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Mycelium Compatibility Groups and Phenotypic Variability Among some Isolates of *Sclerotinia sclerotiorum* the Causal of Stem and Pod Rot of Beans

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Morphological variability among 35 isolates of *Sclerotinia sclerotiorum* causing stem and pod rot of beans collected from various localities of EL-Behera governorate were tested. The isolates varied in colony morphology, mycelial growth rate and number, size, weight and color of sclerotia. Isolates were assessed for mycelial compatibility reactions in culture, accordingly, four types of mycelial reactions were observed. Mycelium compatibility was observed among 594 combinations and more than half (569 reactions) of them showed incompatible reactions between several tested pairs of isolates. Based on mycelial compatibility, vegetative compatibility groups (VCGs) were identified among all the tested isolates. However, total of 569 reactions were classified into three cases, 307 gap, 205 line-gap while, 57 exhibited barrage reactions and, 25 combinations showed compatible reaction.

Key words: *Sclerotinia sclerotiorum* - vegetative compatibility groups.

Sclerotinia sclerotiorum is a necrotrophic, phytopathogenic, filamentous ascomycetuos fungus. It is recognized as an omnivorous plant pathogen with broad host range and worldwide distribution. Over 400 species of plants are susceptible to this pathogen. The majority of these hosts are dicotyledonous, although a number of agriculturally significant monocotyledonous plants also are hosts (Boland & Hall 1994 and Purdy 1979). The initial infection occurs in the late winter or early spring, and the fungal mycelia grow within and between plants. Patches like symptoms of dead plant parts enlarge and coalesce through spring and cause major losses in stands (Bolton *et al.*, 2006). The fungus produces many black structures called sclerotia, which survive from one cropping season to the next. Over-wintered sclerotia may germinate during the summer or may stand dormant for many years (Adams & Ayers 1979). The sclerotium of *S. sclerotiorum* is a pigmented, asexual, multicellular, and firm resting structure composed of condensed vegetative hyphal cells which become interwoven and aggregate together, and it is capable of surviving years in soil (Adams & Ayers 1979; Chet & Henis 1975; Tourneau 1979 and Willetts & Bullock 1992).

Sclerotia germinate myceliogenically or carpogenically depending on environmental conditions in the field and crop canopy (Abawi & Grogan 1979; Steadman 1979 and Bardin & Huang 2001). Carpogenic germination of sclerotia in the soil result in the formation of apothecia which release airborne ascospores for infection of above-ground tissues of plants, causing diseases such as white mold of bean (Boland

& Hall, 1987), pod rot of beans (*Pisum sativum* L.) (Huang & Kokko, 1992), stem blight of canola (Gugel & Morrall, 1986), blossom blight of alfalfa (*Medicago sativa* L.) (Huang, *et al*., 2000), head rot of safflower (*Carthamus tinctorius* L.) (Mündel, *et al*., 1985) and sunflower (*Helianthus annuus* L.) (Huang, 1983). In contrast, myceliogenic germination of sclerotia in the soil results in the production of mycelia which attack under-ground tissues, causing root rot, basal stem rot and wilt of plants (Huang & Dueck, 1980).

Mycelial compatibility, the ability of two strains of filamentous fungi to anastomose and from one continuous colony, is synonymous with vegetative compatibility. Mycelial compatibility groups (MCGs) testing is a phenotypic, macroscopic assay of the self or non-self recognition system controlled by multiple loci common in fungi (Carbone, *et al*., 1999). A distinction must be maintained between vegetative and heterokaryon compatibility unless it is known that two strains not only anastomose but also form a stable heterokaryon. As an easy test for self-recognition, vegetative compatibility has been extremely useful in intraspecific strain comparisons. The deployment of vegetative and heterokaryon compatibility testing, as well as the mechanisms behind these phenomena, have been amply reviewed (Leslie, 1993; Glass & Kuldau 1992; Glass, *et al*., 2000 and Saupe, 2000) and continue to be lively areas of research. Various phenotypic markers such as macroscopic or microscopic evidence (Cortesi, *et al*., 1996; Julian, *et al*., 1996 and Boland, 2004) and morphological characters (Puhalla & Hummel 1983) are employed in understanding the phenomena of vegetative compatibility groups in *S. sclerotiorum*. Compatible pairings formed one confluent colony. Incompatible pairings produced a visible reaction in the interaction zone, such as a red line visible on the colony reverse, or a line of fluffy aerial mycelium or thin mycelium on the colony surface. Microscopically, challenging hyphae in compatible interactions did not necessarily anastomoses but were able to overgrow each other. In incompatible interactions that resulted in macroscopic red reaction lines, deterioration of hyphae was observed within and adjacent to the interaction zone. Genetic regulation of vegetative compatibility is not yet elucidated in *S. sclerotiorum*, but if other ascomycetes such as *Neurospora crassa* and *Podospora anserine* are a guide, it is expected to be multigenic (Glass, *et al*., 2000). The objectives of this study were to investigate and evaluate the variations existed in the associated pathogen recovered from El-Behera governorate and to draw suggestions for its control.

