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## MORPHOLOGICAL VARIABILITY AND MYCELIAL COMPATIBILITY AMONG THE ISOLATES OF *SCLEROTINIA SCLEROTIUM* ASSOCIATED WITH STALK ROT OF SUNFLOWER

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*Sclerotinia stalk rot, caused by Sclerotinia sclerotiorum, is a recurrent disease on sunflower in the North West of Iran. Population structure, morphological variability of S. sclerotiorum was investigated. Population structure was determined by mycelial compatibility group (MCG). For this purpose, 186 isolates (Ardabil Set, 23 isolates, East and West Azerbaijan Sets, 22 and 141 isolates respectively) were grouped in 26 MCGs and 46% were represented by single isolates observed at single locations. Within the West Azerbaijan Set, 19MCGs were identified. Four MCGs were sampled at high frequencies from multiply locations. MCG18 is the highest frequency each locations. In West, East Azerbaijan and Ardabil provinces each province 10MCGs, 1MCGs and 1MCGs consisted of one isolate respectively. MCG18 was the most frequently sampled and widely dispersed MCG and occurred at a frequency of 29, 36 and 62% in the west and East Azerbaijan Sets, respectively. Common MCGs were identified among the West and East Azerbaijan locations sets, but no MCGs within the Ardabil Set were observed with other sets. This study has demonstrated that northwest of Iran populations of S.sclerotiorum from sunflower field crops are made up by various and different MCGs. These populations presented a frequency profile in which many MCGs are recovered once or twice and locally, and few MCGs occurred at high frequency and at far-off places. The isolates showed considerable variation in cultural characteristics through mycelial growth, and sclerotial production in the media plates. Significant differences were found in radial growth, number and weight of sclerotia among MCGs ( $p < 0.001$ ) but did not regardless of their geographic origins. None of the morphological characteristics could be related to the grouping made by mycelial compatibility and no apparent correlation between mycelial growth and sclerotial production among the isolates. The result showed that neither relationship to morphological characteristics nor to mycelial compatibility grouping.*

*Key words: Sclerotinia stalk rot, Helianthus annuus L., morphological variability, pathogen, isolates.*

**Introduction.** *Sclerotinia sclerotiorum* (Lib.) de Bary is a common, widespread pathogen of sunflower (*Helianthus annuus* L.) and non-specific pathogens known, with a host range that extends over more than 360 species in 64 families [1,2]. Originally identified on sunflower in 1861, the fungus has been reported from all sunflower growing regions of the world [3]. *S. sclerotiorum* can attack all parts of the plant. It causes water-soaked lesions on the stem rot in stems of the infected plants. In the soil, sclerotia may germinate into a mycelium, which in-

fects the roots and subsequently causes wilt in sunflower [4]. Sclerotia play an important role in disease cycles as they are the primary structures for their long-term survival and produce inoculate for further infection [5]. Sclerotia germinate either carpogenically or myceliogenically, resulting in two distinct categories of diseases under different environmental conditions. Hyphae developed when sclerotia germinate myceliogenically and can directly attack plant tissues under soil. However, apothecia are produced when sclerotia germinate carpogenically and ascospores can be projected to the air and infect aboveground portions of host plants [6–8]. Yield losses can reach up to 100 %. Incidence of this disease in sunflower fields of Iran has ranged from 3 % to 50 % [9]. 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. Mycelial compatibility groups (MCGs) is used routinely in many laboratories as a quick marker for genotyping *S. sclerotiorum* within populations, and its also have been used to evaluate genetic variability in fungal plant pathogens, such as, *Fusarium oxysporum* Schlecht. [10] and *Phomopsis subordinaria* (Desm.) Traverso [11]. In many species mycelia incompatibility results in the formation of macroscopic reaction lines (barrages) between fungal colonies, interpreted as an agonistic response resulting from the recognition of non-self antigens [12]. In *S. sclerotiorum*, the formation of the incompatible reaction line (evidenced by a dark line or a strip of thin mycelium and discontinuous sclerotia) between pairs of isolates has been observed, which indicated their failure to anastomose [13]. Previous studies have shown that *S. sclerotiorum* populations in canola (*Brassica napus* L. var. *napus*) in

