Diversity of aggressiveness of *Sclerotinia sclerotiorum* (Lib.) de Bary populations in oil plants fields of north and northeast of Iran*

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Abstract

In the Northwest of Iran Sclerotinia stalk rot of sunflower is a serious disease caused by *Sclerotinia sclerotiorum* infection following hyphal germination of its sclerotia. In contrast, in the northern parts of the country, Sclerotinia stem rot is the most destructive disease of canola and the fungus germinates carpogenically to produce airborne ascospores. This study was conducted to compare aggressiveness of isolates of the fungus populations in the above-mentioned areas in sunflower and canola fields. Variation in aggressiveness of isolates was assessed using mycelial plug inoculation technique and the measurement of lesion development was used to compare aggressiveness among isolates. Highly significant differences in aggressiveness were found among MCGs ($P<0.001$) from the north and northwest of Iran. Widely dispersed MCGs such as MCG 56 showed within genotype variability in aggressiveness ($P<0.001$) that was not observed in local MCGs such as MCGs 17, 28, 42, 51and 64 in canola and sunflower fields.

Keywords: *Sclerotinia* rot, MCG, canola, sunflower

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Introduction

Sclerotinia rot caused by the ascomycete fungus, *Sclerotinia sclerotiorum* (Lib.) de Bary is a recurrent disease which has been reported to cause a significant yield loss in canola (*Brassica napus* L.) in Australia (Hind *et al.* 2003) and sunflower (*Helianthus annuus* L.) in China and Canada (Kohli *et al.* 1995). In Iran, Sclerotinia root rot causes serious yield losses of oilseed crops including sunflower and canola. Yield loss in canola varies from 3% to 50% and in sunflower it can be up to 63% (Afshari-Azad 2001; Irani *et al.* 2001). *S. sclerotiorum* persists on or in soil as sclerotia originating from previous epidemics in the same field or introduced through agricultural activities such as tillage, irrigation, manure fertilization and contaminated seeds (Adams and Ayres 1979; Schwartz and Steadman 1978). Sclerotia can germinate to produce either mycelia or, after a perioed of dormancy, apothecia (Adams and Ayres 1979). In Iran, canola is mostly grown in the northern part of the country, whereas sunflower is cultivated in the northwest region. Climatic conditions in these two regions are different. In the north, it is considerably moderate and humid, while in the northwest, it is semi-dry and cold in the winter. In the north, epidemics of Sclerotinia rot on canola are mainly initiated by ascospores released form apothecia (Afshari-Azad, 2001). In contrast, in the northwest, sclerotia germinate myceliogenically to produce hyphae that directly attack plant tissues in the soil (Irani *et al.* 1998). Fungal isolates collected from two different crops and widely separated regions, provide opportunities to study pathotype aggressiveness among populations of different locations (Kull *et al.* 2004).

In the northwest of Iran, no rainfall occurs from April to November, the period of major sunflower production. Therefore, traditionally furrow irrigation is used by farmers because of the low cost and the ease with which it can be operated. Thus, a large volume of soil becomes saturated, which can facilitate germination of sclerotia and increase infection of sunflower. In the north of Iran, on the other hand, precipitation is the only within–season source of moisture for the crop. Conservation tillage used in this region often associated with narrow-row sunflower or canola planting and occurrence of disease is associated with poorly drained areas, high plant densities and vigorous and lodged canopies (Irani *et al.* 1998).

