NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 29, 2012
Print ISSN 1222-4227; Online ISSN 2067-5720

GENETIC AND MORPHOLOGICAL DIFFERENCES AMONG POPULATIONS OF *SCLEROTINIA SCLEROTIORUM* BY MICROSATELLITE MARKERS, MYCELIA COMPATIBILITY GROUPS (MCGS) AND AGGRESSIVENESS IN NORTH OF IRAN

Hossein Barari[1*], Vahid Alavi[1], and Susanna M. Badalyan[2]

[1]Agriculture and Natural Resources Research Center of Mazandaran, Department of Plant Protection, Sari, Iran

[2]Yerevan State University, Laboratory of Fungal Biology and Biotechnology, Yerevan, Armenia

*Corresponding author. E-mail: HosseinBarari1385@yahoo.com

ABSTRACT

***Sclerotinia sclerotiorum* is a cosmopolitan, homothallic fungus and is the most important causal agent of stem rot diseases in field crops of Iran. During 2006-2007, 65 isolates of the fungus were obtained from infected rapeseed, lettuce, bean, tomato, cucumber and wild Sinapis plants in various fields of North provinces of Iran. Genetic diversity between the isolates was investigated by PCR, using five microsatellite primer pairs. These divided the isolates into 9 groups with 25 clear polymorphic alleles. A high level of genetic diversity was observed at about 67.6% between some isolates. By mycelial compatibility grouping (MCG) tests, the isolates were classified into 39 groups of which 26 MCGs were individual. Molecular and phenotypic analyses results of all of the isolates (except MCG1, MCG4 and MCG23) were similar; however the isolates in MCG1, MCG4 and MCG23 groups, with variable microsatellite haplotypes, were morphologically dissimilar. The results shown here were possibly due to high rates of out crossing, as well as to the evolutionary potential within population of the pathogen in different locations in Iran.**

Key words: microsatellites, MCG, *Sclerotinia sclerotiorum*, genetic diversity.

INTRODUCTION

Sclerotinia stem rot is the most destructive and harmful disease of canola, especially in favourable climatic conditions, such as those found on the northern flats of the Caspian Sea (Pakdaman and Mohammadi Goltapeh, 2007). *Sclerotinia sclerotiorum,* an ascomycetous fungus, is dispersed by airborne ascospores and soilborne sclerotia (Boland and Hall, 1994). Yield losses due to stem rot disease, are variable and sometimes reach to maximum level in susceptible plants (Purdy, 1979). 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 colonizes 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 (Atallah et al., 2004). 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, MCG and DNA fingerprinting, have been developed to differentiate *Sclerotinia sclerotiorum* populations. MCG is a phenotypic marker system that is controlled by multiple loci. When members of a MCG pair with each other, in fact they fuse and form one confluent colony. Mycelial incompatibility is described as a failure of different strains to fuse and to form a colony, and is characterized by the formation of a barrage of dead cells between the two incompatible colonies, known as a reaction line (Kohn et al., 1990). DNA fingerprinting technique can also be used to distinguish closely related fungal isolates. Southern hybridisation of restriction digested whole genomic DNA to a cloned probe containing a 4.5 kb repeated dispersed element of nuclear DNA from *Sclerotinia sclerotiorum* was previously used (Kohn et al., 1991) and in

