# Study of Genetic Variations Based on the Morphological Characteristics, within the Population of *Sclerotinia sclerotiorum* from the Major Oilseed Planting Areas in Iran

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**Abstract**

*Sclerotinia sclerotiorum* (Lib.) de Bray, The causal agent of the stem rot disease, is prevalent and poorly managed on oilseed rape in the north of Iran. The genetic variations among of isolates of *S. sclerotiorum* collected from 29 fields in north of Iran was assessed, using morphological characteristics. Classification of the isolates to mycelial compatibility groups (MCGs) is used as a quick marker for genotyping within *S.s* populations. From forty-eight samples isolated from twenty-nine rapeseed fields, thirty-one MCGs were identified, that the most of the isolates within a single MCG were identical; however, the isolates belonging to the MCG4 were morphologically dissimilar. And also these isolates had different color (white, beige and grey) on PDA medium after 21 days. The data suggested that there are high rates of out crossings as well as evolutionary potentials found within the population. This is the first report of genotype variations between *S.s* populations from rapeseed fields in Iran.

**Keywords:** Diversity, MCG, Oilseed, *Sclerotinia Sclerotiorum*

## 1. Introduction

*Sclerotinia sclerotiorum* (Lib.) de Bray an ascomycetous fungus, causing white mould disease on more than 400 agricultural and wild plant species and has a worldwide geographical distribution (Purdy, 1979; Boland & Hall, 1994). *S. sclerotiorum* is described as a homothallic fungus and without conidia that dispersed by airborne ascospores and soilborne sclerotia. The sclerotia are the asexual resting propagules that germinate to produce either hyphae or apothecia (Abawi & Grogan, 1975; Adams & Ayers, 1979; Willets & Wong, 1980; Mitchell & Wheeler, 1990; Hao *et al.,* 2003). *S. sclerotiorum* have shown a high level of intraspecific phenotypic phenotypic variability (Purdy, 1979). Mycelial compatibility groups (MCG) and morphological characteristics is one of the two methods that developed for genotypes of *S. sclerotiorum* populations. When the members of a MCG paired with each other, can fuse to form one confluent colony with no reaction line (Kohn *et al.,* 1990), and mycelial incompatibility was described as a failure of different strains to fuse and to form one colony, and characterized by the formation of a barrage of dead cells between the two incompatible colonies (Kohn *et al.,* 1990). However,

this technique did not allow the identification of heterokarions (Kohn *et al.,* 1991).

There are several reports about the structural variations of *S. sclerotiorum* populations in the world. Thirty-nine colones were identified among 66 isolates on canola (Rapeseed) in Canada from seven locations in Alberta, Saskatchewan and Manitoba states (Kohli *et al.,* 1992) and 50 MCGs identified among 140 isolates from Buenos Aires (Sandra B. Duman *et. al.,* 1997). Limited outcrossing among the *S. sclerotiorum* isolates was observed in North Carolina and California (Kohli & Kohn, 1998). Several works were focused on comparison of the *S. 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 have observed among the wild populations. Sun *et al.* (2005) compared three *S. sclerotiorum* populations collected from Europe, China and Canada .they found that genetic differentiations were highly significant among and within the populations. Although *S. sclerotiorum* causes severe disease in many fields and glasshouse crops, there is not any report in the genetic variations of *S. sclerotiorum* populations in Iran. The objectives of this study were elucidation of the structural and genetic diversity within the populations of *S. sclerotiorum* North of Iran (Gillan, Mazandaran and Golestsan provinces), and measurement of the growth manner of *S. sclerotiorum* haplotypes at different temperature treatments.

## 2. Materials and Methods

### *2.1 Isolates*

Isolates of *Sclerotinia sclerotiorum* were collected from 29 oilseed rape fields in north of Iran (Gilan, Mazandaran and Golestan) fields during 2006-2007 growing season. Samples with the disease symptoms (stem rot, stunting) were collected and sclerotia were removed from the each plant sample. A single sclerotium from the each sample was selected as an isolate. And five isolates from each field were used in this experiment. The sclerotia surface sterilized for 1 min in 70% ethanol or 2 min in 2.5%sodium hypochlorite, rinsed in sterile distilled water, then plated on potato dextrose agar medium (PDA) and incubated at 22.C for two days. Each isolate was purified by transferring the single hyphal tip onto the fresh medium, and generated sclerotia were stored at -20.C until used (Atallah *et al.,* 2004*;* Cubeta *et al.,* 1997).