Information on the genetic diversity and population structure of *S. sclerotiorum* is copious (Carpenter *et al.* 1999; Durman *et al.* 2003; Hambleton *et al.* 2002; Kull *et al.* 2004; Otto-Hanson *et al.* 2011; Wu and Subbarao 2006). Of particular interest are Kohn *et al.* (1991) and Cubeta *et al.* (1997), who documented that populations of *S. sclerotiorum* in canola and cabbage are clonal based on the evidence that a few clonal genotypes predominate in any locality and that the predominant clone is often recovered over many years and extended geographical distances. Further evidence of clonality is supported by strong association among unlinked molecular markers. This signifies extended generations of non recombining populations. Atallah *et al.* (2004), working with *S. sclerotiorum* from potatoes, demonstrated evidence of both clonal and outcrossing populations in Columbia basin of Washington state. This gave strong evidence of what was observed earlier in a limited scale by Kohli and Kohn (1998). Studies of the variability within the population in a given geographical region are important because these types of studies are relevant to the changes occurring in the population. Using mycelial compatibility groupings and aggressiveness tests, Otto-Hanson *et al.* (2011) reported large variation among *S. sclerotiorum* isolates within and between fields from multisite screening nurseries of common bean in the United States

The objectives of the present study were to study the aggressiveness variation among MCGs and assess variability in isolate aggressiveness within MCGs.

Materials and Methods

S. sclerotiorum isolates

Strains of *S. sclerotiorum* used in this study were isolated from canola and sunflower fields in two different agro–climatic conditions in Iran during the years of 2007 and 2008 (Fig. 1). These regions included: Ardabil, Guilan, Mazandaran, West Azerbaijan and East Azerbaijan provinces (Fig. 1). Fields were generally small and their sizes ranged from 0.5-3 hectares. In each field 3 to 30 plants were randomly collected from a designated

Fig.1. Location of sampled fields of sunflower and canola infected by *Sclerotinia sclerotiorum* in five provinces of Iran

5.0-5.0 m^2 area. The sclerotia were air-dried, placed in paper bags and stored at -4°C. They were then surface sterilized using 10% commercial bleach (0.5% NaOCl) for 3 min, washed with sterile water and cultured on potato dextrose agar (PDA). The plates were incubated for 3 days at 25°C in a growth chamber. The grown mycelial tips were then transferred to new PDA plates. Pure cultures were obtained by transferring a single sclerotium and maintained on PDA slants at 4°C for 2–4 weeks.

Isolate aggressiveness among MCGs

Variation in isolates aggressiveness was assessed by using mycelial plug inoculation technique *Brassica napus* cv. Hyola 401 was used for all pathogenicity tests. Seeds were grown in 12-cm-diameter plastic pots containing pasteurized soil, peat and perlite mix (1:1:1) under a 16:8 h day: night photoperiod with a day-time temperature of 20±2°C and a night-time temperature of 14±2°C under humid conditions. The top part of the main stem of 7 to 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 an 8-mm-thick plug of PDA and mycelium. Pipette tips were preloaded and transported in sealed boxes prior to inoculation of plants. The plants were incubated in a mist chamber with the relative humidity maintained over 80%. Disease development was determined and lesion length on the main stem was measured 7 days after inoculation (Zhao *et al.* 2004). Thirty-eight isolates from the north and 26 isolates from the northwest of Iran were selected among MCGs to conduct aggressiveness test. Experimental design for all isolate aggressiveness tests was a randomized, complete block with four replications and four plants per replication and each experiment was conducted twice.

Aggressiveness of MCGs within the Mazandaran and Guilan Sets

Aggressiveness of selected isolates in MCGs 1 and 2 within the Mazandaran set and MCGs 23 and 30 within the Guilan set, was calculated from the isolate aggressiveness tests (Zhao *et al.* 2004).

Additionally, aggressiveness of selected isolates in MCG 17 within the Mazandaran set and MCGs 28 and 37 within the Guilan set, each composed of members from single field locations was determined.