Received 20 May 2011; accepted 06 March 2012

several subsequent studies (Kohli et al., 1995; Cubeta et al., 1997). Microsatellites (Litt and Luty, 1989), also known as simple sequence repeats (SSRs) or short tandem repeats (STRs), are the smallest class of simple repetitive DNA sequences (Tautz et al., 1986; Mc-Donald and Potts, 1997) that are widely dispersed and evenly distributed in the genome of eukaryotes and have been used to study variability within the populations (Sirjusingh and Kohn, 2001). 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 exclusively by asexual means, such as *Sclerotinia sclerotiorum* (Vandenkoornhuyse et al., 2001). There are several reports about the structural variations of *Sclerotinia sclerotiorum* populations in the world. Thirty-nine colonies were identified among the 66 isolates on canola (Rapeseed) in Canada from seven locations in Alberta, Saskatchewan and Manitoba states (Kohli et al., 1992) and 50 MCGs were identified among the 140 isolates from Buenos Aires (Durman et al., 1997). Limited out crossing among the *Sclerotinia sclerotiorum* isolates was observed in North Carolina and California (Kohli and Kohn, 1998). Several works were focused on comparison of the *Sclerotinia sclerotiorum* populations from agricultural and wild plants in Norway (Kohn, 1995), and there was genetic uniformity among the populations on potato and canola; however, greater genetic diversity was observed among the wild populations. Three *Sclerotinia sclerotiorum* populations collected from Europe, China and Canada, were compared and genetic differentiations were highly significant among and within the populations (Sun et al., 2005).

Despite the fact that *Sclerotinia sclerotiorum* causes major diseases in many fields and glasshouse crops, there were no reports about the genetic diversity of the *Sclerotinia sclerotiorum* populations in Iran. The objectives of this study were elucidation of the structural and genetic diversity within the populations of *Sclerotinia sclerotiorum* on the field crops in North of Iran (Gilan, Mazandaran and Golestsan provinces), and measurement of aggressiveness of *Sclerotinia sclerotiorum* haplotypes.

MATERIAL AND METHODS

Isolates

Isolates of Sclerotinia sclerotiorum were collected from 52 rapeseed, lettuce, bean, tomato, and cucumber fields in Gilan, Mazandaran and Golestsan, North provinces of Iran, during 2006-2007 growth season. Samples were collected from the infected plants and the sclerotia were removed from the each plant sample. 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) then incubated at 22 ºC for two days. Each isolate was purified by transferring the single hyphal tip onto the fresh medium, and generated sclerotia were stored at -20ºC until use (Atallah et al., 2004; Cubeta et al., 1997; Willets and Wong, 1980).

DNA extraction

DNA was extracted using the rapid mini-preparation method (Liu Don et al., 2000), 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 was 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 was 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 was vortexed briefly and spun at 10,000 *g* 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 10,000 *g* for 2 min., the supernatant was discarded, and the pellet was air dried. Finally, the pellet of 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 (Hopwood et al., 1997).

Microsatellite primers, PCR conditions, 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 $(TACA)_{10}$ (Sirjusingh and Kohn, 2001). The PCR reaction mixture included 10-20 ng 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, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 100 µg mL^{-1} gelatine, 0.05% tween 20 and 0.05% Nonidet P-40). The final reaction volume was adjusted to 50 µL with deionized H_2O. All of the reagents were obtained 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 denaturising 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 (Nicholson et al., 1997). All polymorphic alleles were identified from each microsatellite primer combination and bands representing alleles were scored as present (1) or absent (0). Nei's genetic distance matrix (Nei and Li, 1979) was prepared and bootstrap analysis with 2000 replications was performed to generate a dendrogram of unweighted pair-group mean analysis (UPGMA; Sokal and Michener, 1958) using the TREECON 1.3b program (Van de Peer and de Wachter, 1994).

Mycelial compatibility group determination

For evaluation of interform MCG variability, five isolates from the each field were paired together. 0.5 cm diameter mycelial plugs were obtained from the edge of two-day-old 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 colouring per litre of culture medium. Petri dishes were incubated in the dark at 22°C for 14 days. Pairings of the 65 isolates were performed in a pyramid design, where groups of 10 isolates were 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 ensure accurate results.

Aggressiveness

Rapeseed plants were used for aggressiveness tests by *Sclerotinia sclerotiorum* fungus isolated from different crops. Blossom colonization was achieved by placing autoclaved blossoms on top of a growing colony of *Sclerotinia sclerotiorum* on PDA for 3 to 4 days. Each isolate was represented five times. Each colonized blossom was lightly wrapped with gauze to hold the flower on the stem and to preserve moisture. Plants were subjected to an intermittent mist for 24 hours. Misting was stopped after 24 hours, but the plants were kept at above 95% relative humidity for 48 hours. Ambient temperature was 16 ± 2ºC at night and 27 ± 2ºC during the day. On the third day post-inoculation, plants were transported to the greenhouse and kept at approximately 25°C during the day and 18°C at night for 3 additional days, and lesion

lengths were measured using Vernier callipers.