### *2.2 Mycelial compatibility group determination*

To determine the interafarm MCG variability, five isolates from the each field were paired together (totally 145 isolates). For evaluation of interfarm MCG variability, 48 isolates (two or three isolates from the each field) were paired to the each other. Agar disc with five millimeter diameter from two-day PDA culture of the each isolate was placed on PDA medium containing 75µl/L of red food coloring (Wilton Red food coloring, USA) (kohn *et al.,* 2006), and the agar disc from the other isolate was placed beside it, with 3 cm distance from each other. These isolates were performed in a pyramid design, where groups of 10 isolates were paired on PDA and incubated in the dark at room temperature. To determine possible difference in MCGs among 145 isolates of *S. Sclerotiorum* all isolates from the same farm (intrafarm MCG variability) were paired on PDA containing 75µl/L of red food coloring (Wilton Red food coloring, USA) (kohn *et al.,* 2006). Agar discs with a diameter of 5 mm were placed 3 cm apart on PDA in 9-cm Petri dishes, one pairing per dish, and incubated in the dark at room temperature.

From every farm one, two or three representative isolates were selected (at all:48 isolates) and these isolates were performed in a pyramid design, where groups of 10 isolates were paired on PDA using the above described procedure to determine of different MCGs present among isolated samples from different farms (interfarm MCG variability).

### *2.3 Temperatures treatments*

The radial growth of the S. sclerotiorum isolates were assessed at five different temperatures, including the optimum temperature (22.C) and others 10 and 5.C below and above (12, 17, 27 and32.C). PDA plates were inoculated with a 5 mm diameter plug of the colonized agar and incubated in the darkness at the various temperatures. Colony diameters were measured daily and the values averaged until the colony neared the edge of the dish. In a completely randomized design, four Petri dishes replicates for each isolate were used in four times separately. Data were analyzed by ANOVA using MSTAT-C program, and mean values were compared at the P at 0.05 level using Duncan's multiple range test The color of each colony was noted 21 days after the initial growth on PDA.

## 3. Results

### *3.1 Morphological variability among S. sclerotiorum isolates*

The growth rate of the isolates differed significantly: the isolates grew faster at 22.C, followed by 27.C, 17.C,

and 12.C, respectively. Isolates grew normally at 12, 17, 22 and 27 .C, but failed to grow at 32 .C. Colonies filled the 8-cm diameter petri dishes in 3 days at 22 .C. ANOVA for the growth rate data showed highly significant P-values among the isolates at all temperature treatments (Table 1). The color of the colonies on PDA growth medium varied among the isolates. There were three main colony colors: brown to dark brown pigmentation restricted to the region around the inoculation disc, beige pigmentation across the entire colony and white (no pigmentation; Fig. 1).Five isolates (R28, R30, R33, R36 and R51), exhibited brown pigmentation around the fungal disc and four isolates (R34, R38, R58 and R64) produced white colonies without pigment. The colony color of the rest of the isolates was beige (Fig. 1, Table 1).

*3.2 Mycelial compatible groups variability among S. sclerotiorum isolates*

Assessments of compatibility were based on mycelia continuity between the interacting colonies without formation of either a strip of thin mycelium or aerial mycelium, and the uniform distribution of sclerotia in the plate. Evaluation of compatibility was based on the failure of the two colonies to fuse, which were infected by the formation of a strip of this mycelium or aerial mycelial at the interaction zone (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) (Fig. 2). 31 MCGs were determined among the 48 studied isolates. The 23 isolates established independent MCGs. The isolate belong to the independent MCG, was compatible only with own. MCG4 and MCG23 consisted of six and four isolates, respectively; MCG1, MCG5and MCG18 included three isolates and three MCGs included only two isolates. The remaining 23 isolates were compatible only with themselves (Table1).

