

Genetic and Morphological Diversities in *Sclerotinia sclerotiorum* Isolates in Northern Parts of Iran

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Abstract: *Sclerotinia sclerotiorum* is the most important causal agent of stem rot diseases on the field crops in Iran. During 2006-2007, totally 65 isolates of the fungus were obtained from infected rapeseed, lettuce, bean, tomato, cucumber and wild sinapsis plants in various fields of North provinces in Iran. Genetically diversities between the isolates were investigated by PCR, using five microsatellite primer pairs and those divided to 9 groups with 25 clear polymorphic alleles. A high level of genetic diversity was observed about 67%, between the some isolates. By mycelial compatibility grouping tests, the isolates were settled into 39 groups that 26 MCGs were individual. Molecular and phenotypic analyses results of the most of isolates were similar; however the isolates in MCG1, MCG4 and MCG23 groups, with variable microsatellite haplotypes, were morphologically dissimilar. The results shown that there were possibly high rates of out crossing as well as evolutionary potential within population of the pathogen in different collecting locations. This is the first study in genetical variations of *S. sclerotiorum* populations in Iran.

Key words: Microsatellites % MCG % *Sclerotinia sclerotiorum* % Genetic diversity

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is a cosmopolitan, homothallic and necrotrophic fungus, causes disease on more than 400 host species. *S. sclerotiorum*, an ascomycetous fungus, is dispersed by airborne ascospores and soilborne sclerotia [1]. Stem rot is the most important disease, caused by the fungus on the field plants, specially on rapeseed. Yield losses due to stem rot disease, is vary and sometimes reach to maximum level in susceptible plants [2].

Epidemics are initiated when airborne ascospores land on open blossoms, attached to the canopy. Contaminated flowers fall on stems and on the ground and then fungal mycelia rapidly colonize the petals. Stems or leaves contacting colonized petals acquire the disease. Flower removal or fungicide applications at initial full bloom have drastically reduced disease incidence [3]. Infrequently, plants are infected at soil level by mycelia growing from sclerotia, asexual resting propagules, in close proximity of the plant crown.

Two independent mechanisms, mycelial compatibility group (MCG) and DNA fingerprinting, have been developed to differentiate of *S. sclerotiorum* populations.

Mycelial compatibility group (MCG) is a phenotypic marker system that controlled by multiple loci. When the members of a MCG paired with each other, in fact those fuse and form one confluent colony, although mycelial incompatibility is described as a failure of different strains to fuse and to form a colony, then characterized by the formation of a barrage of dead cells between the two incompatible colonies, known as a reaction line [4]. DNA fingerprinting technique can also be used to distinguish closely related fungal isolates. Southern hybridization of restriction digested whole genomic DNA to a cloned probe containing a 4.5 kb repeated dispersed element of nuclear DNA from *S. sclerotiorum* was previously used [5] and in several subsequent studies [6,7]. Microsatellites [8], also known as simple sequence repeats (SSRs) or short tandem repeats (STRs), are the smallest class of simple repetitive DNA sequences [9,10] that widely dispersed and evenly distributed in the genome of eukaryotes and have been used to

study variability within the populations [11,12]. The advantages of microsatellites over other markers are their high specificity, reproducibility, polymorphism and co-dominance.

These markers have been used to detect potential out crossing in fungi previously thought to perpetuate asexually exclusively, such as *S. sclerotiorum* [13]. There are several reports about the structural variations of *S. sclerotiorum* populations in the world. Thirty-nine colons were identified among the 66 isolates on canola (Rapeseed) in Canada from seven locations in Alberta, Saskatchewan and Manitoba states [14] and 50 MCGs identified among the 140 isolates from Buenos Aires [15]. Limited out crossing among the *S. sclerotiorum* isolates was observed in North Carolina and California [16]. Several works were focused on comparison of the *S. sclerotiorum* populations from agricultural and wild plants in Norway [17,18] and there was genetic uniformity among the populations on potato and canola; however, greater genetic diversity have observed among the wild populations. Three *S. sclerotiorum* populations collected from Europe, China and Canada, were compared and genetic differentiations were been highly significant among and within the populations [19].

Despite *S. sclerotiorum* have caused the major diseases in many fields and glasshouse crops, there was not any report about the genetic diversity of the *S. sclerotiorum* populations in Iran. The objectives of this study were elucidation of the structural and genetic diversity within the populations of *S. sclerotiorum* on the field crops in North of Iran (Gillan, Mazandaran and Golestsan provinces) and measurement of the growth manner of *S. sclerotiorum* haplotypes at different temperatures.