Data was analysed by one-way ANOVA using PROC GLM in SAS. Isolate was the treatment and lesion length was the observational units. The data was not normally distributed and a rank transformation was used for the ANOVA test.

RESULTS

Genetic diversity among the *Sclerotinia sclerotiorum* isolates

The microsatellite primers exhibited 25 clear polymorphic 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 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,* which were then 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

[a]Sirjusingh and Kohn, 2001
[b]Atallah et al., 2004

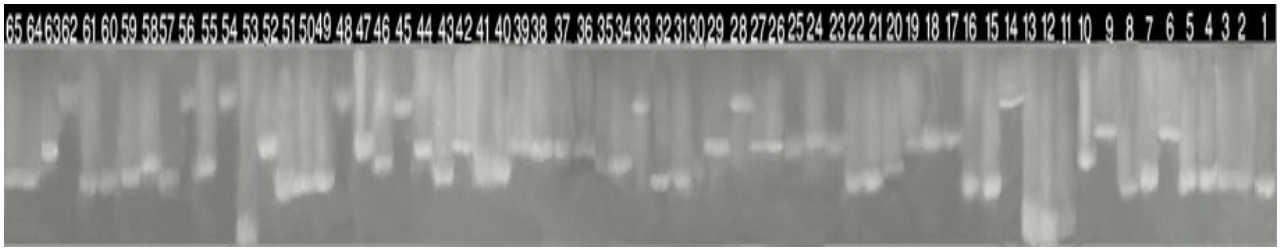

Figure 1. Microsatellite analysis of *Sclerotinia sclerotiorum* isolates amplified with the $(CATA)_{25}$ primer. PCR amplicons were separated on a 2.6% agarose gel in 0.5 X TBE. Bands were stained with ethidium bromide and visualized on a UV-Transeluminator