Aggressiveness of MCGs within the Ardabil, West Azerbaijan and East Azerbaijan Sets

Aggressiveness of selected isolates in MCG 39, within the Ardabil set, MCG 48 and MCG 61within the West Azerbaijan set and also

Table 1. Aggressiveness of 38 and 26 *Sclerotinia sclerotiorum* isolates of different mycelial compatibility groups in north and northwest of Iran, respectively

Isolate	MCG*	Lesion length (cm)	Isolate	MCG*	Lesion length (cm)
M142	17	6.62	AW1	46	8.15
G35	29	6.55	AW90	57	7.19
G78	36	6.50	AW21	49	7.12
M1	1	6.49	AW166	64	6.68
M151	19	6.46	AW162	62	6.56
M66	3	6.45	AW121	61	6.44
G35	26	6.33	AW122	60	6.19
M131	15	6.31	AW164	63	6.14
M140	16	6.02	AW1 20	59	5.92
G2	23	5.98	AW3	48	5.60
M120	12	5.74	AW62	55	5.32
M129	14	5.67	AW72	56	5.28
G79	37	5.66	AW2	47	5.23
G1	22	5.65	A25	41	4.85
G28	27	5.62	AW61	54	4.80
M125	13	5.61	AW30	50	4.80
M73	7	5.58	A1	39	4.71
M153	21	5.51	AW60	53	4.59
G66	31	5.50	AW51	51	4.56
G34	30	5.49	A14	40	4.55
M108	8	5.42	AE1	42	4.46
G30	28	5.31	AE4	43	4.42
M71	5	5.31	AE2	45	4.41
M64	9	5.19	AW59	52	4.39
M72	6	5.13	AW107	58	4.36
M152	20	5.09	AE3	44	4.08
M55	2	5.08	LSD(0.05)	-	1.11
M114	10	5.07			
G77	35	5.02			
M118	11	5.00			
G6	24	4.95			
G61	33	4.94			
G67	34	4.91			
G9	25	4.86			
M68	4	4.69			
M150	18	4.62			
G85	38	4.46			
G60	32	4.29			
LSD(0.05)	-	1.13			

*MCG: Mycelial compatibility group assigned for this research; ** the isolate collection number is preceded by a letter to indicate set: AW- West Azerbaijan, A - Ardabil, AE - East Azerbaijan, M- Mazandaran, G- Guilan

aggressiveness of selected isolates in MCG 56 composed of isolates from multiple locations (In West Azerbaijan and East Azerbaijan), was compared. Additionally, aggressiveness of isolates in East Azerbaijan set MCG 42, West Azerbaijan set MCG 51 and MCG 64 each composed of members from single field locations was determined. Aggressiveness tests were conducted as previously stated.

Statistical analysis

All statistical analyses were performed by PROC GLM in SAS. Data were analyzed using Student's T-test and means were compared by least significant differences (LSD) at P=0.05. The experiment was repeated at least twice for isolates showing reduced lesion sizes (Littell *et al.* 2006).

Results

According to the results based on mycelia plug inoculation technique isolates aggressiveness varied ($P \leq 0.001$) within the five sets. In the pathogenicity comparison, isolates collected from sunflower plants were more aggressive than those of canola (Table 1). Highly significant aggressiveness differences were found among the

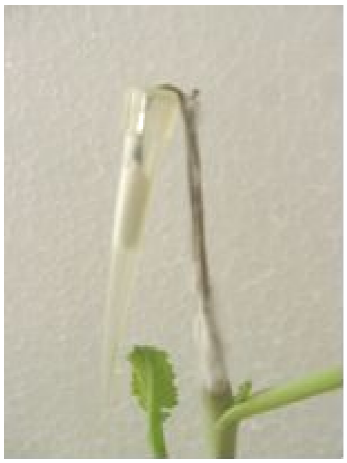

Fig. 2. Stem of canola plant inoculated with an isolate of *S. sclerotiorum* for cut stem test

38 and 26 isolates of north and northwest of Iran, respectively.

Isolate aggressiveness variability among MCGs.