MATERIALS AND METHODS

Isolates: Isolates of *Sclerotinia sclerotiorum* were collected from 52 rapeseed, lettuce, bean, tomato and cucumber fields in Gillan, Mazandaran and Golestsan, north provinces of Iran, during 2006-2007 growing seasons. Samples were collected from the infected plants and the sclerotia were removed from the each plant sample. The single sclerotium was selected as an isolate. The sclerotia were surface sterilized for 1 min in 70% ethanol or 2 min in 2.5% sodium hypochlorite, rinsed in sterile distilled water, then plated on potato dextrose agar medium (PDA) and incubated at 22°C for two days. Each isolate was purified by transferring the single hyphal tip onto the fresh medium, and generated sclerotia were stored at -20°C until used [3,6,20].

DNA Extraction: DNA was extracted using the rapid mini-preparation method [21], with some modifications. This procedure included the following steps. (i) To a 1.5-ml Eppendorf tube containing 500 µl of lysis buffer (400 mM Tris-HCl [pH 8.0], 60 mM EDTA [pH 8.0], 150 mM NaCl, 1% sodium dodecyl sulfate), a small lump of the mycelia is added by using a sterile toothpick, then the tube was left at room temperature for 10 min. (ii) After adding 150 µl of potassium acetate (pH 4.8; which is made of 60 ml of 5 M potassium acetate, 11.5 ml of glacial acetic acid and 28.5 ml of distilled water), the tube is vortexed briefly and spun at 10,000g for 1 minute. (iii) The supernatant was transferred to another 1.5-ml Eppendorf tube and centrifuged again as described above. After transferring the supernatant to a new 1.5-ml Eppendorf tube, an equal volume of isopropyl alcohol was added. The tube was mixed by inversion briefly. (iv) The tube was spun at 10,000g for 2 min. the supernatant was discarded and the pellet was air dried. Finally the pellet as purified DNA was diluted in 1X TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) to a working concentration of 10-20 ng/µl and stored at 5°C [22].

Microsatellite Primers, PCR Implication, Separation of PCR Products and Data Analysis: Five sets of microsatellite primers were used in this study, including (AGAT)₁₄, (AAGC)₄, (CATA)₂₅, (CA)₉, (GT)₁₀ and (TACA)₁₀ [12]. The PCR reaction mixture included 10-20ng of the purified DNA and the reaction buffer (100 µM each of dATP, dCTP, dGTP and dTTP, 200 nM of microsatellite primer and 0.8 units of Taq polymerase, 10mM Tris-HCl pH 8.3, 1.5 mM MgCl₂, 50 mM KCl, 100 µgmLG¹ gelatine, 0.05% tween 20 and 0.05% Nonidet P-40). The final reaction volume was adjusted to 50 µL with deionized H₂O. All of the reagents were prepared from Fermentas Inc. USA. Amplification was carried out using initial denaturation at 95°C for 8 min, followed by 35 cycles primer annealing at 59°C (for all microsatellite primers) and extension at 72°C for 60 s, with a 5 min extension at 72°C on the final cycle. The PCR products were separated on denaturing agarose gel (2.6% w/v). Gels were stained with ethidium bromide, visualized under UV light and digitally documented with the gel documentation UVP-V system. The gel was run at 90 W for 90 min [9]. All polymorphic alleles were identified from the each microsatellite primer combination and bands representing alleles were scored as present (1) or absent (0). Nei's genetic distance matrix [23] was prepared and bootstrap analysis with 2000 replications was performed to generate a dendrogram of unweighted pair-group mean analysis (UPGMA) [5], using the treecon 1.3b program [24].

Mycelial Compatibility Group Determination: For evaluation of interfarm MCG variability, five isolates from the each field were paired together. 0.5cm diameter mycelial plugs were obtained from the edge of the two days colonies on PDA. Three mycelial plugs were paired in 6.5-cm-diameter petri dishes containing PDA amended with 75 µl of Wilton’s red food coloring per liter of culture medium [3-5], Petri dishes were incubated in the dark at 22°C for 14 days. Pairings of the 65 isolates paired in all-pairwise combinations. Three replications were made for each pairing. Only incompatible isolates were paired subsequently. Compatible isolates were distinguished by the fusion of mycelia, without an accumulation of red dye in the fusion zone. Incompatible reactions produced a barrage recognized by an obvious red line on the bottom side of petri dishes or by the formation of aerial mycelia along the barrage line. Pairings that yielded questionable reactions were repeated to insure accurate results.

