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PATHOGENICITY VARIATION AND MYCELIAL COMPATIBILITY GROUPS IN *SCLEROTINIA SCLEROTIORUM*

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Abstract: Population variability of *S. sclerotiorum*, the causal agent of Sclerotinia stalk rot of sunflower, was determined by mycelial compatibility grouping (MCG) and pathogenicity variation comparison. To study mycelial compatibility grouping and pathogenicity variability, isolates of *S. sclerotiorum* were collected from sunflower fields in East, West Azerbaijan and Ardebil provinces of Iran. Among 186 isolates tested, 26 MCGs were identified and 46% were represented by single isolates. There were differences among MCGs comparing mycelial growth rate, sclerotial production on PDA and aggressiveness cause disease. Significant differences were detected in number of sclerotia, dry weight of sclerotia, mycelial growth rate and aggressiveness among MCGs ($p < 0.001$) regardless of their geographic origins. There was generally a poor correlation ($r = 0.21$, $p \leq 0.05$) between sclerotia weight and number of sclerotia produced on PDA and also to the mycelial growth rate at 24 ($r = 0.35$, $p \leq 0.05$) and 48h ($r = 0.39$, $p \leq 0.05$). Our studies in comparison of the detached leaf and cut-stem methods showed that the highest rank correlations ($r = 0.78$ $p \leq 0.01$), while aggressiveness of two inoculation methods (stem and leaf detached) were not correlated to colony diameter growth or the other two factors. Variation in isolates aggressiveness may be important considerations in disease management systems.

Key words: Sclerotinia stalk rot, *Helianthus annuus* L., vegetative incompatibility, agressiveness

INTRODUCTION

Sclerotinia white and stalk rot caused by the *Sclerotinia sclerotiorum* (Lib.) de Bary is a common and major disease and significant cause of yield loss in Australian canola (*Brassica napus* L.), oil seed crops (Sprague and Stewart-Wade 2002; Hind *et al*. 2003) and sunflower (*Helianthus annuus* L.) in China and Canada (Kohli *et al*. 1995; Li 2004). The fungus infects as many as 408 plant species including many important crops, such as apeseeds, sunflower and soybean, and many vegetables (Boland and Hall 1994). In Iran sclerotinia stalk rot, caused by *S. sclerotiorum*, is a serious disease and causes yield loss of sunflower up to 63% (Irani *et al*. 2001).

S. sclerotiorum is an ascomycetous necrotroph fungus dispersed as airborne ascospores or soilborne sclerotia. Epidemics are initiated when ascospores land on open blossoms on the canopy. Contaminated flowers fall on stems and on the ground and fungal mycelia rapidly colonize the blooms. Stems or leaves contacting colonized blossoms acquire the disease. Differentiation of *S. sclerotiorum* strains based on morphological differences in sclerotia, mycelial growth, and ascospores have been reported in previous studies (Morrall *et al*. 1972; Marukawa *et al*. 1975; Price and Colhoun 1975; Kohn *et al*. 1991; Li *et al*. 2008).

The lack of variation in virulence among *S. sclerotiorum* isolates from defined geographical areas has also been reported in a number of studies on agricultural populations (Alvarez and Molina 2000; Atallah *et al*. 2004; Auclair *et al*. 2004). Differences in virulence may be detected when comparing isolates from widely separated geographical regions, but there has been no conclusive evidence to suggest host specialization among isolates of *S. sclerotiorum* (Kull *et al*. 2004). Generally, it is important to understand the epidemiology and genetic diversity of the pathogen population regionally to control plant diseases by fungicides or resistant cultivars. The use mycelial compatibility groups (MCGs) as a valuable tool to measure population diversity of fungi. This technique is also a quick marker in many laboratories for genotyping *S. sclerotiorum* within populations (Schafer and Kohn 2006).

Mycelial\(vegetative) compatibility/incompatibility is a self-/non-self-recognition system controlled by multiple loci, but knowledge of the underlying genetic mechanisms is limited in most filamentous fungi (Glass and Kaneko 2003). Clonality is described as the repetitive recovery of the same genotypes over an extended period of time and a large geographic area (Kohn 1995). A single clone was repeatedly sampled over 4 years, across 2,000 km (Anderson and Kohn 1995; Kohn 1995). A clonal popula-

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tion of *S. sclerotiorum* was identified in the "canola belt" of Canada with a restricted number of clones accounting for a large part of the population; a number of other genotypes were recovered infrequently (Kohli *et al*. 1992; Kohli *et al*. 1995; Cubeta *et al*. 1997).