Canada and cabbage (*Brassica oleracea* L. var. *capitata*) in the United States are clonal and that isolates could be separated into distinct mycelial compatibility groups [14,15]. Moreover, genetic analyses have shown that isolates of *S. sclerotiorum* are homogenous with limited variability in the 18S and 28S rDNA regions [14] and the only useful genetic markers appear to be within a dispersed repetitive element [14]. In these studies, a clone was defined as a genotype maintained in a single mitotic lineage [16,17]. It has been established that field population of *S. sclerotiorum* are clonal and that several clones may infect each field [18]. Somewhat more recombination is evident in subtropical compared with temperate populations [19,20]. In temperate regions, clonal lineages persist over time, with new fingerprints and MCGs arising through mutation and infrequent out-breeding. Consistent with previous studies on *S. sclerotiorum*, there are more fingerprints than MCGs, an indication that transposition leading to new fingerprints occurs more frequently than mutational events resulting in new MCGs [21]. These results indicate that much of the sclerotinia stem rot in the fields that were sampled was caused by a set of widely occurring *S. sclerotiorum* clones that likely predate the cultivation of soybean in the region. The observation of multiple fingerprints within groups of mycelially compatible isolates indicates that new genotypes are evolving. On the other hand, the observed persistence of clonal lineages would be expected given the long-term viability of the asexually produced sclerotia in the soil and the potential dispersal of inoculums with the movement of seed infested with sclerotia. Whether selection is increasing the frequency of clones that may have evolved locally in association with soybean cannot be determined, especially considering that edible bean and other susceptible hosts

have long been cultivated in this geographical region.

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

### **Materials and methods**

**Isolates.** A set of geographically diverse isolates of *S. sclerotiorum* used in the study were collected from infected sunflower fields in the districts by a distances of more than 30 km in the Ardabil, West and East Azerbaijan provinces of north and north west of Iran in growing seasons of the years 2007 to 2008. In each field several plants with symptoms of *Sclerotinia* stalk rot were randomly collected from a designated 5.0-5.0 m<sup>2</sup> area, air-dried, placed in paper envelopes, and stored at -4°C. For isolation, a single sclerotium or infected host tissue surface-sterilized using 10 % commercial bleach (0.5 % NaHCl) for 3 min, washed sterile water, and then incubated on potato dextrose agar (PDA) (Difco) plates. The plates were incubated for 3 days at 25°C and growth chamber (12 h photoperiod). Pure cultures were obtained by transfer of a single sclerotium and maintained on PDA slants at 4°C for 2–4 weeks [22].

### **Mycelial compatibility grouping.**

Mycelial compatibility grouping was as described by Schafer and Kohn [23]. Isolates were paired on modified potato dextrose agar (PDA) (Difco) amended with 175 µl/liter of McCormick's red food coloring (McCormick Corp., Dallas, TX). After 4 and 7 days pairings were performed in a pyramid design and scored as incompatible or compatible. Reactions were scored as incompatible if a red line was observed in the encounter zone between the two developing colonies.

Reactions were scored as compatible if the two developing colonies merged to form one mycelium with no interaction zone. All isolates were paired in groups of 10, including self-self pairings, until all isolates were assigned to an MCG or determined to be incompatible with representatives of all MCGs, including MCGs with only one isolate. In *S. sclerotiorum*, self-self incompatibility has not been observed; self-self pairings were used as a positive (compatible) control and repeat the pairings on PDA without red food coloring.

**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 on to fresh PDA(30 g/L) at 25°C and radial growth (colony diameter, cm) measured after 24 and 48 h. After 25 days, sclerotial production (total number and dry weight of sclerotia per plate/g) were evaluated. Four replications with four plates per replication were used for each isolates.

**Data analysis.** Data of lesions was analyzed by one-way ANOVA using PROC GLM in SAS. Individual isolates functioned as treatments and lesion length constituted the observational units. Results were compared using Student's unpaired *t* test.

### **Results and discussion**

**Genetic variation of MCG.** Mycelial compatibility group were determined for three Sets of *S.sclerotiorum* isolates; Ardabil (23 isolates), East Azerbaijan (19 isolates), West Azerbaijan (144 isolates). Among 186 isolates tested, 26MCGs were identified (table 1) within the sunflower fields in three provinces and 46 % were represented by single isolates observed at single locations. The West Azerbaijan Set, in which 144 isolates were, grouped 19MCGs contained 10MCGs each only consisting of single isolates. In Ardabil and East Azerbaijan each provinces 1MCGs

**Table 1.** Number of isolates and mycelia compatibility groups (MCGs) observed in each *S. sclerotiorum* set

Set <sup>a</sup>	No. of isolates <sup>b</sup>	No. of MCGs <sup>c</sup>	MCGs identified <sup>d</sup>
Ardebil	23	3	1, 2, 3
East Azerbaijan	22	5	4, 5, 6, 7, 18
West Azerbaijan	141	19	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26

<sup>a</sup>Name of set or field collection of isolates.