Temperatures Treatments: The radial growth of the *S. sclerotiorum* isolates were assessed at five different temperatures, including the optimum temperature (22°C) and others 10 and 5°C below and above (12, 17, 27 and 32°C). PDA plates were inoculated with a 5 mm diameter plug of the colonized agar and incubated in the darkness at the various temperatures. Colony diameter was measured daily and the values averaged until the colony neared the edge of the dish. In a completely randomized

design, four Petri dishes replicates for each isolate were used in four times separately. Data were analyzed by ANOVA using MSTAT-C program and mean values were compared at the *P* # 0.05 level using Duncan's multiple range test. The color of each colony was noted 21 days after the initial growth on PDA.

RESULTS

Genetic Diversity among the *Sclerotinia Sclerotiorum* Isolates:

The microsatellite primers exhibited 25 clear polymorphism alleles from the 65 fungal samples. The number of polymorphic alleles per locus ranged from 2 to 8 (Table 1). In order to determine the genetic relationships among of populations of fungal isolates , a separate matrix was used to the data obtained from the 44 polymorphic alleles and the 65 isolates of *S. sclerotiorum* were clustered (Figures 1 and 2).

Table 1: Number of alleles recognized for each microsatellite marker set in the *Sclerotinia sclerotiorum* population used in the present study compared with those from previous studies

Repeat motifs	Allele Number	Previous reports	Other species
(CA) ₉	2	4 ^a 2 ^b	No
(GT) ₁₀	4	5 ^a 2 ^b	No
(CATA) ₂₅	6	10 ^a 3 ^b	Yes
(AGAT) ₁₄ (AAGC) ₄	8	8 ^a 4 ^b	Yes
(TACA) ₁₀	5	7 ^a 4 ^b	Yes

^aSirjusingh and Kohn, 2001

^bAtallah *et al.*, 2004



Fig. 1: Microsatellite analysis of *Sclerotinia sclerotiorum* isolates amplified with the (CATA)₂₅ primer. PCR amplicons were separated on a 2.6% agarose gel in 0.5 X TBE. Bands were stained with ethidium bromide and visualized on a UV-Transeluminator

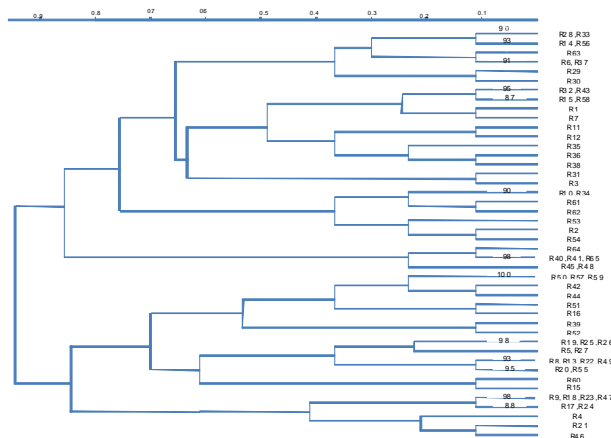


Fig. 2: Unweighted pair-group mean analysis dendrogram of genetic distance among the 65 fungal isolates based on Nei's coefficients. The numbers given above the lines indicate the bootstrap values of 2000 replicates

Table 2: MCG, colony colour and growth rate of *Sclerotinia sclerotiorum* isolates at different temperatures on PDA