Materials and Methods:

1. Sampling and isolation of the causal fungus:

During the 2009-2010 growing season, samples showing stem and pod rot of beans were collected from four localities of El-Behera governorate, *i.e.* Abo-Homous, El-Delngat, Etay El-Baroud and El-Mhamodia. Isolation of the causal fungus was conducted according to the normal practices.

The recovered isolates were purified by growing single sclerotia from each colony, after surface sterilization using 0.5% sodium hypochlorite (NaOCL) for 3 min, washed with sterile water and then incubated on potato dextrose agar (PDA) medium slants for three days. Hyphal tips were cut and transplanted at least three times to obtain the genetically identical cultures. The pathogen was identified using the key of Kohn (1979).

2. Morphological variability of the causal fungus:

Single sclerotial cultures of all recovered isolates were preserved on PDA medium. Isolates were investigated for morphological and cultural characteristics via, radial growth on PDA (cm), number of sclerotia developed in Petri dishes, size of sclerotia (mm) and weight of sclerotia (mg). Plates containing 20 ml of PDA, a 5 mm PDA plug removed from the margin of an actively growing colony of the individual isolate was centered on PDA with four replicates for each isolates. Plates were sealed with parafilm and incubated at 25± 2°C in the dark. After of 5 days, the diameter of colony was measured. All plates were investigated continually for 25 days and dry sclerotial yields were measured. Size and weight of sclerotia of each isolates were recorded and classified according to Abida Akram *et al*. (2008).

3. Mycelial compatibility of the causal fungus:

Mycelial compatibility between all isolates was done as described by Kohn *et al*. (1991), in which all isolates were paired in combination including self-self pairing. Mycelial plugs (5-mm in diameter) of each isolate were cut with the aid of sterile cork borer at the growing margin of 5-day old culture plates at 25± 2°C and transferred to the opposite ends of PDA plate (6 cm apart) . The compatibility and incompatibility were scored 7 days after incubation at 25± 2°C according to the criteria adopted by Kohn *et al.* (1990), and Cubeta *et al*. (1997). Incompatibility was recognized as minor when the reaction line of the paired isolates was characterized by hyphal tufts on the colony surface, and more pronounced when there is a demarcation line on the colony reverse and the presence of discontinuity or lysis of the hyphal tips. On the other hand, complete compatibility was characterized by completely overlapping growth of both mycelia and then growing in one colony. Degrees of incompatibility were weighed on an arbitrary scale of 0 - 3 where 0 means complete compatibility and 3 means complete incompatibility (Powell and Vargas 2001).

4-Statistical analysis:

This was conducted using SAS program (SAS Inc., 2000). Least significant differences (LSD) were used to separate mean differences and to rank isolates.

Results

1. Fungal isolates associated with stem and pod rot of beans:

Thirty five *S. sclerotiorum* were recovered from beans plants showed stem and pot rot collected from different localities in El-Behera governorate. These were Abo-Homous, El-Delngat, Etay El-Baroud and El-Mhamodia where sets of isolates of 8, 13, 9 and 5 were recovered, respectively (Table 1).

2. Variability in the recovered isolates of S. sclerotiorum:

2.1. Colony color:

On the basis of colony color, the isolates were classified into three groups: white color, beige and brown color. Data in Table (1) showed that, the isolates, SS-6, SS-10, SS-13, SS-14, SS-17, SS-18, SS-19, SS-20, SS-22, SS-23 and SS-30 were white, while, isolates, SS-1, SS-2, SS-3, SS-4, SS-5, SS-7, SS-8, SS-9, SS-11, SS-12, SS-15, SS-16,

SS-21, SS-28, SS-32, SS-33 and SS-35 were beige and isolates, SS-24, SS-25, SS-26, SS-27, SS-29, SS-31 and SS-34 were brown (Figure 1, 3 & Table 1).