Previous studies have demonstrated that *S. sclerotiorum* populations on canola in Canada and cabbage in the United States are clonal. Clonal lineages have also been isolated from Ontario and Quebec from *Sclerotinia* strains isolated from soybean (Hambleton *et al*. 2002). However, isolates could be separated into distinct mycelial compatibility groups (MCGs) (Kohn *et al*. 1991; Cubeta *et al*. 1997). Individual isolates of *S. sclerotiorum* are also classified into clonal lineages by the use of two or more independent markers such as MCGs and microsatellites (González *et al*. 1998; Carbone *et al*. 1999; Sirjusingh and Kohn 2001; Auclair *et al*. 2004). However, MCGs or microsatellite markers have not been associated with specific virulence characteristics or ecological adaptations of the pathogen.

The main objectives of the present study were to determine the presence of different MCGs among a set of isolates of *S. sclerotiorum* from sunflower fields in differential provinces of Iran. We also characterized each MCG according to virulence and morphology on solid medium.

MATERIALS AND METHODS

Fungal isolates

One hundred eighty-six isolates of *S. sclerotiorum* were obtained from naturally infected sunflower from three fields in North and North west of Iran in growing seasons of 2007–2008 (Table 1). In each field, several plants with symptoms of Sclerotinia stalk rot were randomly collected. Samples were then air-dried, placed in paper bags, and stored at –4°C. For fungal isolation, a single sclerotium or infected host tissue was surface-sterilized using 10% commercial bleach (0.5% NaHCl) for 3 min, washed with sterile water, and transferred onto Potato Dextrose Agar (PDA) culture medium. The plates were then incubated for 3 days at 25°C in an incubator with 12 h photoperiod. Pure cultures were then obtained by transfering a single sclerotium and maintained on PDA slants at 4°C for 2–4 weeks.

Mycelial compatibility grouping

Mycelial compatibility grouping was performed as described by Schafer and Kohn (2006). Isolates were paired on modified potato dextrose agar (PDA) amended with 175 µl/l of McCormack's red food coloring. A 3-mm-diameter plug of 3-day-old PDA culture from each of three isolates was placed on the same Petri dish and arranged as an equilateral triangle (≈4 cm from each other). After incubation at 25°C for 7 days, the reaction between each isolate pair was evaluated. If two isolates could grow together without any obvious line between them, they were considered compatible with each other. Otherwise, if a reaction line of either hyphal tufts or red barrage zone of sparse growth was observed between paired isolates, they were considered incompatible (Kohn *et al*. 1990).

Cultural variation

Mycelial plugs (5-mm-diameter mycelial disc) of each isolate were taken from the growing margins of colonies grown on PDA for 3 days and inoculated onto fresh PDA (30 g/l) at 25°C and radial growth (colony diameter) was measured after 24 and 48 h. After 25 days, sclerotia production (total number and dry weight of sclerotia per plate/g) were evaluated. Four replications with four plates per replication were used for each isolate.

Pathogenicity assessment on rapeseed cut stem

Brassica napus cv. Hyola 401 was grown in 12-cm-diameter plastic pots with a pasteurized soil, peat, and perlite mix (1:1:1) under a 16:8 h day: night photoperiod with a day-time temperature of 22°C and a night-time temperature of 16°C under humid conditions. Main stems of 8-week-old flowering plants were horizontally severed with a sterile razor blade and the open end of a 1,000-µl pipette tip was pushed into the margin of a 3-day-old colony to acquire a 8-mm-thick plug of PDA and mycelium. Pipette tips were preloaded and transported in sealed pipette tip boxes prior to inoculation of plants. Inoculated plants were incubated in a mist chamber with the relative humidity maintained over 80%. Disease development was determined, and lesion length (cm) on the main stem was measured 7 days after inoculation (Zhao *et al*. 2004).

Pathogenicity assessment on detached rapeseed leaf

Seeds of *B. napus* cv. Hyola 401 was germinated in 12-cm-diameter pots containing an equal mixture (1:1:1:1) of peat: soil: sand: vermiculite and grown under greenhouse conditions at 18±1.5°C (day) and 25±1.5°C (night) for a 16 h day length. The same size true and second leaves from 30 day old seedlings of *B. napus* cv. Hyola 401 were used to assess pathogenicity of isolate in a plastic container. Each plastic container (22x10 cm^2) was covered with towels and then moistened with 50 ml sterile distilled water to maintain humidity and then was put within each container a piece of unlit (8x8 cm). Glass tube (6 cm in length) were filled with tap water, capped, and placed in pans with one tube placed under each plastic container. The end of each petiole was wrapped with a little towel and then was pushed through the glass tube cap until the cut end reached the water. If the leaf did not stay flat, labeling tape was used to hold it down.