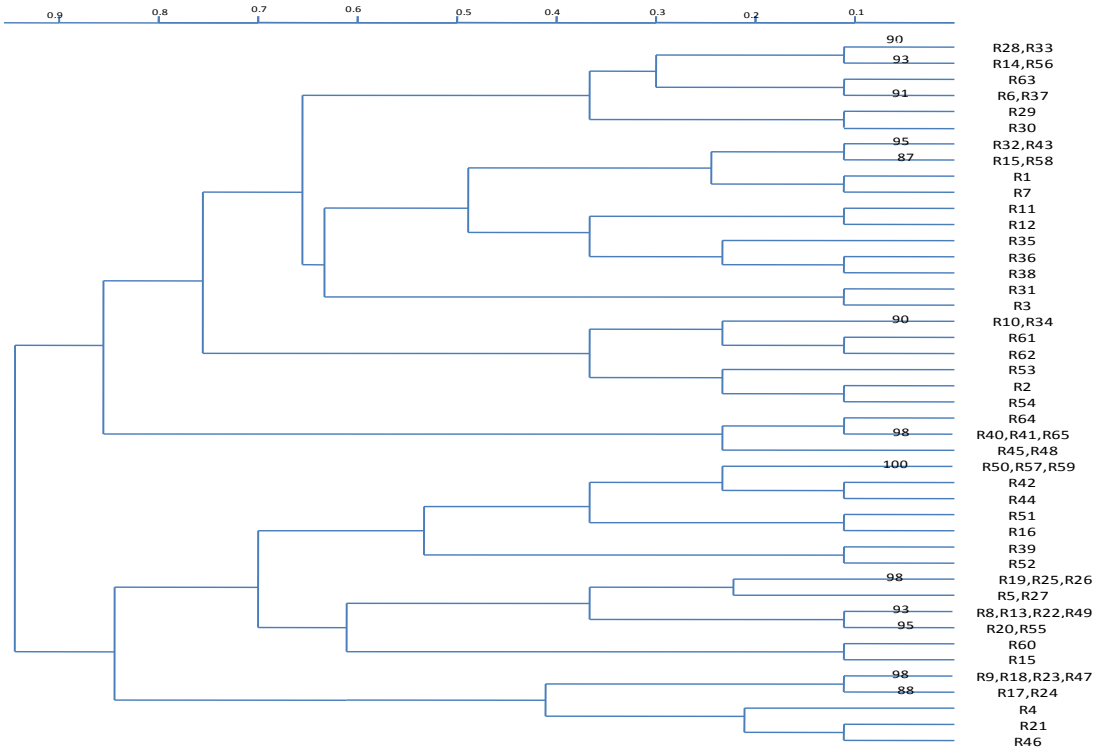

Figure 2. Unweighted pair-group mean analysis dendrogram of genetic distance among the 65 fungal isolates based on Nei's coefficients. The numbers given above the lines indicate the bootstrap values of 2000 replicates

Mycelial compatible group's variability among *Sclerotinia 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 was reflected by the formation of a strip of this mycelium or aerial mycelia at the interaction zone (Kohli et al., 1992). Mycelia incompatibility can also be indicated by the formation of a dark line along the interaction zone associated with the red food dye (Kohli et al., 1992) (Figure 3). 39 MCGs were determined among the 65 studied isolates. 26 isolates were established as independent MCGs. The isolate that belonged to the independent MCGs, was compatible only with itself. MCG4 and MCG1,4 and 23 consisted of eight and four isolates, respectively. MCG18 included three isolates and eight MCGs included only two isolates (Figure 4). The remaining 26 isolates were compatible only with themselves.

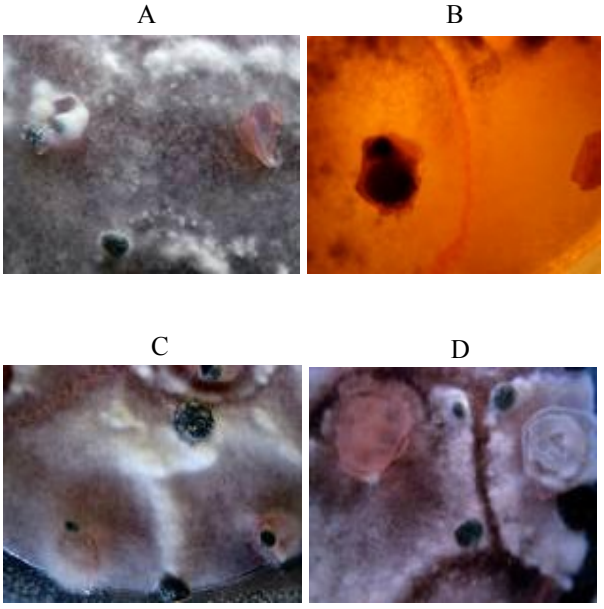

Figure 3. Interaction of *Sclerotinia sclerotiorum* isolates on PDA media amended with Wilton's red food color: (A) compatible reaction, (B) Incompatible reaction, (C) Incompatible reaction (aerial mycelium), (D) Incompatible reaction

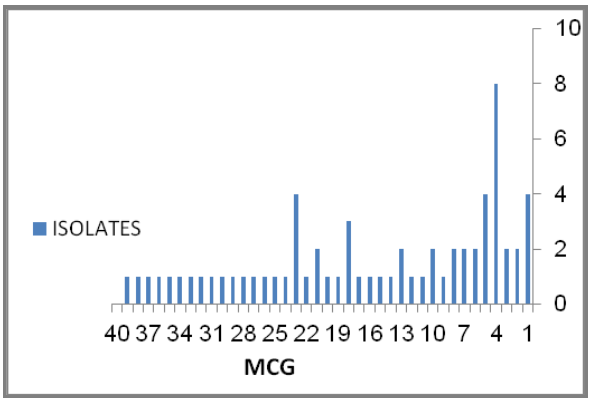

Figure 4. Histogram of frequencies of the 39 mycelial compatibility groups (MCGs): eight MCGs included two isolates, one included three and eight and three MCGs included fourisolates. The 26 MCGs were formed by individual isolates