2.2. Radial growth:

Based on radial growth recovered after 5 days, the isolates were found to be classified into three groups: very fast, *i.e.* the isolate completely colonized the dish (9-cm in diameter), intermediate, *i.e.* the isolates colonies diameter ≥5 cm < 9 cm, and slow growing isolates, *i.e.* isolates colony diameter < 5 cm. Data after 5 days of incubation revealed that most of the isolates: SS-1, SS-2, SS-3, SS-4, SS-5, SS-6, SS-7, SS-8, SS-9, SS-10, SS-11, SS-12, SS-13, SS14, SS-16, SS-18, SS-19, SS-21, SS-22, SS-23, SS-25, SS-26, SS-27, SS-28, SS-29, SS-33, SS-34 and SS-35 represented the fast growing isolates. Isolates SS-20, SS-24, SS-30 and SS-31 were intermediate, while isolates SS32, SS15 and SS-17 showed slow radial colony growth (Figure 3 & Table 1).

2.3. Number of sclerotia:

Number of sclerotia produced by *S. sclerotiorum* isolates, SS-4, SS-8, SS-9, SS-11, SS-12, SS-14, SS-15, SS-21, SS-25, SS-28 and SS-35 ranked as high producers of sclerotia, *i.e.* ≥30 sclerotia/plate, while isolates SS-1, SS-2, SS-3, SS-5, SS-6, SS-7, SS-10, SS-13, SS-16, SS-23, SS-24, SS-26, SS-27, SS-29, SS-30, SS-31, SS-32, SS-33 and SS-34 were intermediate, *i.e.* >14 < 30 sclerotia/plate and isolates SS-17, SS-18, SS-19, SS-20 and SS-22 showed least number of sclerotial formation, *i.e*. ≤14 sclerotia/plate (Figure 3 & Table 1).

2.4. Size of sclerotia:

Size of sclerotia of isolates, SS-1, SS-5, SS-7, SS-8, SS-10, SS-16, SS-17, SS-18, SS-19, SS-20, SS-22, SS-24, SS-15, SS-26, SS-33, SS-23, SS-27, SS-29, SS-30, SS-31, SS-32 and SS-35 showed taller diameter 4.0 to 5.6 mm, on the other hand, isolates, SS-2, SS-3, SS-4, SS-9, SS-12, SS-14, SS-21, SS-25, SS-28 and SS-34 were intermediate while, SS-6, SS-11 and SS-13 ones monifested least size of sclerotia (Figure 2 , 3 & Table 1).

2.5. Weight of sclerotia:

According to the weight of sclerotia, the isolates were categorized in three groups: isolates having heavy sclerotia (sclerotial weight more than 20 mg), isolates having intermediate sclerotia (sclerotial weight ≥12 mg < 20 mg) and isolates with low weight of sclerotia (sclerotial weight less than12 mg). Thus isolates, SS-4, SS-7, SS-16, SS-17, SS-18, SS-19, SS-20, SS-22, SS-23, SS-24, SS-27, SS-30, SS-31 and SS-32 were heavy weight isolates, and SS-1, SS-2, SS-3, SS-5, SS -8, SS-9, SS-10, SS-13, SS-15, SS-21, SS-25, SS-26, SS-28, SS-29, SS-33, SS-34, SS-35 were intermediate weight isolates, while SS-6, SS-11, SS-12 and SS-14 exhibited low sclerotial weight isolates (Figure 3 & Table 1).

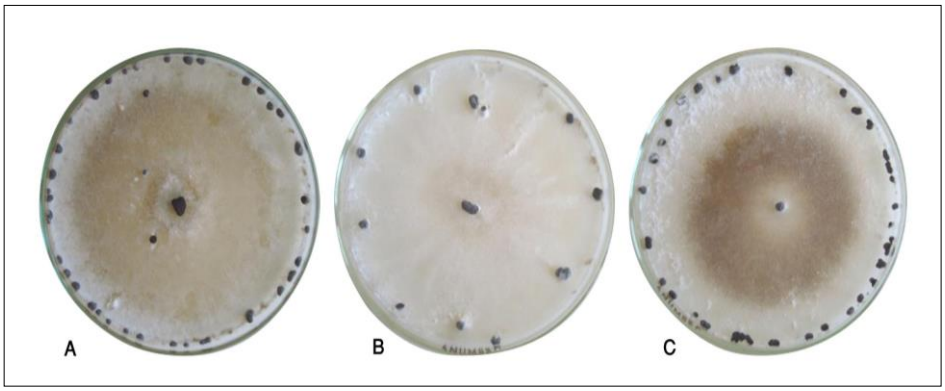

Fig. 1: Colony color types of *Sclerotinia sclerotiorum* isolates recovered from different fields in EL-Behera governorate. (A) Beige. (B) White. (C) Brown

Colony color types in *Sclerotinia sclerotiorum* isolates were recorded after7 days of incubation at 25± 2°C.