Mycelial cultures were established from stored stock cultures as previously described. Using aseptic technique, 8 mm^2 plugs were cut 1 cm back from the advancing margin of mycelial growth on a 48-h-old PDA culture maintained in the dark at 20±2°C. Mycelial plugs were placed fungus-side down centered on one side of the middle of leaf between the main leaf vein and the leaf edge and gently pressed to ensure good contact with the leaf surface. Plastic containers containing inoculated leaves were incubated in a mist chamber with the relative humidity maintained over 80% and at 20±2°C. After 48 h, both lesion length and width were measured. The lesion length and width were used to calculate the lesion area of an ellipse in square centimeters. The experiment was performed twice (Kull *et al*. 2003).

Data analysis

Statistical analyses were performed by using PROC GLM program in SAS statistical software. Data were analyzed using Student's T-test and a two-tailed and P-value less than 0.05 was considered significant. The experiment was repeated twice for isolates showing reduced lesion sizes. To determine the correlations among aggressiveness, mycelial growth and sclerotial production of MCGs, the means of these factors were also used for estimation of the correlation coefficient (r) for their different combinations.

RESULTS

Genetic variation of MCG

Mycelial compatibility group were determined for three sets of *S. sclerotiorum* isolates; Ardabil (23 isolates), East Azerbaijan (22 isolates) and West Azerbaijan (141 isolates) as are shown in table 1. Among 186 isolates tested, 26 MCGs were identified within the sunflower fields in three provinces and 46% were represented by single isolates observed at single locations (Table 2). The West Azerbaijan set, which included 141 isolates, 19 MCGs groups were identified and contained 10 MCGs each only consisting of single isolates (Table 1). In Ardabil and East Azerbaijan in each province 1 MCGs consisted of one isolate respectively and others were more than one isolate. The largest MCGs were MCG 18, MCG 23, MCG 17 and MCG 10 representing 14.6%, 11.95%, 9.78% and 9.23% of the sunflower isolates, respectively (Table 2). This MCGs were sampled at high frequencies from multiply locations. MCG18, the highest frequency MCG sampled, included isolates which was detected in West and East Azerbaijan provinces and its common MCGs were identified among the West and East Azerbaijan locations sets, but no MCGs within the Ardabil Set were observed with other sets (Table 1).

Table 1. Geographic location, host, and number of isolates and mycelia compatibility groups (MCGs) observed in each *S. sclerotiorum* set

Set[a]	Host plant	Year of collection	No. of isolates[b]	No. of MCGs[c]	MCGs identified[d]
Ardebil	sunflower	2007	23	3	1, 2, 3
East Azerbaijan	sunflower	2008	22	5	4, 5, 6, 7, 18
West Azerbaijan	sunflower	2008	141	19	8, 9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26

[a]name of set or field collection of isolates; [b]number of isolates used for MCG determination from each set; [c]number of MCGs detected in each set; [d]specific MCGs detected in each set

Table 2. Mycelial compatibility group (MCG) designation for 186 *S. sclerotiorum* isolates

MCG[a]	Isolate code[b]
1	A1, A4, A6, A9, A11, A13, A15, A16, A19, A20, A21, A22, A24
2	A14
3	A25, A27, A30, A34, A36, A38, A39, A40, A44
4	AE1, AE5, AE6, AE8, AE9, AE19
5	AE4
6	AE3, AE7
7	AE2, AE10, AE11, AE12, AE13, AE14, AE15, AE16, AE17, AE18
8	AW1
9	AW2
10	AW3, AW4, AW5, AW6, AW7, AW8, AW9, AW10, AW11, AW12, AW13, AW14, AW15, AW16, AW17, AW18, AW19
11	AW21, AW22, AW23, AW24, AW25, AW26, AW27, AW28, AW29
12	AW30
13	AW51, AW52, AW53, AW54, AW55, AW56, AW57
14	AW59
15	AW60
16	AW61
17	AW62, AW63, AW64, AW65, AW66, AW67, AW68, AW69, AW70, AW71, AW30, AW31, AW32, AW33, AW35, AW36, AW37
18	AW72, AW73, AW74, AW75, AW76, AW77, AW78, AW79, AW80, AW81, AW82, AW83, AW84, AW85, AW86, AW87, AW88, 6 isolates from the Salmas(AW31-36), 2 isolates from the Orumieh (AW91-92), 3 isolates from the East Azerbaijan (Tasoj) (AE19-21)
19	AW90, AW93, AW94, AW95, AW96, AW97, AW98, AW99, AW100, AW101, AW102, AW103, AW104, AW105, AW106
20	AW107, AW108, AW9, AW110, AW111, AW112, AW113, AW114, AW115, AW 116
21	AW1 20
22	AW122
23	AW121, AW125, AW126, AW128, AW130, AW133, AW134, AW135, AW136, AW137, AW138, AW139, AW142, A1W44, AW148, AW150, AW155, AW156, AW158, AW159, AW160, AW161
24	AW162
25	AW164
26	AW166, AW169, AW170, AW171, AW172, AW173, AW174, AW179, AW175