## 4. Discussion

The colony diameters of the isolates belonging to the same groups generally were statistically significant in agreement with each other at all temperature treatment. Similarly the growth of two isolates belonging to MCG21 (R45, R48) was similar at all temperature points, except at 12.C, where R45 grew faster than R48 (Table 1). More variation in mean growth rate was observed in isolates from MCG4, The colony diameters of the R20, R22, R49 and R55 were statistically similar at all temperature points tested; However the growth rate of the remaining isolates were statistically different (Table 1). Our presumption about link of different colors and variation genetic within the population confirmed. The colony colors of the isolates within the same MCG were similar, except MCG4 and MCG23. MCG4 consisted of 6 isolates; the color of the isolates R30 was brown, while R64 was white and the other 4 isolates differed from the previous isolates as beige of their colonies was observed in the culture medium (Table 1).

Populations of *S. sclerotiorum* from twenty-eight fields in north of Iran were a heterogeneous mix of MCGs. This corroborates reports of *S. sclerotiorum* MCG 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 MCGs, appears similar irrespective of host crop and field location. This is the first report demonstrating the morphological variation within population of *S. sclerotiorum* in Iran.

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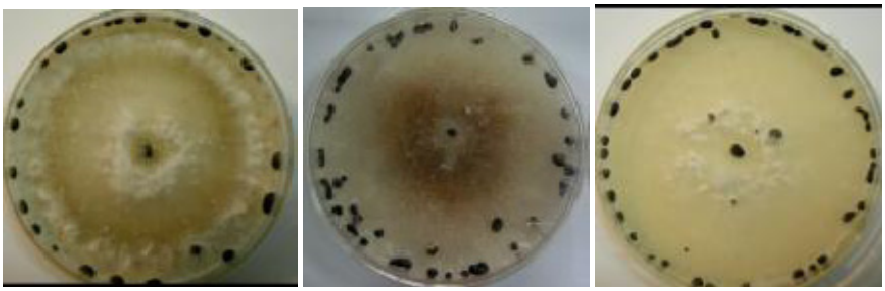
Table 1. MCG, colony color and growth rate of *Sclerotinia sclerotiorum* isolates at different temperatures on PDA.