Variation in aggressiveness of isolates was assessed using cut stem method inoculation technique. The initial disease symptoms in cut stem method were typical water-soaked lesions 3 days after inoculation. Water soaked lesions were visible from the point of inoculation downward (Fig. 2). When the margins of lesions reached the stem nodes, leaves wilted and died the next day. Highly significant differences in aggressiveness were found among the 38 and 26 isolates of north and north west of Iran, respectively ($P<0.001$) (Table 1). The differences in mean lesion lengths were greatest when inoculated with isolates AW1 (MCG 46) of sunflower Also, the West Azerbaijan isolates were significantly more aggressive than those of the other provinces (Table 1). Isolate aggressiveness varied among MCGs (Table 1) and these pathogenic differences of isolates and MCGs werenot found related to their geographic origin.

MCG aggressiveness within the Mazandaran and Guilan Sets.

Aggressiveness values for isolates within MCGs composed of members from multiple locations (MCG 2 and 30) were significantly different ($p=0.0047$ and $p=0.0024$, respectively). But there were no significant rating differences found in MCG 1 and 23 ($p=0.1575$ and $p=0.4281$, respectively). Isolate aggressiveness within locally observed MCGs composed of members from a single location (MCGs 17 and 28) did not differ ($p=0.05$). However, the isolates within the MCG 37 were highly different ($p=0.0004$). The isolate M 4 belonging to MCG 1 had the highest mean stem lesion length (most aggressive) and was significantly different from all other isolates of MCGs, with a rating of 6.83 cm. MCG 1 was composed of many isolates from Mazandaran fields. Isolate G51 had the lowest mean stem lesion length (least aggressive) with a mean rating of 1.22 cm (Table 2).

MCG aggressiveness within the Ardabil, West and East Azerbaijan Sets

There were significant differences within the aggressiveness ratings of 25 isolates of Ardabil, West and East Azerbaijan ($P<0.001$). MCGs were selected and relative aggressiveness of isolates within these MCGs was assessed. Variation in aggressiveness was highly significant ($P<0.001$) for isolates in MCG 56 and also MCG 48 ($P=0.0020$) but there were no significant rating differences found to MCG 61 ($P=0.0460$) in the northwest of Iran. Isolates aggressiveness within MCGs composed of members from a single location (MCGs 42, 51and 64) did not differ. Selected isolates from MCG 56, the most geographically diverse and frequently sampled MCG, had mean lesion area values from 4.46 to 8.15cm (Table 2). These two extreme values were exhibited by isolates collected in West Azerbaijan (Uromia, Salmas, Khoy) and East Azerbaijan sunflower fields.

Discussion

This study was conducted to compare aggressiveness of isolates of the fungus populations in two different agro–climatic conditions in Iran. Overall results of this study showed highly significant differences in aggressiveness among MCGs from the north and northwest of Iran.

A lack 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

Table 2. Aggressiveness of 25 *S. sclerotiorum* isolates from Guilan and Mazandaran canola fields and 25 *S. sclerotiorum* isolates from Ardabil, West Azerbaijan and East Azerbaijan sunflower fields

Isolate	MCG*	Lesion length(cm)	Isolate	MCG*	Lesion length(cm)
M**4	1	6.83	AW75	56	8.15
G27	23	6.72	AW172	64	7.19
Ga57	30	6.46	AW161	61	7.12
G33	28	6.02	AW171	64	6.68
G43	30	6.01	AW173	64	6.56
G5	23	5.97	AW5	48	6.44
G32	28	5.84	AW160	61	6.19
G7	23	5.79	AW74	56	6.13
M59	2	5.64	AW57	51	5.92
M8	1	5.62	AE6	42	5.60
G23	23	5.53	AW53	51	5.31
M148	17	5.50	AW130	61	5.27
M146	17	5.25	AW31	56	5.22
M91	2	5.19	AE20	56	4.85
M147	17	5.13	AW7	48	4.80
M81	2	5.13	AW91	56	4.79
G31	28	4.99	A20	39	4.59
M60	2	4.91	AW16	48	4.56
G81	37	4.81	AE5	42	4.55
G83	37	4.28	AW76	56	4.46
M56	2	4.21	AW19	48	4.42
M50	1	4.03	A19	39	4.41
M58	2	2.41	A22	39	4.39
G84	37	2.00	AW55	51	4.36
G51	30	1.22	AE19	42	4.07
LSD(0.05)	-	1.77	LSD(0.05)	-	1.12