Isolates	Host	Site	MCG s ^a	Colony color ^b	Colony growth at different temperature(mm) ^c				
					12°C	17°C	22°C	27°C	32°C
R4	Lettuce	Dashtenaz	1	Beige	7.0jk	12.5c	57.8jkl	43.8mno	2.4 ab
R19	Rapeseed	Amol	1	Beige	9.3mn	20.1c	70.0abcde	50.3q	6.9qrs
R25	Rapeseed	Rezvanshahr	1	Beige	9.6mnr	19.8c	60.9hij	50.1q	6.4pqr
R26	Rapeseed	Bandar Anzali	1	Beige	9.0m	20.0c	72.0abc	48.9pq	6.8 qr
R5	Lettuce	Kiakolla	2	Beige	4.0f	19.1c	59.0jkl	53.3qrs	8.uv
R27	Rapeseed	Bandar Anzali	2	Beige	4.4fg	5.5b	60.1ijk	57.1stu	8.8vw
R6	Lettuce	Kiakolla	3	Beige	6.8ij	14.1c	58.0jkl	50.5q	8.2 uv
R37	Rapeseed	Nokandeh	3	Beige	6.6ij	12.6c	60.1ijk	50.6q	7.6 tu
R8	Lettuce	Amol	4	Beige	6.5ij	13.8c	56.9jklm	40.5m	5.3jk
R13	bean	Juibar	4	Beige	6.8ij	14.5c	57.8jkl	40.6m	4.8ij
R20	Rapeseed	Amol-hular	4	Beige	6.8ij	16.6c	58.1jkl	39.5lm	5.0ijk
R22	Rapeseed	Babol	4	Beige	7.1jk	11.6c	56.9jklm	43.8mno	4.8 ij
R30	Rapeseed	Ghaemshahr	4	Brown	2.5cd	16.8c	68.0cdef	38.5klm	3.4 cd
R49	Rapeseed	Kordkoy	4	Beige	6.6ij	17.6c	68.1cdef	53.8qrs	7.6 tu
R55	Rapeseed	Semeskandeh	4	Beige	7.1jk	12.3c	67.6cdef	66.8wx	2.6 ab
R64	Rapeseed	Bayekolla	4	White	2.35cd	17.1c	60.4ij	41.4mn	5.3jkl
R9	Lettuce	Amol	5	Beige	3.0de	16.8c	43.1rst	28.7ghi	1.9 ab
R18	Rapeseed	Amol	5	Beige	1.2b	6.9b	60.2ijk	40.6m	4.8 ij
R23	Rapeseed	Chardangeh	5	Beige	0.9ab	6.9b	60.2ijk	41.0mn	5.4 kl
R47	Rapeseed	Behshahr	5	Beige	1.2b	6.8b	61.8ijk	40.5mn	5.3jkl
R10	Bean	Kiakolla	6	White	11.6pq	18.8c	59.0jkl	39.1lm	4.3 fg
R34	Rapeseed	Shirgah	6	White	11.5pq	19.8c	57.8jkl	40.6mn	4.3 fg
R14	bean	Babol	7	Beige	7.0jk	13.1c	60.2ijk	50.3q	10.0 y
R56	Rapeseed	Semeskandeh	7	Beige	7.6k	14.4c	61.1hij	48.6pq	10.0y
R17	tomatto	Juibar	8	Beige	3.7f	11.5c	43.1rst	31.6ij	6.4pq
R24	Rapeseed	Rezvanshahr	8	Beige	2.9de	16.8c	43.1rst	28.7ghi	1.9 ab
R21	Rapeseed	Babol	9	Beige	1.3b	11.1c	48.1pq	39.0lm	5.1 jk
R28	Rapeseed	Bandar anzali	10	Brown	7.4jk	14.4c	66.8efg	50.5q	5.4 lm
R33	Rapeseed	Juibar	10	Brown	7.4jk	14.6c	67.2defg	50.3q	5.4 lm
R29	Rapeseed	Juibar	11	Beige	1.6bc y	12.1c	63.0ghi	55.2s	9.1 xy
R31	Rapeseed	Juibar	12	Beige	1.2b	6.5b	42.5st	33.0ijk	8.1uv
R32	Rapeseed	Juibar	13	Beige	3.1de	7.6b	57.5jkl	44.6no	9.1xy
R43	Rapeseed	Bandarturkaman	13	Beige	3.3e	6.2b	57.1jklm	43.8mn0	8.7vw
R35	Rapeseed	Shirgah	14	Beige	13s	21.8c	51.3nop	49.1pq	3.6de
R36	Rapeseed	Arateh	15	Brown	7.6k	19.4c	73.0ab	50.9pqr	4.9 ij
R38	Rapeseed	Kordekhail-Sari	16	White	0.6a	3.1a	43.1rst	28.7ghi	0.9 a
R39	Rapeseed	Shast kalateh	17	Beige	5.9hi	19.4c	66.0efg	66.1wx	x 2 ab
R40	Rapeseed	Shast Kalateh	18	Beige	6.6ij	15.6c	69.0cdef	50.6qr	4.3 fg
R41	Rapeseed	Kordkoi	18	Beige	6.4ij	15.8c	67.1defg	50q	4.5ghi
R65	Rapeseed	Bayekolla	18	Beige	6.8ij	19.9c	65.1fgh	53.3qrs	4.3 fg
R42	Rapeseed	Kordkoi	19	Beige	7.5k	20.1c	70.0abcde	51.0qr	1.3 a
R44	Rapeseed	Kordkoi-zare	20	Beige	1.6bc	8.4b	36u	31.1ij	4.5ghi
R45	Rapeseed	Kordkoi-zare	21	Beige	7.0j	14.0c	57.5jkl	50.3q	10.0 y
R48	Rapeseed	Behshahr	21	Beige	4.5fg	15.5c	59.0jkl	48pq	9.1 xy
R46	Rapeseed	Behshahr	22	Beige	4.6fg	5.4a	40.1	30.3i	6.3pq
R50	Rapeseed	Kordkoi-Kar.	23	Beige	7.4jk	20.1c	54.5lmno	41.1mn	1.8 a
R57	Rapeseed	Hullar	23	Beige	6.6ij p	12.3c	52.8mno	46.1no	3.4 cd
R58	Rapeseed	Hullar	23	White	1.8bc	8.3b	44.4qrst	32.8ijk	4.6 hi
R59	Rapeseed	Hullar	23	Beige	6.6ij	15.6c	51.1nop	43.3mno	4.3 fg
R51	Rapeseed	Kordkoi-Kar.	24	Brown	4.0f	5.9c	54.5lmno	40.3m	3.5 de
R52	Rapeseed	Galugah	25	Beige	9.0m	18c	45.5qr	30.6hij	2.4 ab
R53	Rapeseed	Suteh	26	Beige	12.1qr	18.6c	43.1rst	31.6ij	6.4pq
R54	Rapeseed	Semeskandeh	27	Beige	10.0no	18.0c	65.0fgh	11.9ab	4.9 ij
R60	Rapeseed	Galugah	28	Beige	11.6pq	19.8c	74.0a	60.1u	2.0 a