Aggressiveness variability among *Sclerotinia sclerotiorum* isolates

Visible symptoms occurred on stems six days post-inoculation (Figure 5). Isolates were significantly different in their ability to infect and spread on rapeseed stem ($p<0.05$), resulting in different lesion sizes. When the same analyses was performed for MCGs, significant differences of pathogenic variability was observed among MCGs ($p<0.001$), where the lesion size varied from 0.33 cm (MCG33 from Kordkoi) to 27.33 cm (MCG11 from Juibar) (Table 2) in 48 h. These pathogenic differences of isolates and MCGs were not found to be related to their geographic origin.

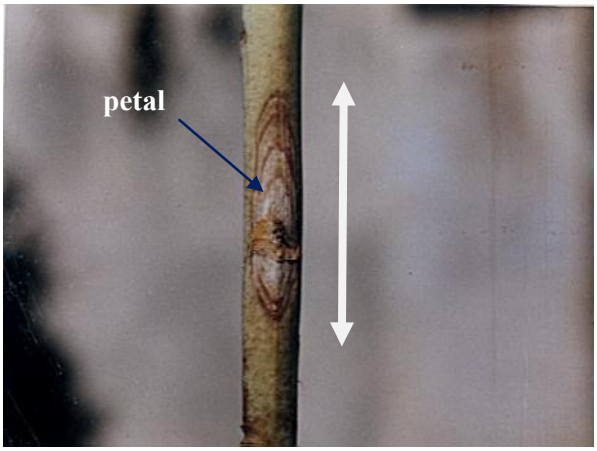

Figure 5. Symptom of white rot of rapeseed, 6 days after inoculation

Table 2. Lesion size of different mycelial compatibility groups of *S. sclerotiorum*

MCG	Lesion diameter on stem (cm)	MCG	Lesion diameter on stem (cm)	MCG	Lesion diameter on stem (cm)
1	14.87	14	15.17	27	0.67
2	19.67	15	13.33	28	3.67
3	15.00	16	20.67	29	3.00
4	4.67	17	16.11	30	16.00
5	14.00	18	20.00	31	9.00
6	7.17	19	10.17	32	13.00
7	26.67	20	15.12	**33**	**0.33**
8	2.00	21	18.08	34	2.00
9	2.00	22	14.17	35	7.33
10	4.00	23	6.00	36	19.33
11	**27.33**	24	24.67	37	1.33
12	16.67	25	2.00	38	2.33
13	16.67	26	2.00	39	16.67

DISCUSSION

In the present study, we found that microsatellite markers were very efficient in identifying genetic variation among the isolates. Five of these marker sets identified polymorphism among the Iranian isolates. When we compared our findings with previous work (Atallah et al., 2004), we found one more alleles at $(TACA)_{10}$, two more alleles at $(GT)_{10}$, three more alleles at $(CATA)_{25}$ and four more alleles at $(AGAT)_{14}(AAGC)_4$ loci (Atallah et al., 2004; Sirjusingh and Kohn, 2001). Sexton and Howlett (2004) identified more alleles at seven microsatellite loci than reported by Atallah et al. (2004).

We found 44 different clones (haplotypes) among the 65 isolates representing the population, indicating a high rate of variability in the region (up to 67.6%). 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. Atallah et al. (2004) showed a 92% of variability among the isolates. 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. 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, and 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, Table 3). 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 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. (2004).

Variations in aggressiveness of *S. sclerotiorum* have been investigated previously. On the basis of a detached celery petiole assay, 50 MCGs identified from 160 Argentinean isolates (Durman et al., 2003), were found not to differ in aggressiveness among MCGs. By using a limited-term, plug

inoculation technique, Kull et al. (2004) reported that aggressiveness varied between isolates and MCGs from different locations, but not in MCGs produced from isolates originating from infections in single fields. Present results indicate that significant differences in aggressiveness are present between MCGs, regardless of the isolate origins. To evaluate pathogenity of *S. sclerotiorum* dispersed in a region, or to screen resistant cultivars, these results suggest that more representative isolates should be included.