| Isolates | Site | MCG s [a] | Colony color [b] | Colony growth at different temperature(mm) [c] | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 12˚C | 17˚C | 22˚C | 27˚C | 32˚C |
| R19 | Amol | 1 | Beige | 9.3mn | 20.1c | 70.0abcde | 50.3q | 6.9 qrs |
| R25 | Rezvanshahr | 1 | Beige | 9.6mn | 19.8c | 60.9hij | 50.1q | 6.4pqr |
| R26 | Bandar Anzali | 1 | Beige | 9.0m | 20.0c | 72.0abc | 48.9pq | 6.8 qr |
| R27 | Bandar Anzali | 2 | Beige | 4.4fg | 55.5b | 60.1ijk | 57.1stu | 8.8 vw |
| R37 | Nokandeh | 3 | Beige | 6.6ij | 12.6c | 60.1ijk | 50.6q | 7.6 tu |
| R20 | Amol-Hular | 4 | Beige | 6.8ij | 16.6c | 58.1jkl | 39.5lm | 5.0 ijk |
| R22 | Babol | 4 | Beige | 7.1jk | 11.6c | 68.0cdef | 43.8mno | 3.4 cd |
| R30 | Ghaemshahr | 4 | Brown | 2.5cd | 16.8c | 56.9jklm | 38.5klm | 4.8 ij |
| R49 | Kordkoy | 4 | Beige | 6.6ij | 17.6c | 68.1cdef | 53.8qrs | 7.6 tu |
| R55 | Semeskandeh | 4 | Beige | 7.1jk | 12.3c | 67.6cdefg | 66.8wx | 2.6 ab |
| R64 | Bayekolla | 4 | White | 2.35cd | 17.1c | 60.4ij | 41.4mn | 5.3 jkl |
| R18 | Amol | 5 | Beige | 1.2b | 6.9c | 60.2ijk | 40.6m | 4.8 ij |
| R23 | Chardange | 5 | Beige | 0.9ab | 6.9c | 60.2ijk | 41.0mn | 5.3 kl |
| R47 | Behshahr | 5 | Beige | 1.2b | 6.8c | 61.8ijk | 40.5mn | 5.3 jkl |
| R34 | Shirgah | 6 | White | 11.5pq | 19.8c | 57.8jkl | 40.6m | 4.3 fg |
| R56 | Semeskandeh | 7 | Beige | 7.6k | 14.4c | 61.1hij | 48.6pq | 10.0 y |
| R24 | Rezvanshahr | 8 | Beige | 2.9de | 16.8c | 43.1rst | 10.5a | 1.9 ab |
| R21 | Babol | 9 | Beige | 1.3b | 11.1c | 48.1pq | 39.0lm | 5.1 jk |
| R28 | Bandar Anzali | 10 | Brown | 7.4jk | 14.4c | 66.8efg | 50.5q | 5.4 lm |
| R33 | Juibar | 10 | Brown | 7.4jk | 14.6c | 67.2defg | 50.3q | 5.4 lm |
| R29 | Juibar | 11 | Beige | 1.6bc | 12.1c | 63.0ghi | 55.2s | 9.1 xy |
| R31 | Juibar | 12 | Beige | 1.2b | 6.5c | 42.5st | 33.0ijk | 8.1 uv |
| R32 | Juibar | 13 | Beige | 3.1de | 7.6c | 57.5jkl | 44.6no | 9.1 xy |
| R43 | B.turkaman | 13 | Beige | 3.3e | 6.2c | 57.1jklm | 43.8mno | 8.7 vw |
| R35 | Shirgah | 14 | Beige | 13s | 21.8c | 51.3nop | 49.1pq | 3.6 de |
| R36 | Arateh Gh. | 15 | Brown | 7.6k | 19.4c | 73.0ab | 50.9pqr | 4.9 ij |
| R38 | Kordkhail-sari | 16 | White | 0.6a | 3.1c | 43.1rst | 28.7ghi | 0.9 a |
| R39 | Shast kalateh | 17 | Beige | 5.9hi | 19.4c | 66.0efg | 66.1wx | 2 ab |
| R40 | Shast kalateh | 18 | Beige | 6.6ij | 15.6c | 69.0bcdef | 50.6qr | 4.3 fg |
| R41 | Kordkoi | 18 | Beige | 6.4ij | 15.8c | 67.1defg | 50q | 5.7 no |
| R65 | Bayekolla | 18 | Beige | 6.8ij | 19.9c | 65.1fgh | 53.3qrs | 4.3 fg |
| R42 | Kordkoi | 19 | Beige | 7.5k | 20.1c | 70.0abcde | 51.0qr | 1.3 a |
| R44 | Kordkoi-zrre. | 20 | Beige | 1.6bc | 8.4c | 36u | 31.1ij | 4.5 ghi |
| R45 | Kordkoi-zrre. | 21 | Beige | 7.0j | 14.0c | 57.5jkl | 50.3q | 10.0 y |
| R48 | Behshahr | 21 | Beige | 4.5fg | 5.5c | 59.0jkl | 48pq | 12.1 z |
| R46 | Behshahr | 22 | Beige | 4.6fg | 5.4c | 40.1t | 30.3i | 6.3 pq |
| R50 | Kordkoi-kar. | 23 | Beige | 7.4jk | 20.1c | 54.5lmno | 41.1mn | 1.8 a |
| R57 | Hullar | 23 | Beige | 6.6ij | 5.4c | 52.8mno | 46.1nop | 3.4 cd |
| R58 | Hullar | 23 | White | 1.8bc | 8.3c | 44.4qrst | 32.8ijk | 4.6 hi |
| R59 | Hullar | 23 | Beige | 6.6ij | 15.6c | 51.1nop | 43.3mno | 4.3 fg |
| R51 | Kordkoi-kar. | 24 | Brown | 4.0f | 5.9c | 54.5lmno | 40.3m | 3.5 de |
| R52 | Galu gah | 25 | Beige | 9.0m | 18c | 45.5qrs | 30.6hij | 2.4 ab |
| R53 | Suteh | 26 | Beige | 12.1qr | 18.6c | 43.1rst | 31.6ij | 6.4 pq |
| R54 | Semeskandeh | 27 | Beige | 10.0no | 18.0c | 65.0fgh | 11.9ab | 4.9 ij |
| R60 | Galugah | 28 | Beige | 11.6pq | 19.8c | 74.0a | 60.1u | 2.0 a |
| R61 | Dasht-e-naz | 29 | Beige | 2.0c | 8.3c | 44.5qrst | 30.2i | i4.6 hi |
| R62 | Dasht-e-naz | 30 | Beige | 14.5u | 20.5c | 50.0op | 33.1ij | ij9.4 y |
| R63 | Garakhail | 31 | Beige | 11.3pq | 20.6c | 55.5klmn | 36.8kl | 5.6 mn |