<sup>b</sup> Number of isolates used for MCG determination from each set.

<sup>c</sup> Number of MCGs detected in each set.

<sup>d</sup>Specific MCGs detected in each set.

consisted of one isolate respectively and others were more one isolates. The largest MCGs were MCG18, MCG23, MCG17 and MCG10 representing 14.6 %, 11.95 %, 9.78 % and 9.23 % of the sunflower isolates, respectively. This MCGs were sampled at high frequencies from multiply locations. MCG18, the highest frequency MCG sampled, included isolates was detected in West and East Azerbaijan provinces. Common MCGs were identified among the West and East Azerbaijan locations sets, but no MCGs within the Ardabil Set were observed with other sets. In most pairings, mycelial incompatibility 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 mycelia growth, separated one mycelium from the other, distinct band of hyphal lyses in the reaction zone (table 2).

**Variability in growth characters.** All isolates were morphologically characterized on solid medium. The colony of isolates on PDA ranged from white to a gray to a dark brown, with light tan being the most common. Differences among MCGs were observed by comparing their differences in all the morphological characters such as colony diameter (cm), number of sclerotia, and dry weight of sclerotia (g). Based on radial growth, the isolates were classified

into very fast growing, intermediate and slow growing. There were significant differences between different MCGs in relation to the colony diameter measured after 24 and 48 h of incubation. The average growth rates in 48h 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 showed 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) per plates. Significant differences occurred in number of sclerotia, and weight of sclerotia (g) of MCGs on PDA ( $P < 0.001$ ) (table 4). None of the morphological characteristics could be related to the grouping made by mycelial compatibility groups.

Our observation confirmed that populations of *S. sclerotiorum* from west Azerbaijan fields were a heterogeneous mix of MCGs. This agree with previous reports of *S. sclerotiorum* MCG population structure on different crops and locations such as canola in Canada [24], Norwegian vegetable crops [25], sunflower in Manitoba [26], cabbage in North Carolina [15] and soybean in Argentina [27] and Canada [28]. In our studies, 26 MCGs were identified and nearly half (46 %) belonged to a single mycelial compatibility group. Both west and eastern Azerbaijan fields contained two or

**Table 2.** Mycelial compatibility group (MCG) designation for 355 *Sclerotinia sclerotiorum* isolates

MCG <sup>a</sup>	Isolate code <sup>b</sup>
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

<sup>a</sup> MCG: Mycelial compatibility groups.

<sup>b</sup>The isolate collection number is preceded by a letter to indicate set: AW=West Azerbaijan, A=Ardabil, AE=Eest Azerbaijan.

three high frequency MCGs and a larger proportion of low frequency MCGs. Four MCGs were sampled at multiple locations. In our study, MCG 18, the highest frequency MCG sampled, included isolates was detected in West and East Azerbaijan

provinces. Common MCGs were identified among the West and East Azerbaijan locations sets, but no MCGs within the Ardabil Set were observed with other sets and no movement of propagules. This is not surprising. West IM and Middle IM are two

**Table 3.** Analysis of variance for number of sclerotia production, diameter growth, lesions size leaf and stem among mycelial compatibility groups of *S. sclerotiorum*

Parameters	Sun of squares	df	Mean square	F	p-level
Number of Sclerotia	15504.658	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 <sup>2</sup> )	1094.262	25	43.770	15.46	< 0.001
Stem Lesion (cm)	118.328	25	4.733	7.56	< 0.001

**Table 4.** Correlation analysis among number of Sclerotia, Sclerotia dry weight, diameter growth (24 and 48) in Mycelial Compatibility Groups (MCGs) of *S. sclerotiorum*\*

Parameters	Number of sclerotia	Sclerotia dry weight	Diameter growth (cm/24)	Diameter growth (cm/48)
Number of Sclerotia	1.000	0.205*	0.349*	0.387*
Sclerotia dry weight (g)	0.205*	1.000	-0.311	-0.280
Diameter growth (cm/24)	0.346*	-0.311	1.000	0.894**
Diameter growth (cm/48)	0.387*	-0.280	0.894**	1.000
Leaf lesion (cm <sup>2</sup> )	0.076	0.088	-0.046	-0.028
Stem lesion (cm)	-0.033	0.001	-0.114	-0.120

\* Correlation is significant at  $P \leq 0.05$ . \*\* Correlation is significant at  $P \leq 0.01$ . Entry: Correlation coefficient (r)

major sunflower production areas in many years. Although the two areas are approximately 30 km apart, 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. Frequent seed exchanges have been occurring and the same varieties have been planted in both areas for many years, indicating that the host selection pressures are identical in both pathogen populations. All of these factors might result in no differentiation between two pathogen populations. In my study, MCG analysis indicated that MCG18 were both indigenous and mobile, highly dispersed genotype.

