetic Variability in Field Populations of *Sclerotinia sclerotiorum*. *Phytopathol.*, **81**: 480-485.
26. Kull, L. S. and Pedersen, W. L. 2004. Mycelial Compatibility Grouping and Virulence of *Sclerotinia sclerotiorum*. *Plant Dis.*, **88**: 325-332.
27. Leslie, J. F. 1993. Fungal Vegetative Compatibility. *Ann. Rev. Phytopathol.*, **31**: 127-150.
28. LeToumeau, D. 1979. Morphology, Cytology and Physiology of *Sclerotinia* Species in Culture. *Phytopathol*., **69**: 887-890.
29. Li, Z., Zhang, M., Wang, Y., Li, R. and Dilantha, W. G. 2008. Mycelial Compatibility Group and Pathogenicity Variation of *Sclerotinia sclerotiorum* from

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China, Canada, and England. *Plant Pathol. J.*, **7**: 131-139.
30. Malvarez, G., Carbone, I., Grunwald, N. J., Subbarao, K. V., Schafer, M. and Kohn, L. M. 2007. New Populations of *Sclerotinia sclerotiorum* from Lettuce in California and Peas and Lentils in Washington. *Phytopathol.*, **97**: 470-483.
31. Marciano, P., Dileena, P. and Magro, P. 1983. Oxalic Acid, Cell Wall Degrading Enzymes and pH in Pathogenesis and Their Significance in the Virulence of Two *Sclerotinia sclerotiorum* Isolates on Sunflower. *Physiol. Plant Pathol.*, **22**: 339-345.
32. Mert-Turk, F., Ipek, M., Mermer, D. and Nicholson, P. 2007. Microsatellite and Morphological Markers Reveal Genetic Variation within of Population of *Sclerotinia sclerotiorum* from Oilseed Rape in the Canakkale Province of Turkey. *Phytopathol.*, **155**: 182-187.
33. Morrall, R. A. A., Duczek, L. J. and Sheard, J. W. 1972. Variation and Correlations within and between Morphology, Pathologenicity and Pectolytic Enzyme Activity in *Sclerotinia* from Saskatchewan. *Can. J. Bot.*, **50**: 767-786.
34. Nelson, B. D., Hems, T. C. and Olson, J. A. 1991. Comparison of Laboratory and Field Evaluations of Resistance in Soybean to *Sclerotinia sclerotiorum*. *Plant Dis.*, **75**: 662-665.
35. Price, K. and Colhoun, J. 1975a. A Study of Variability of Isolates of *Sclerotinia sclerotiorum* (Lib.) de Bary from Different Hosts. *Phytopathol. Z.*, **83**: 159–166.
36. Purdy, L. H. 1955. A Broader Concept of the Species *Sclerotinia sclerotiorum* Based on Variability. *Phytopathol.*, **45**: 421-427.
37. Purdy, L. H. 1979. *Sclerotinia sclerotiorum*: History, Disease and Symptomatology, Host Range, Geographic Distribution and Impact. *Phytopathol.*, **69**: 875–80.
38. Rollins, J. A. and Dickman, M. B. 2001. pH Signaling in *Sclerotinia sclerotiorum*: Identification of a *pacC/R1M1* Homolog. *App. Environ. Microbiol.*, **67**: 75–81.
39. Schafer, R. S. and Kohn, L. M. 2006. An Optimized Method for Mycelial Compatibility Testing in *Sclerotinia sclerotiorum*. *Mycologia*, **98**: 593-597.
40. Sexton A. C. and Howlett, B. J. 2004. Microsatellite Markers Reveal Genetic Differentiation among Populations of *Sclerotinia sclerotiorum* from Australian Canola Fields. *Current Genetics*, **46:** 357-365.
41. Sexton, A. C., Whitten, A. R. and Hawlett, B. J. 2006. Population Structure of *Sclerotinia sclerotiorum* in an Australian Canola Field at Flowering and Stem-infected Stages of the Disease Cycle. *Genome*, **49**: 1408-1415.
42. Wegulo, S. N., Yang, X. B. and Martinson, C. A. 1998. Soybean Cultivar Response to *Sclerotinia sclerotiorum* in Field and Environmental Studies. *Plant Dis.*, **82**: 1264-1270.
43. Hao, J., Peltier, A. J., Meng, J., Osborn, T. C. and Grau, C. R. 2004. Evaluation of Sclerotinia Stem Rot Resistance in Oilseed *Brassica napus* Using a Petiole Inoculation Technique under Greenhouse Conditions. *Plant Dis.*, **88**: 1033-1039.

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گروههای سازگاری میسلیومی و تنوع بیماریزایی جمعیت های *Sclerotinia sclerotiorum* (Lib.) de Bary روی کلزا در استان گلستان

ا. کریمی، ن. صفایی و م. شمس بخش

چکیده

به منظور بررسی ساختار ژنتیکی و تنوع بیماریزایی *Sclerotinia sclerotiorum*، عامل پوسیدگی سفید ساقه کلزا، با استفاده از گروههای سازگاری میسلیومی (MCG) و مقایسه ویرولانس

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جدایه ها از تعداد ۵۷ جدایه منتخب جمع آوری شده از سه منطقه جغرافیایی مختلف در استان گلستان استفاده گردید. در بین ۵۷ جدایه مورد بررسی، ۳۵ گروه سازگاری میسلیومی تعیین شد که ۴۲/۸۶ درصد آنها از یک جدایه تشکیل شده بودند و همه در منطقه علی آباد قرار داشتند. تعداد MCGها در سه منطقه مورد مطالعه متفاوت بود. ۲۵/۷۱، ۲۸/۵۷، و ۴۵/۷۲درصد MCGها به ترتیب متعلق به کلاله، هاشم آباد و علی آباد بود. در منطقه کلاله، نه MCG شناسایی شد که همه از دو جدایه تشکیل شده بودند. ده MCG در منطقه هاشم آباد شناسایی شد که هشت MCG از دو جدایه تشکیل شده بودند و بقیه هر کدام شامل سه جدایه بودند. شانزده MCG در منطقه علی آباد مشاهده شد، به استثنای یک MCG که متشکل از دو جدایه بود بقیه از یک جدایه تشکیل شده بودند. همچنین، هیچ MCG بین مناطق مورد مطالعه مشترک نبود. شاخص تنوع شانون MCG ها برای کل مناطق ۰/۸۶ بود. تقسیم تنوع کل به دو جزء تنوع درون و بین جمعیت ها نشان داد که ۹۵/۴۵ درصد تنوع مربوط به تنوع بین جمعیت ها است. تنوع در ویرولانس جدایه ها با استفاده از روش مایه زنی دمبرگ ارزیابی شد. ویرولانس جدایه ها در سه منطقه متفاوت بود ($P= 0.01$). در بیشتر موارد جدایه های متعلق به یک MCG از نظر ویرولانس با یکدیگر تفاوت داشتند. نتایج این مطالعه نشان می دهد که جمعیت های *S sclerotiorum*. مورد بررسی هتروژنوس بوده و مخلوطی از چندین MCG است و لذا در سیستم های مدیریت این بیماری در این مناطق باید ساختار جمعیت این بیمارگر و تنوع در ویرولانس جدایه ها در نظر گرفته شود.

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