Table 2: Continued

R61	Rapeseed	Dashtenaz	29	Beige	2.0c	8.3b	44.5qrst	30.2i	4.6 hi
R62	Rapeseed	Dashtenaz	30	Beige	14.5u	20.5c	50.0op	33.1ij	9.4 y
R63	Rapeseed	Garakhail	31	Beige	11.3pq	20.6c	55.5klmn	36.8kl	5.6mn
R15	Wild sinapis	Juibar	32	Brown	6.4ij	15.6c	57.5jkl	38.5klm	4.8 ij
R16	Wild sinapis	Kordkoichardeh3	33	Brown	7.1j	19.1c	54.0lmno	41.1mn	4.3 fg
R1	Cucumber	Bahnamir	34	Brown	7.4jk	14.6c	59.0jkl	48.6pq	4.3 f
R2	Cucumber	Bahnamir	35	Beige	3.1de	14.4c	66.0efg	53.3qrs	4.5ghi
R3	Cucumber	Juibar	36	White	2.9de	21.8c	56.9jklm	30.3i	6.1pq
R7	Lettuce	Juibar	37	Brown	7.5jk	11.8c	43.1rst	11.5ab	4.9 ij
R11	bean	Kiakolla	38	Beige	5.9hi	14.6c	52.3no	33.1ij	5.4 lm
R12	bean	Kiakolla	39	Beige	3.3e	13.5c	59.2jkl	47.7pq	3.5 de
				LSD	1.287	1.902	2.055	2.820	0.845

a)MCG, mycelial compatibility groups

b) Colors are recorded 21 days after sub culturing on PDA

c) The numbers represent the colony diameters (inoculation discs subtracted) of the isolates on PDA medium 2 days post-transfer. Mean values within a column followed by the same letter are not significantly different at the $P = 0.05$ level.

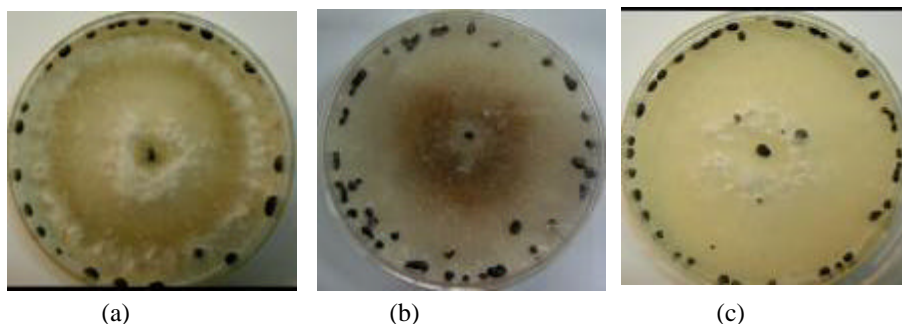


Fig. 3: The colony colors of *Sclerotinia sclerotiorum* isolates on PDA media, 3 weeks after culturing.(A) beige pigmentation (B)Brown pigmentation; (c) no pigmentation (white).