[a]MCG: mycelial compatibility groups; [b]the isolate collection number is preceded by a letter to indicate set:
AW – West Azerbijan, A – Ardabil, AE – Eest Azerbijan

Most pairings isolates that could grow together without any obvious line between them, were considered compatible with each other (Fig. 1). In the incompatible reactions most pairings, was not detected by the presence of a red reaction line, instead, there was usually an interaction zone of sparsely mycelium, thin band of mycelia, or when relatively clear zone, devoid of significant mycelial growth, separated one mycelium from the other with distinct band of hyphal lyses in the reaction zone (Fig. 2).

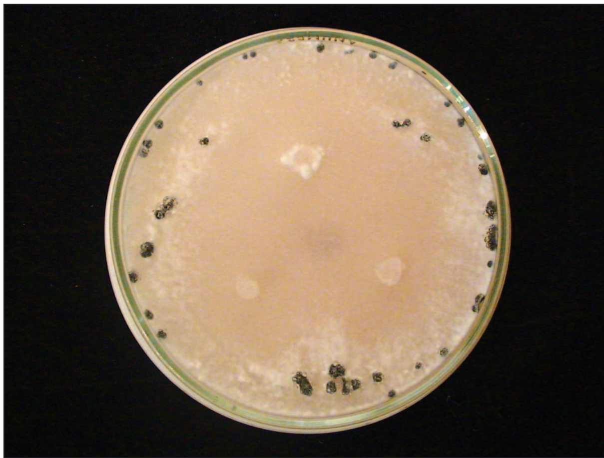
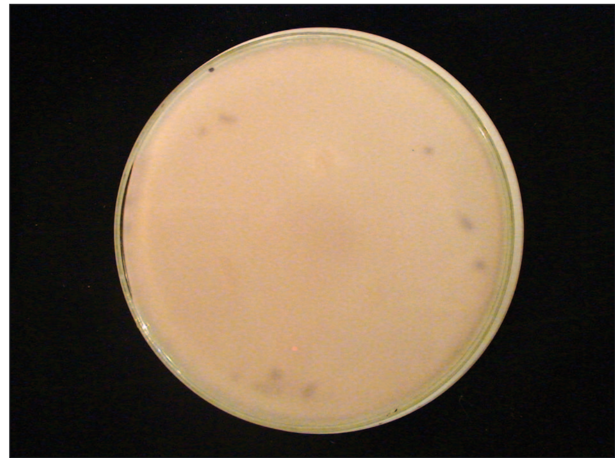

Fig. 1. Compatible reactions (clonal) among isolates could grow together without any obvious line between them

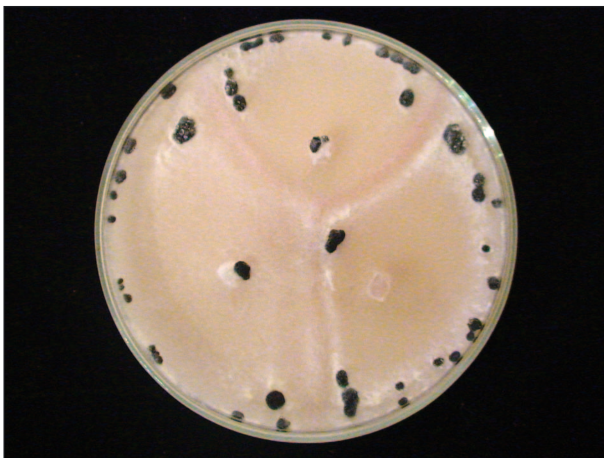
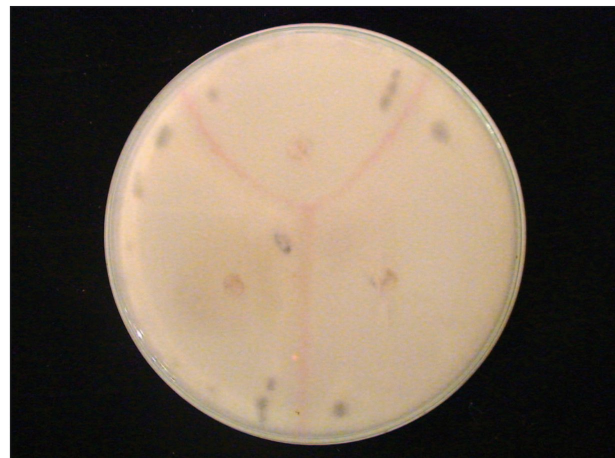
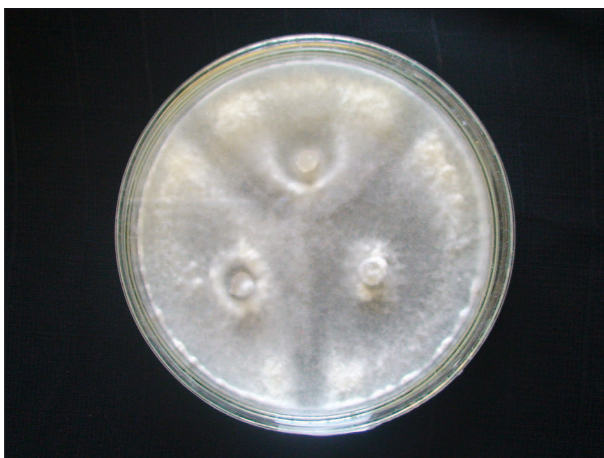