Table 3. MCG of *Sclerotinia sclerotiorum* isolates at different temperatures on PDA

Isolates	Host	Site	MCGs	Isolates	Host	Site	MCGs
R4	Lettuce	Dashtenaz	1	R35	Rapeseed	Shirgah	14
R19	Rapeseed	Amol	1	R36	Rapeseed	Arateh	15
R25	Rapeseed	Rezvanshahr	1	R38	Rapeseed	Kordekhail-Sari	16
R26	Rapeseed	Bandar Anzali	1	R39	Rapeseed	Shast kalateh	17
R5	Lettuce	Kiakolla	2	R40	Rapeseed	Shast Kalateh	18
R27	Rapeseed	Bandar Anzali	2	R41	Rapeseed	Kordkoi	18
R6	Lettuce	Kiakolla	3	R65	Rapeseed	Bayekolla	18
R37	Rapeseed	Nokandeh	3	R42	Rapeseed	Kordkoi	19
R8	Lettuce	Amol	4	R44	Rapeseed	Kordkoi-zare	20
R13	Bean	Juibar	4	R45	Rapeseed	Kordkoi-zare	21
R20	Rapeseed	Amol-hular	4	R48	Rapeseed	Behshahr	21
R22	Rapeseed	Babol	4	R46	Rapeseed	Behshahr	22
R30	Rapeseed	Ghaemshahr	4	R50	Rapeseed	Kordkoi-Kar.	23
R49	Rapeseed	Kordkoy	4	R57	Rapeseed	Hullar	23
R55	Rapeseed	Semeskandeh	4	R58	Rapeseed	Hullar	23
R64	Rapeseed	Bayekolla	4	R59	Rapeseed	Hullar	23
R9	Lettuce	Amol	5	R51	Rapeseed	Kordkoi-Kar.	24
R18	Rapeseed	Amol	5	R52	Rapeseed	Galugah	25
R23	Rapeseed	Chardangeh	5	R53	Rapeseed	Suteh	26
R47	Rapeseed	Behshahr	5	R54	Rapeseed	Semeskandeh	27
R10	Bean	Kiakolla	6	R60	Rapeseed	Galugah	28
R34	Rapeseed	Shirgah	6	R61	Rapeseed	Dashtenaz	29
R14	bean	Babol	7	R62	Rapeseed	Dashtenaz	30
R56	Rapeseed	Semeskandeh	7	R63	Rapeseed	Garakhail	31
R17	Tomatto	Juibar	8	R15	Wild sinapis	Juibar	32
R24	Rapeseed	Rezvanshahr	8	R16	Wild sinapis	Kordkoichardeh3	33
R21	Rapeseed	Babol	9	R1	Cucumber	Bahnamir	34
R28	Rapeseed	Bandar anzali	10	R2	Cucumber	Bahnamir	35
R33	Rapeseed	Juibar	10	R3	Cucumber	Juibar	36
R29	Rapeseed	Juibar	11	R7	Lettuce	Juibar	37
R31	Rapeseed	Juibar	12	R11	Bean	Kiakolla	38
R32	Rapeseed	Juibar	13	R12	Bean	Kiakolla	39
R43	Rapeseed	Bandarturkaman	13				

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 (Kohli et al., 1992), Norwegian vegetable crops (Carpenter et al., 1999), sunflower in Manitoba (Kohli et al., 1995), cabbage in North Carolina (Cubeta et al., 1997), and soybean in Argentina (Durman et al., 2001) and Canada (Hambleton et al., 2002). The population structure of *S. sclerotiorum*, based on the MCGs, appears similar irrespective of host crop and field location.