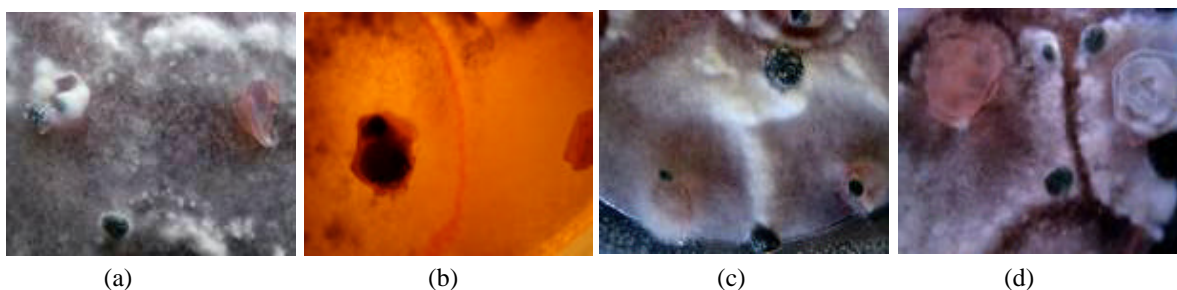


Fig. 4: Interaction of *Sclerotinia sclerotiorum* isolates on PDA media amended with Wilton's red food color: (A) compatible reaction, (B) Incompatible reaction (red line), (C) Incompatible reaction (aerial mycelium), (D) Incompatible reaction

Morphological Variability among *S. Sclerotiorum* Isolates:

The growth rate of the isolates differed significantly. The isolates grew faster at 22°C, followed by 27, 17 and 12°C, respectively. Isolates grew normally at 12, 17, 22 and 27°C, but failed to grow at 32°C. The colonies filled the 8-cm diameter petri dishes in 3 days at 22°C. ANOVA for the growth rate data showed highly significant P -values among the isolates at all temperature treatments (Table 2). The color of the colonies on PDA growth

medium varied among the isolates. There were three main colony colors: brown to dark brown pigmentation restricted to the region around the inoculation disc, beige pigmentation across the entire colony and white (no pigmentation, Figure 3). Five isolates (R28, R30, R33, R36 and R51), exhibited brown pigmentation around the fungal discs and four isolates (R34, R38, R58 and R64) produced white colonies without any pigments. The colony color of the rest of the isolates was beige (Figure 3, Table 2).

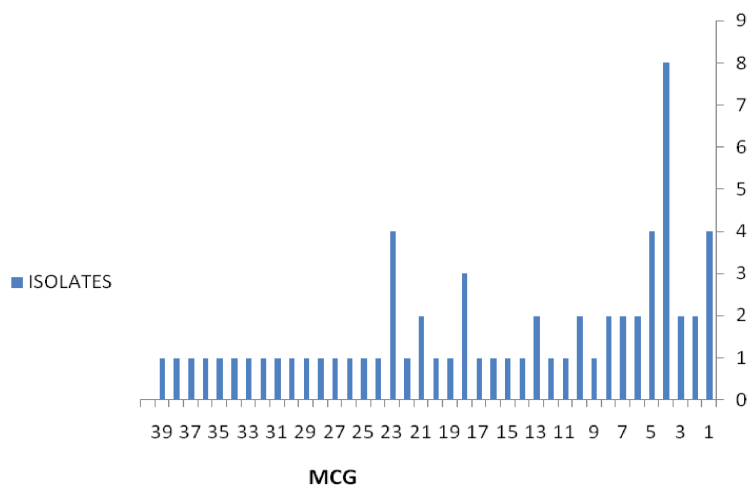


Fig. 5: Histogram of frequencies of the 39 mycelial compatibility groups (MCGs): eight MCGs included two isolates, One included three and eight and three MCGs included four isolates. The 26 MCGs were formed by individual isolates

Mycelial Compatible Groups Variability among *S. Sclerotiorum* Isolates:

Assessments of compatibility were based on mycelia continuity between the interacting colonies without formation of either a strip of thin mycelium or aerial mycelium and the uniform distribution of sclerotia in the plate. Evaluation of compatibility was based on the failure of the two colonies to fuse, which were infected by the formation of a strip of this mycelium or aerial mycelial at the interaction zone [14]. Mycelia incompatibility can also be indicated by the formation of a dark line along the interaction zone associated with the red food dye [14] (Figure 4). 39 MCGs were determined among the 65 studied isolates. The 26 isolates were established independent MCGs. The isolate belong to the independent MCG, was compatible only with own. MCG4 and MCG23 consisted of six and four isolates, respectively. MCG1, MCG5 and MCG18 included three isolates and three MCGs included only two isolates. The remaining 26 isolates were compatible only with themselves (Figure 5).