Fig. 2. Incompatible reactions among isolates with the presence of red reaction line, band of narrow fluffy aerial hyphae with barrage zone of sparse growth and narrow fluffy aerial hyphae where the mycelium meet respectively

Variability in growth characteristics

All isolates were morphologically characterized on solid medium. The colony color of isolates on PDA ranged from white to a gray or a dark brown, with light tan being the most common. Variation among MCGs were observed by comparing their differences in all morpho-

logical characteristics such as colony diameter, number of sclerotia, and dry weight of sclerotia. Based on the radial growth, the isolates were classified into 3 groups including very fast growing, intermediate and slow growing. There were significant differences among different MCGs in relation to the colony diameter measured after 24 and 48 h of incubation. The average growth rates in 48 h varied from 2.39 cm (isolate AW169) to 4.48 cm (isolate AW2) (Table 3). Significant variability was found in radial growth among MCGs after 24 and 48h of incubation ($p < 0.001$). There were obvious variability in sclerotia dry weight and number among MCGs. Dry weight of sclerotia produced on PDA ranged from 0.12 g (isolate A4) to 0.30 g (isolate AE4). Significant differences was observed in the number of sclerotia, and sclerotia weight of MCGs on PDA ($p < 0.001$) (Table 4). None of the morphological characteristics was related to the grouping made by mycelial compatibility groups.

Table 3. Number and weight of sclerotial, diameter growth, cut stem, and detached leaves lesions size of different mycelial compatibility groups of *S. sclerotiorum*

MCG	Number of scleortia [per/plate]	Sclerotia dry weight [g/plate]	Diameter growth [cm/24 h ±SE]	Diameter growth [cm/48 h ±SE]	Detached leaf Lesion area[x] [cm/48 h±SE]	Cut stem Lesion length[w] [cm^2/7d ±SE]
1	33.56±1.92	0.12±0.01	1.69±0.05	3.85±0.06	8.14±0.11	4.71±0.30
2	34.00±1.07	0.27±0.01	1.40±0.03	3.35±0.03	8.80±0.30	4.55±0.28
3	59.25±0.74	0.28±0.01	1.40±0.04	3.57±0.05	9.06±0.32	4.85±0.30
4	34.69±0.82	0.29±0.00	1.13±0.03	3.31±0.07	6.86±0.78	4.46±0.24
5	70.44±2.79	0.30±0.02	1.65±0.04	4.44±0.06	8.14±1.09	4.42±0.21
6	27.25±1.78	0.13±0.00	1.61±0.03	3.88±0.04	5.92±0.84	4.08±0.21
7	39.25±1.42	0.27±0.01	0.90±0.06	2.72±0.20	7.44±0.32	4.41±0.35
8	44.63±0.77	0.18±0.00	1.28±0.05	3.59±0.09	18.96±0.98	8.15±0.66
9	63.00±1.32	0.29±0.00	1.87±0.03	4.48±0.03	6.23±1.50	5.23±0.50
10	57.56±1.56	0.21±0.01	1.62±0.02	3.56±0.05	8.79±0.65	5.60±0.33
11	58.69±1.78	0.18±0.01	1.93±0.02	4.46±0.04	17.95±2.79	7.12±0.50
12	32.50±0.98	0.13±0.06	1.95±0.03	4.42±0.06	7.75±0.33	4.80±0.20
13	36.00±1.53	0.23±0.00	1.98±0.02	4.47±0.03	8.06±0.37	4.56±0.17
14	32.81±0.81	0.29±0.01	1.44±0.04	3.55±0.04	6.64±0.20	4.39±0.14
15	35.38±1.05	0.24±0.01	1.62±0.01	3.74±0.04	7.44±0.74	4.59±0.31
16	55.44±0.94	0.19±0.01	1.50±0.02	3.52±0.04	7.55±0.88	4.80±0.24
17	29.44±0.66	0.18±0.00	1.42±0.09	3.68±0.07	9.44±0.49	5.32±0.45
18	50.25±1.98	0.13±0.01	1.50±0.03	3.65±0.08	9.54±0.43	5.28±0.37
19	33.19±1.10	0.23±0.01	1.59±0.04	3.58±0.06	14.41±0.63	7.19±0.65
20	26.25±0.67	0.19±0.00	0.60±0.08	2.60±0.07	6.72±0.30	4.36±0.34
21	34.06±0.94	0.21±0.01	1.60±0.04	3.91±0.06	8.90±0.43	5.92±0.55
22	41.06±0.95	0.28±0.01	1.18±0.01	2.50±0.15	9.19±0.42	6.19±0.44
23	42.00±0.49	0.29±0.01	1.39±0.04	3.42±0.05	10.54±0.36	6.44±0.51
24	26.69±1.77	0.27±0.00	0.89±0.02	2.75±0.08	12.12±0.33	6.56±0.48
25	33.06±0.77	0.16±0.00	1.36±0.02	3.50±0.05	11.09±0.65	6.14±0.39
26	34.94±1.80	0.29±0.01	0.54±0.05	2.39±0.07	12.52±0.80	6.68±0.49