*MCG: Mycelial compatibility group assigned for this research; ** the isolate collection number is preceded by a letter to indicate set: AW, - West Azerbaijan,; A, - Ardabil,; AE - Eest Azerbaijan, M,- Mazandaran,; G,- Guilan

North American isolates on potato. Auclair *et al.* (2004) tested four Canadian clonal lineages and did not find any association between genotype and aggressiveness on soybean. Also Durman *et al.* (2001) using detached celery petiole assay found no significant differences in aggressive among 160 Argentinean isolates on soybean and sunflower. Wu and Subbarao (2006) suggested that the low variation in aggressiveness among isolates from agricultural areas reported in some studies may result from comparing isolates that were not collected from widely separated geographical regions.

Durman *et al.* (2003) and Kohli *et al.* (1992) attributed the variation in aggressiveness to the changing environments where the coexistence of several MCGs would be favored over a more clonal population. In contrast, Kull *et al.* (2004) reported that aggressiveness varied between isolates and MCGs from different locations in North and South America.

Li *et al.* (2008) found highly significant differences in aggressiveness among MCGs from different locations on sunflower in China, Canada and England. Otto-Hanson *et al.* (2011) using mycelial compatibility groupings and aggressiveness tests reported large variation among *S. sclerotiorum* isolates within and between fields from multisite screening nurseries of common bean in the United States. These differences in aggressiveness may be detected when comparing isolates from widely separated geographical regions. Their results also confirm that these differences do not relate to the geographic origins of MCGs.

In the present study highly significant differences in aggressiveness were found between isolates in different MCGs in the north and northwest of Iran regardless of the isolates origins.

When the isolates were grouped into their respective MCGs highly significant differences were again found among the MCGs ($P<0.001$). Differences in aggressiveness may be detected when comparing isolates from widely separated geographical regions. These data supports the hypothesis that the differences in *S. sclerotiorum* aggressiveness are due to the clonal groups, not the individual isolates. Our results confirm this

conclusion and additionally indicate that these differences do not relate to the geographic origins of MCGs. MCGs may exist at high frequencies with wide geographic distribution. Widely dispersed MCGs such as MCG 56 showed within genotype variability in aggressiveness that was not observed in local MCGs such as MCGs 17, 28, 42, 51and 64 in canola and sunflower fields.

Kull *et al.* (2004) identified differences in aggressiveness in soybean field isolates from DeKalb and Watseka in Illinois a soybean field in Argentina and in a "diverse set" comprised of isolates from different hostes and locations. They also observed isolate aggressiveness variation (P=0.01) within the "diverse", Argentinae, DeKalb and Watseka sets. Widely dispersed MCGs showed within genotype variability in aggressiveness that was not observed in local MCGs. Additionally, individual MCGs within the DeKalb and Watseka Sets differed in isolate aggressiveness.

In the present research, isolates AW1 (MCG46) and AW75 (MCG56) of sunflower had the highest rating mean in mycelial plug inoculation (most aggressive) and were significantly different from all other MCGs, with their rating of 8.15 cm were composed of only one unique West Azerbaijan field isolate. MCG 30 had the lowest rating mean of 1.22 cm (least aggressive). MCG 30 is the unique Guilan isolate (G51). Further research comparing genetics and aggressiveness across populations from different host species growing in close geographical regions would be of interest. In conclusion, significant difference in aggressiveness was found not only among MCGs but also within MCGs. Insight into the population structure and variation in the virulence of *S. sclerotiorum* in Iran will be valuable for disease management strategies and screening for resistance to this broad host range pathogen.

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