DISCUSSION

In the present study, we found that microsatellite markers were very competent in identifying genetic variation among the isolates. five of these marker sets exposed polymorphism among the Iranian isolates. When we compared our findings with the previous work [3], we found two more alleles at $(GT)_{10}$ and $(TACA)_{10}$, three more alleles at $(CATA)_{25}$ and four more alleles at $(AGAT)_{14}$ and $(AAGC)_4$ loci. In contrast, we found that microsatellite $(TACA)_{10}$ locus was monomorphic in our populations

while four and seven alleles were reported previously [3,12]. Sexton and Howlett (2004) identified more alleles at seven microsatellite loci than reported by Atallah *et al.* [3,25].

We found 44 different clones (haplotypes) among the 65 isolates representing the population indicating a high rate of variability in the region (67%). Sexton and Howlett (2004) collected isolates from four oilseed rape fields and found that genotypic diversity ranged from 36% to 80% of the maximum in the four populations [2]. Atallah *et al.* (2004) showed that 92% of the variability among the isolates [3]. Kohli and Kohn (1998) reported that genetic diversity was between 10% and 29% in four Canadian canola and North Carolina cabbage fields, respectively, especially the former indicating a high level of clonality [16]. Although genotypic diversity was observed to some extent in most of the studies from different countries and continents, it is possible that the genotypes may not share identical alleles.

Mycelial compatibility grouping, a phenotypic marker system controlled by multiple loci, was often associated with groups of identical or closely related microsatellite haplotypes, except for MCG1, MCG4 and MCG23. MCG1 included 4 isolates (R4, R19, R25, R26) that were all compatible when paired on PDA media. R19, R25 and R26 shared identical microsatellite alleles, R4 differed from the three other isolates for four microsatellites and clustered closely with different isolates at the UPGMA dendrogram (Figure 2). We confirmed the pairing of the isolates belonging to this MCG in two further replications, as the molecular data did not agree with the pairing.

The microsatellite markers also revealed polymorphisms among the isolates of MCG4 and MCG23. These results support the hypothesis that isolates within a single MCG and sharing the same microsatellite alleles may be clonal; however, given the non-clonal nature of MCG1, MCG4 and MCG23, it will be important to examine a large number of additional isolates to confirm this for the 10 MCGs. The data from this study demonstrated that mycelial incompatibility in *S. sclerotiorum* occurred between the field populations and that a field population of *S. sclerotiorum* was composed of more than one MCG. Mycelial compatibility groups, like vegetative compatibility groups, were thought to be determined by the alleles at several loci in the genome. Sexton and Howlett (2004) identified more alleles at seven microsatellite loci than reported by Atallah *et al.* [3,25].

The colony diameters of the isolates belonging to the same groups generally were statistically significant in agreement with each other at the all temperature treatments. Similarly the growth of two isolates belonging to MCG21 (R45, R48) was similar at the all temperature points, except at 12°C, where R45 grew faster than R48 (Table 2). More variation in mean growth rates was observed in the isolates from MCG4. The colony diameters of the R20, R22, R49 and R55 Were statistically similar at the all tested temperature points, However the growth rates of the remaining isolates were statistically different (Table 2). The results were confirmed our presumption about link of different colors and genetic variations within the populations. The colony colors of the isolates within the same MCG were similar, except MCG1, MCG4 and MCG23. The isolates belong to MCG4, had different color types (Table 2). Populations of *S. sclerotiorum* from 52 fields in north of Iran were a heterogeneous mix of MCGs. This corroborates reports of *S. sclerotiorum* MCGs population structure on canola in Canada [14], Norwegian vegetable crops [26], sunflower in Manitoba [7] cabbage in North Carolina [6] and soybean in Argentina [15] and Canada [22]. The population structure of *S. sclerotiorum*, based on the MCGs, appears similar irrespective of host crop and field location.

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This is the first report of the genetically and morphological variations within population of *S. sclerotiorum* in Iran.