MCG – mycelial compatibility groups; SE – Standard error; [w]data for the cut stem inoculation method are lesion length in cm; [x]data for the detached leaf inoculation method are lesion area in cm^2

Table 4. Analysis of variance for number of sclerotial production, diameter growth, lesions size leaf and stem among mycelial compatibility grorups of *S. sclerotiorum*

Parameters	Sun of squares	df	Mean square	F	p-level
Number of scleortia	15,504.658	25	620.186	84.13	< 0.001
Sclerotia dry weight	0.360	25	0.014	20.46	< 0.001
Diameter growth [mm/24]	14.076	25	0.563	85.67	< 0.001
Diameter growth [mm/48]	37.048	25	1.481	68.64	< 0.001
Leaf lesion [cm^2]	1094.262	25	43.770	15.46	< 0.001
Stem lesion [cm]	118.328	25	4.733	7.56	< 0.001

df – degree of freedom; F – value

Pathogenicity assessment

Variation in isolates pathogenicity and aggressiveness was assessed using cut stem and detached rapeseed leaf methods inoculation technique. After 24 h, lesions on detached leaves became visible under the plug as water soaking and leaf necrosis, which expanded out from the plug after 36 h. At 48 h, the water-soaking and necrotic regions reached the leaf margin in some leaves (Fig. 3A). Comparing relative aggressiveness among isolates inoculated onto detached leaves, isolates AW1 and AW24 were identified as the most aggressive, AW174 and AW162 were intermediately aggressive, and AE3 and AW2 were the least aggressive.

Cut stem-inoculated plants showed typical water-soaked symptoms of Sclerotinia stem rot 3 days after inoculation. Water soaked lesions were visible from the point of inoculation downward (Fig. 3B). When the margins of lesions reached stem nodes, leaves wilted and died the next day. Virulence showed relationship neither to morphological characteristics nor to mycelial compatibility grouping.