Carpogenic germination of S. *sclerotiorum* sclerotia is affected greatly by soil moisture and temperature. Hao et al. (2003) and Clarkson et al. (2004) reported that apothecium production by *S. sclerotiorum* is largely confined to 10-15°C soil temperature

and -0.03 or -0.07 MPa soil moisture. Stem rot symptoms caused by *S. sclerotiorum* in North of Iran can be observed in mid-May but predominantly occur in early June. The average air temperature of the last 3 years was 14.2°C in April and 20.4°C in May. The rainfall for the same time period was 46.5 mm in April and 21.6 mm in May and relative humidity 82 and 79.3 percentage (data from State Meteorological Station based in Gharakhail). It has been reported that 15-25°C air temperature and high humidity (optimum >95% RH; but also possible as low as 50-60%) are required for ascospore germination (Clarkson et al., 2004). Although we did not test for outcrossing, environmental conditions may facilitate outcrossing.

CONCLUSIONS

The isolates were collected from fields in a geographical region of Iran where diverse crops are also cultivated. Given the high genetic diversity of this fungus, we suggest that the environmental conditions in the region may favour sexual recombination within *Sclerotinia sclerotiorum*. Oilseed rape is a new crop to this area and the high level of polymorphism may reflect the movement of *Sclerotinia sclerotiorum* onto this crop from several wild plant hosts.

In conclusion, this is the original report on the genetic variation within a population of *Sclerotinia sclerotiorum* in Iran.

Molecular data and morphological characters demonstrated that the population is genetically diverse.

Acknowledgements

We are thankful for technical support of Agriculture research center and natural resources of Mazandaran, Iran and Laboratory of Fungal Biology and Biotechnology of Yerevan State University, Armenia.

REFERENCES

Atallah, Z., Larget, B., Johnson, D., 2004. *High genetic diversity, phenotypic uniformity and evidence of outcrossing in Sclerotinia sclerotiorum in the Columbia basin of Washington state.* Phytopathology, 94: 737-742.

Boland, G., Hall, R, 1994. *Index of plant hosts of Sclerotinia sclerotiorum*. Canadian Journal of Plant Pathology, 2: 93-108.

Carpenter, M.A., Frampton. C., Stewart, A., 1999. *Genetic variation in New Zealand populations of the plant pathogen Sclerotinia sclerotiorum.* N. Z. J. Crop Hortic. Sci., 27: 13-21.

Clarkson, J.P., Phelps, K., Whipps, J.M., Young, C.S., Smith, J.A., Watling, M., 2004. *Forecasting Sclerotinia disease on lettuce: towards developing a prediction model for carpogenic germination of sclerotia*. Phytopathology, 94: 268-279.

Cubeta, M.A., Cody, B.R., Kohli, Y. & Kohn, L., 1997. *Clonality in Sclerotinia Sclerotiorum on infected cabbage in eastern North Carolina.* Phytopathology, 87:1000-1004.

Durman, S.B., Menendez, A.B.M. & Godeas, A.M., 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 27-28.

Hambleton, S., Walker, C.., Kohn, L.M., 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.

Hao, J.J., Subbarao, K.V., Duniway, J.M., 2003. *Germination of Sclerotinia minor and S. sclerotiorum sclerotia under various soil moisture and temperature combinations.* Phytopathology, 93: 443-450.

Hopwood, A., Oldroyd, N., Fellows, S., Ward, R., Owen, S-A., Sullivan, K., 1997. *Rapid quantification of DNA samples extracted from buccal scrapes prior to DNA prolifing*. Biotechniques, 23: 18-20.

Kohli, Y., Morrall, R., Anderson, J. & Kohn, L., 1992. *Local and trans-Canadian clonal distribution of Sclerotinia sclerotiorum on canola*. Phytopathology, 82: 875-880.

Kohli, Y., Brunner, L.J., Yoel, H., Milgroom, M.G., Anderson, J.B., Morrall, R.A.A., & Kohn, L.M., 1995. C*lonal dispersal and spatial il mixing in populations of the plant pathogenic fungus, Sclerotinia sclerotiorum.* Mol. Ecol., 4: 69-77.