a) MCG, mycelial compatibility groups

b) Colors are recorded 21 days after sub-culturing on PDA (see Fig. 1).

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



A B C

Figure 1 The colony colors of *Sclerotinia sclerotiorum* isolates in PDA 21 days after culture.(A) beige pigmentation (B)Brown pigmentation; (c) no pigmentation (white)

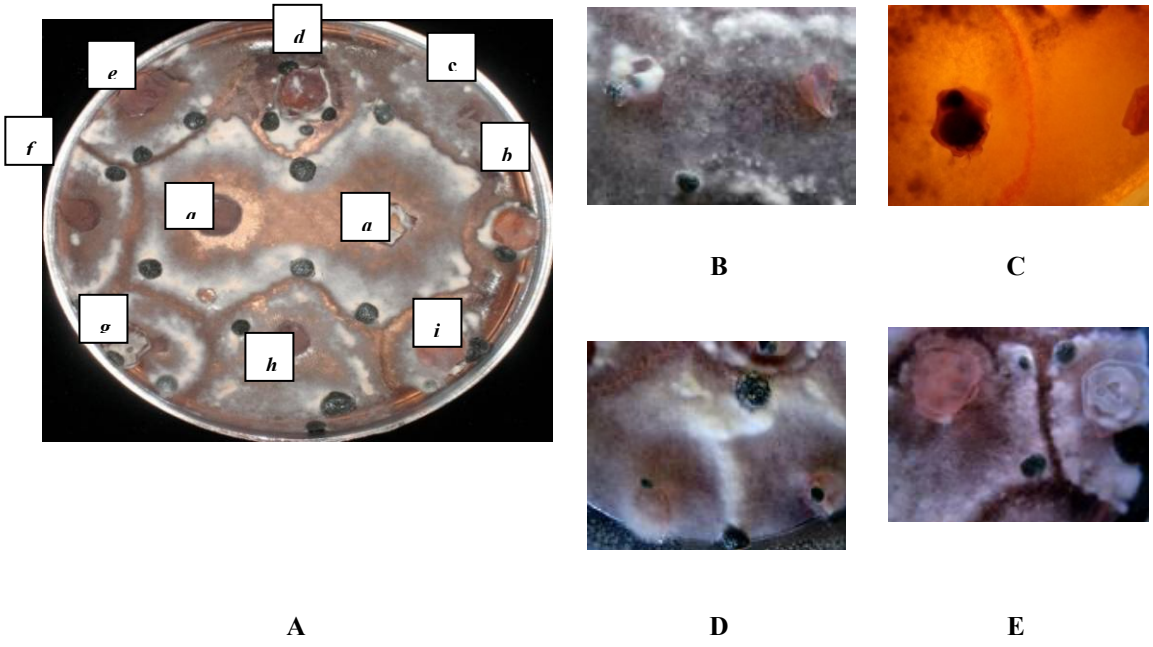



Figure 2. (A) Various vegetative interactions among *Sclerotinia sclerotiorum* isolates on potato dextrose agar amended with red food color after 1 week of incubation: self compatible(a-a), typical barrage zones of incompatible reactions between isolates(a-b,b-c,c-d,d-e.ef, f-g,g-h,h-i,i-b and a-f) (B) compatible reaction (C) Incompatible reaction(red line), (D) ) Incompatible reaction(aerial mycelium) (E) ) Incompatible reaction

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