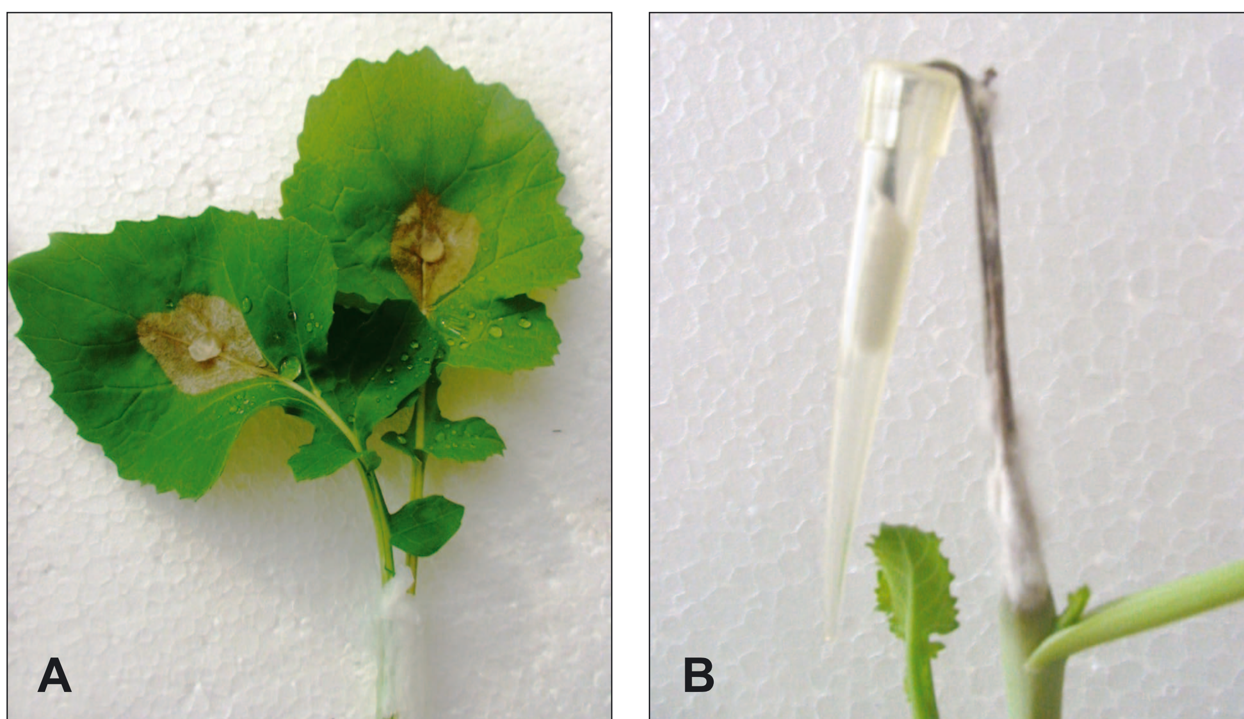

Fig. 3. Leaf and stem of canola plant inoculated with an isolate of *S. sclerotiorum* for the detached leaf and cut stem test respectively

Correlation analysis between virulence and morphological characteristics

Correlation analysis was conducted among the combinations of diameter, the yields of sclerotia, leaf and stem lesions. Results indicated that virulence of the two inoculation methods weren't correlated to colony diameter growth but there was generally poor correlation between number of sclerotia and diameter growth in 24 h ($r = 0.35$, $p < 0.05$), 48 h ($r = 0.39$, $p < 0.05$). There was also a poor correlation ($r = 0.21$, $p < 0.05$) between sclerotia weight and number of sclerotia produced by the isolates. Both methods had the highest rank correlations ($r = 0.78$, $p < 0.01$). Results also showed no relationship to morphological characteristics or mycelial compatibility grouping. In addition, no relationship was found between virulence and morphological characteristics or mycelial compatibility grouping (Table 5).

Table 5. Correlation analysis among number of scleortia, sclerotia dry weight, diameter growth (24 and 48), leaf and stem lesions in Mycelial Compatibility Groups (MCGs)

Parameters	Number of scleortia	Sclerotia dry weight	Diameter growth [cm/24]	Diameter growth [cm/48]	Leaf lesion [cm²]	Stem lesion [cm]
Number of Scleortia	1.000	0.205*	0.349*	0.387*	0.076	–0.033
Sclerotia dry weight [g]	0. 205*	1.000	–0.311	–0.280	0.088	0.001
Diameter growth [cm/24]	0.346*	–0.311	1.000	0.894**	0.046	–0.114
Diameter growth [cm/48]	0.387*	–0.280	0.894**	1.000	–0.028	–0.120
Leaf lesion [cm²]	0.076	0.088	–0.046	–0.028	1.000	0.780**
Stem lesion [cm]	–0.033	0.001	–0.114	–0.120	0.780**	1.000

*correlation is significant at $p \leq 0.05$; **correlation is significant at $p \leq 0.01$. Entry: correlation coefficient (r)

DISCUSSION

Overall results of this study showed that the populations of *S. sclerotiorum* on sunflower from the North West of Iran were a heterogeneous mix of MCGs. This agrees with previous reports of *S. sclerotiorum* MCGs population structures on different crops (Cubeta *et al*. 1997; Carpenter *et al*. 1999; Hambleton *et al*. 2002; Durman *et al*. 2003; Kull *et al*. 2004; Li *et al*. 2008). This study also indicated that North and North West populations of *S. sclerotiorum* from sunflower fields contained various and different MCGs. These populations presented a frequency profile in which many MCGs are recovered once or more and locally, and few MCGs occurred at high frequency. Similarly, this frequency trend was observed in Canadian studies of *S. sclerotiorum* on canola (Kohli *et al*. 1995; Hambleton *et al*. 2002) and on soybean in Argentina (Durman *et al*. 2001).