Kohli, Y. & Kohn, L., 1998. *Random association among alleles in clonal populations of Sclerotinia sclerotiorum.* Fungal Genet. Biology, 23: 139-149.

Kohn, L.M., Carbone, I. & Anderson, J., 1990. *Mycelial interactions in Sclerotinia sclerotiorum.* Exp. Mycology, 14: 255-267.

Kohn, LM., Stasoviski, E., Carbone, I., Royers, J.& Anderson, J., 1991. *Mycelial incompatibility and molecular markers identify genetic variability in field populations of Sclerotinia sclerotiorum.* Phytopathology, 81: 480-485.

Kohn, LM., 1995. The *clonal dynamic in wild and agricultural plantpathogen populations*. Canadian Journal of Botany, 73: 1231-1240.

Kull, L. S., Pedersen, W. L., Palmquist, D., and Hartman, G. L. 2004. *Mycelial compatibility grouping and aggressiveness of Sclerotinia sclerotiorum.* Plant Dis., 88:325-332

Litt, M. & Luty, J.M., 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.

Liu, D., Coloe, S., Baird, R. and Pedersen, J., 2000. *Rapid mini-preparation of fungal DNA for PCR.* Journal of Clinical Microbiology., Jan., p.471.

McDonald, D.B., Potts, W.K., 1997. *DNA microsatellites as genetic markers for several scales.* In: Mindell DP (ed.), Avian molecular evolution and systematics. Academic Press, San Diego: 29-49.

Nei, M., Li W.H., 1979. *Mathematical models for studying genetic variation in terms of restriction endonucleases.* Proc. Natl. Acad. Sci., USA, 76: 5268-5273.

Nicholson, P., Rezanoor, H.N., Simpson, D.R. & Joyce, D., 1997. *Differentiation and quantification of the cereal eyespot fungi Tapesia yallundae and Tapesia acuformis using a PCR assay.* Plant Pathol., 46: 842-856.

Pakdaman, B. S., and Mohammadi Goltapeh, E., 2007. *In vitro studies on the integrated control of rapeseed white stem rot disease through the application of herbicides and Trichoderma species.* Pakistan Journal of Biotlogical Sciences, 10: 7-12

Purdy, L., 1979. *Sclerotinia sclerotiorum – history, diseases and symptomatology, host range, geographic distribution, and impact*. Phytopathology, 69: 875-880.

Schafer, M.R. & Kohn, L.M. 2006. *An optimized method for mycelial compatibility testing in Sclerotinia sclerotiorum.* Mycologia, 98(4): 593-597.

Sexton, A.C., Howlett, B.J., 2004. *Microsatellite markers reveal genetic differentiation among populations of Sclerotinia sclerotiorum from Australian canola fields.* Curr. Genet., 46: 357-365.

Sirjusingh, C, Kohn, L.M., 2001. *Characterization of microsatellites in the fungal plant pathogen, Sclerotinia sclerotiorum*. Mol. Ecol. Notes, 1: 267-269.

Sokal, RR. & Michener, CD., 1958. *A statistical method for evaluating systematic relationships*. Univ. Kansas Sci. Bull. 38: 1409-1438.

Sun, J.M., Irzykowski, W., Jedryczka, M. & Han, F.X., 2005. *Analysis of the structure of Sclerotinia sclerotiorum (Lib.) de Bary populations from different regions and host plants by random amplified polymorphic DNA markers.* Journal Integr. Plant Biology, 47: 385-395.

Tautz, D., Trick, M., Dover, G.A., 1986. *Cryptic simplicity in DNA is a major source of genetic variation*. Nature, 322: 652-656.

Van de Peer, Y., de Wachter, R., 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.

Vandenkoornhuyse, P., Leyval, C., & Bonnin, I., 2001. *High genetic diversity in arbuscular mycorrhizal fungi: Evidence for recombination events.* Heredity, 87: 243-253.

Willets, H. & Wong, J., 1980. *The biology of Sclerotinia sclerotiorum, S. trifoliorum and S. minor with emphasis on specific nomenclature*. Botany Rev., 46: 101-165.