Movement of genotypes from the point of introduction with infested agricultural materials might be expected to result in a nonrandom distribution of genotypes within the locality, as compared to random patterns of dispersal of airborne ascospore inoculum of *Sclerotinia sclerotiorum* [26]. MCG18 was detected in sunflower fields in several fields in west Azerbaijan province such as Khoy, Salmas and Orumia, No common MCGs were observed between Ardabil and any other location indicating little or no movement of propagules, selection for specific MCG genotypes, or a small sampling size. In West and Eastern Azerbaijan provenances carpogenic germination by *S.sclerotiorum* has been rarely observed in nature, and the fungus mainly infects plants via eruptive germination of sclerotia [29].

### Conclusions

This study has demonstrated that Azerbaijan populations of *S. sclerotiorum*

from field crops are made up by various and different MCGs. These populations presented a frequency profile in which many MCGs are recovered once or twice and locally, and few MCGs occurred at high frequency and at far-off places. Due to the heterogeneity, on the analyzed variables, among the Iranian isolates within each MCG, any imposed groups based on morphological characters would not be easily related to the mycelial compatibility grouping. All isolates were morphologically characterized on solid medium. The isolates varied in colony morphology, mycelial growth rate, sclerotium production and sclerotial weight.

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#### МОРФОЛОГІЧНА МІНЛИВІСТЬ І СУМІСНІСТЬ МІЦЕЛІВ ІЗОЛЯНТІВ *SCLEROTINIA SCLEROTIORUM*, ЩО Є ПРИЧИНОЮ СТЕБЛОВОЇ ГНИЛІ У СОНЯШНИКА

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Стеблова гниль *Sclerotinia sclerotiorum* є однією зі шкідливих хвороб соняшника на північному заході Ірану. У результаті дослідження були вивчені морфологічна мінливість і структура по-

пуляції *Sclerotinia sclerotiorum*. Структура популяції визначалася за допомогою сумісності мицеліїв. Було виділено 186 ізолянтів (Ардабиль – 23, Східний і Західний Азербайджан – 22 і 141 відповідно). Під час дослідження було встановлено, що на відміну від інших MCG лише ізолянти MCG56 було виділено у соняшника, вирощуваного в східній і західній частині Азербайджану. Отримані нами результати показали, що схожі ізолянти зустрічалися в Мазандаранському, Гіланському і Ардабильському районах Ірану.

**Ключові слова:** *Sclerotinia* стеблова гниль, *Helianthus annuus* L., морфологічна мінливість, патоген, ізолянт.

#### МОРФОЛОГИЧЕСКАЯ ИЗМЕНЧИВОСТЬ И СОВМЕСТИМОСТЬ МИЦЕЛИЕВ ИЗОЛЯНТОВ *SCLEROTINIA SCLEROTIORUM*, ЯВЛЯЮЩЕГОСЯ ПРИЧИНОЙ СТЕБЛЕВОЙ ГНИЛИ У ПОДСОЛНЕЧНИКА

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Стеблевая гниль *Sclerotinia sclerotiorum* является одной из вредоносных болезней подсолнечника на северо-западе Ирана. В результате исследования были изучены морфологическая изменчивость и структура популяции *Sclerotinia sclerotiorum*. Структура популяций определялась с помощью совместимости мицелиев. Были выделены 186 изолянтов (Ардабиль – 23, Восточный и Западный Азербайджан – 22 и 141 соответственно). Во время исследования было установлено, что в отличие от других MCG только лишь изолянты MCG56 были выделены у подсолнечника, выращиваемого в Восточной и Западной части Азербайджана. Полученные нами результаты показали, что похожие изолянты встречались в Мазандаранском, Гиланском и Ардабильском районах Ирана.

**Ключевые слова:** *Sclerotinia* стеблевая гниль, *Helianthus annuus* L., морфологическая изменчивость, патоген, изолянт.