Clone is defined as a group of isolates (> 1 isolate) that share a unique DNA fingerprint and are mycelially compatible with each other, but incompatible with isolates of other clones (Kohn *et al*. 1990; Kohn *et al*. 1991; Kohli *et al*. 1992). Results of previous studies on population structure of the fungus in areas where canola, sunflower, and cabbage and other crops are grown have shown that this pathogen populations are mainly clonal, contrary to expectations for a sexually reproducing fungus. Somewhat more recombination is evident in subtropical compared with temperate populations (Carbone *et al*. 1999; Carbone and Kohn 2001). An early study on the population genetic structure of *S. sclerotiorum* using DNA fingerprinting found identical fingerprints only among isolates from the same field. Carpenter *et al*. (1999) found identical fingerprints only among isolates from the same field and some highly similar isolates indicated dispersal across the 90 km between Leith and Rakaia fields, but no recent dispersal was evident between Takaka and the other populations. Similarly, in a study of *S. sclerotiorum* isolates on cabbage in North Carolina indicated that MCGs or clones were shared between fields about 75 km apart, but no common MCGs or clones were detected between North Carolina and Canada or between North Carolina and Louisiana (Cubeta *et al*. 1997).

In contrast, the results of Canadian studies showed identical clones over distances up to 2000 km (Kohn 1995). This difference may be related to the scale and duration of farming, in that canola has been cultivated over extensive areas for more than 20 years, providing a continuous supply of inoculums and new host material, thus facilitating the spread of the fungus. Additionally, the apparent differences in dispersal may be associated with variation in the frequency of outcrossing and meiotic recombination (Carpenter *et al*. 1999).

In our study, only MCG 18 was detected in the sunflower fields in East and West Azerbaijan but no common MCGs shared among Ardebil and other provinces. It is not surprising to find shared MCG among West and East Azerbaijan provinces because these two regions are the major sunflower production areas in North West of Iran. Furthermore the two areas are approximately 100 km apart, and sunflower fields are actually contiguous between two regions, providing a pathway of cross infection for pathogen isolates from the two areas. In addition, both production areas share the same growth season, similar weather conditions, soil structures and agricultural practices, suggesting that no barrier exists in ecological adaptation of the pathogen isolates in either of these areas and also in this two regions the epidemiology of this disease is similar and primary inoculums usually occurres by germination of sclerotia and can directly attack plant tissues under soil and basal stem resulting in wilt. All of these factors may have resulted in no differentiation between two pathogen populations. In the present study, MCG analysis indicated that MCG 18 were both indigenous and mobile, highly dispersed genotype.

A lake of variation in aggressiveness among isolates from different geographical areas has been noted in a number of studies on agricultural population. Atallah *et al.* (2004) found no significant differences in aggressiveness among 35 North American isolates on potato. Auclair *et al*. (2004) tested representing four Canadian clonal lineages and did not find any association between genotype and virulence on soybean and also Durman *et al*. (2001) on the basis of a detached celery petiole assay found no significant differences in aggressiveness among 160 Argentinean isolates on soybean, sunflower. In contrast, Kull *et al*. (2004) reported that aggressiveness varied between isolates and MCGs from different locations from North and South America, but not in MCGs produced from isolates originating from infections in single fields also Li *et al*. (2008) found significant differences in aggressiveness among MCGs from different locations in sunflower from China, Canada and England.

In this study significant differences in pathogenicity were observed among different MCGs, regardless of the isolates origins. Differences in virulence may be detected when comparing isolates from widely separated geographical regions. Differences in the morphology of *S. sclerotiorum* strains based on morphological differences in sclerotia production and mycelial growth in the solid medium by some researchers have been reported previously (Morrall *et al*. 1972; Marukawa *et al*. 1975; Price and Colhoun 1975; Kohn *et al*. 1991; Li *et al*. 2003). However, similar differences among Iranian strains of *S. sclerotiorum* in our study have been shown. We, however, found no correlation between pathogenicity and colony diameter (data not shown). This agrees with the results of previous reports by Garrabrandt *et al*. (1983) and Li *et al*. (2008). In conclusion, our results about pathogen aggressiveness showed neither relationship to morphological characteristics nor to mycelial compatibility grouping. None of the morphological characteristics were related to the grouping made by mycelial compatibility.

Further studies comparing genetics and virulence across populations from different host species growing in close geographical proximity would be of interest. Insight into the population structure and variation in the virulence of *S. sclerotiorum* will be valuable for the formulation of disease management strategies. That including screening for resistance to this broad spectrum pathogen which is considered an important disease causal agent and yield reducing factor in sunflower fields around the world including Iran.

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