REFERENCES

1. Boland, G. and R. Hall, 1994. Index of plant hosts of *Sclerotinia sclerotiorum*. Canadian J. Plant Pathol., 2: 93-108.
2. Purdy, L., 1979. *Sclerotinia sclerotiorum* - history, diseases and symptomatology, host range, geographic distribution and impact. Phytopathol., 69: 875-880.
3. Atallah, Z., B. Larget and D. Johnson, 2004. High genetic diversity, phenotypic uniformity and evidence of outcrossing in *Sclerotinia sclerotiorum* in the Columbia basin of Washington state. Phytopathol., 94: 737-742.
4. Kohn, LM., I. Carbone and J. Anderson, 1990. Mycelial interactions in *Sclerotinia sclerotiorum*. Exp Mycol., 14: 255-267.
5. Kohn, LM., E. Stasoviski, I. Carbone, J. Royers and J. Anderson, 1991. Mycelial incompatibility and molecular markers identify genetic variability in.eld populations of *Sclerotinia sclerotiorum*. Phytopathol., 81: 480-485.
6. Cubeta, M.B. Cody, Y. Kohli and L. Kohn, 1997. Clonality in *Sclerotinia Sclerotiorum* on infected cabbage in eastern North Carolina. Phytopathol., 87: 1000-1004.
7. Kohli, Y., L.J. Brunner, H. Yoel, M.G. Milgroom, J.B. Anderson, R.A.A. Morrall and L.M. Kohn, 1995. Clonal dispersal and spatiiu il mixing in populations of the plant pathogenic fungus, *Sclerotinia sclerotiorum*. Mol. Ecol. 4:69-77.
8. Litt, M. and J.M. Luty, 1989. A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am. J. Hum. Genet., 44: 397-401.
9. McDonald, D.B. and W.K. Potts, 1997. DNA microsatellites as genetic markers for several scales. In: D.P. Mindell, (eds.). Avian molecular evolution and systematics. Academic Press, San Diego, pp: 29-49.
10. Tautz, D., M. Trick and G.A. Dover, 1986. Cryptic simplicity in DNA is a major source of genetic variation. Nature, 322: 652-656.
11. Gaggiotti, O.E., O. Lange, K. Rassmann and C. Gliddon, 1999. A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. Mol. Ecol., 8: 1513-1520.
12. Sirjusingh, C. and L.M. Kohn, 2001. Characterisation of microsatellites in the fungal plant pathogen, *Sclerotinia sclerotiorum*. Mol. Ecol. Notes., 1: 267-269.

13. Vandenkoornhuyse, P., C. Leyval and I. Bonnin, 2001. High genetic diversity in arbuscular mycorrhizal fungi: Evidence for recombination events. *Heredity*, 87: 243-253.
14. Kohli, Y., R. Morrall, J. Anderson and L. Kohn, 1992. Local and trans-Canadian clonal distribution of *Sclerotinia sclerotiorum* on canola. *Phytopathol.*, 82: 875-880.
15. Durman, S.B., A.B.M. Menendez and A.M. Godeas, 2001. Mycelial compatibility groups in *Sclerotinia sclerotiorum* from agriculture fields in Argentina. Proc. XIth Int. Sclerotinia Workshop. C. Young and K. Hughes. eds YORK. UK, pp: 27-28.
16. Kohli, Y. and L. Kohn, 1998. Random association among alleles in clonal populations of *Sclerotinia sclerotiorum*. *Fungal Genet Biol.*, 23: 139-149.
17. Kohn, L.M., 1995. Clonal dispersal and spatial mixing in populations of the plant pathogenic fungus, *Sclerotinia sclerotiorum*. *Mol. Ecol.*, 4: 69-77.
18. Kohn, L.M., 1995. The clonal dynamic in wild and agricultural plantpathogen populations. *Canadian J. Bot.*, 73: 1231-1240.
19. Sun, J.M., W. Irzykowski, M. Jedryczka and F.X. Han, 2005. Analysis of the structure of *Sclerotinia sclerotiorum* (Lib.) de Bary populations from different regions and host plants by random amplified polymorphic DNA Markers. *J. Integr Plant Biol.*, 47: 385-395.
20. Willets, H. and J. Wong, 1980. The biology of *Sclerotinia sclerotiorum*, *S. trifoliorum* and *S. minor* with emphasis on specific nomenclature. *Botany Rev.*, 46: 101-165.
21. Liu, D., S. Coloe, Baird R. and J. Pedersen 2000. Rapid mini-preparation of fungal DNA for PCR. *J. Clinical Microbiol. Jan.*, pp: 471.
22. Hambleton, S., C. Walker and L.M. Kohn, 2002. Clonal lineages of *Sclerotinia sclerotiorum* previously known from other crops predominate in 1999-2000 from Ontario and Quebec. *Can. J. Plant Pathol.*, 24: 309-315.
23. Nei, M. and W.H. Li, 1979. Mathematical models for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA.*, 76: 5268-5273.
24. Van de Peer, Y. and R. De Wachter, 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput Appl. Biosci.*, 10: 569-570.
25. Sexton, A.C. and B.J. Howlett, 2004. Microsatellite markers reveal genetic differentiation among populations of *Sclerotinia sclerotiorum* from Australian Canola Fields. *Curr Genet*, 46: 357-365.
26. Carpenter, M.A., C. Frampton and A. Stewart, 1999. Genetic variation in New Zealand populations of the plant pathogen *Sclerotinia sclerotiorum*. *N.Z. J. Crop Hort. Sci.*, 27: 13-21.