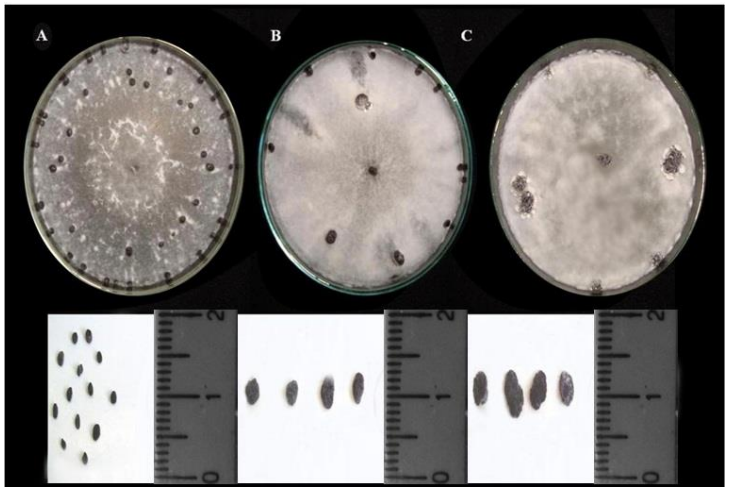

Fig. 2: Sclerotial size of *Sclerotinia sclerotiorum* isolates recovered from different fields in EL-Behera governorate.

(A) Small sclerotia. (B) Intermediate sclerotia. (C) Big sclerotia.

- Values are average of three replicates.

3. Mycelial compatibility reaction types:

Four types of mycelial compatibility reactions were recognized among the 35 tested isolates. They were referred as gap, line-gap, barrage, and compatible reaction types, and are comparable to the types described Brasier (1984 & 1986). The morphological features of these reaction types are shown in (Fig. 4). In the compatible reactions, two paired colonies merged uniformly or there was a slight mycelia thickening along the interaction zone, and no dark or brown line appeared red on the back of the PDA plates (Fig. 4.A). The gap reaction was characterized by wide gap (3-10 mm) between the two paired colonies and two dark lines on the back of the PDA plate (Fig. 4.C). The line-gap reaction was characterized by a narrow gap (1mm) along the interaction zone, witch looked like a dark line, and there were one or two brown lines on the back of the PDA plate (Fig. 4.B) .The barrage reaction did not form the gap or line gap at the interaction zone and was characterized by a thick, white mycelial barrage about 3-5 mm wide between the two paired colonies, and a dark zone on the back of the PDA plate (Fig. 4.D). A common feature of the three in compatible reactions was the formation of a thick mycelium zone between the two paired colonies (Fig. 4) after hyphal contact, hyphal death was observed on the thick mycelium zone within 2-3, 3-5 and 6-8 days for gap, line-gap and the barrage reactions respectively. Hyphal death eventually led to the formation of wide (gap) or narrow (line-gap) reactions along the interaction zone, but no line or gap appeared in the barrage reaction up to 14 days after inoculation. In the compatible reactions, no thick mycelial zone or only a slight mycelial thickening appeared between the paired colonies and no hyphal death was observed along the interaction zone within 14 days. There were 594 pairings of the 35 isolates and out of all, 25 combinations showed a compatible reaction (4.2% of all combinations) where mycelia of the two isolates intermingled at the zone of interaction. Based on mycelial compatibility, 569 mycelial compatibility groups were found among all the isolates. Among 569 reactions were 307 combinations showed gap reactions (51.6 % of all the combinations), 205 combinations showed line-gap reactions (34.5 % of all the combinations), while 57 combinations showed barrage reactions (9.59 % of all the combinations) (Fig. 5).

Table (1): Characteristics of *Sclerotinia sclerotiorum* isolate causing stem and pod rot of beans collected from various locations in EL-Behera governorate, grown on PDA medium.

olates	Location	Colony color	Radial growth (cm)	Av. no. of sclerotia	Ave. size of sclerotium (mm)	Ave. wt. of sclerotium (mg)
SS1	Abo-Homous	Beige	9	19.50	4.007	19.25
SS2	Abo-Homous	Beige	9	23.25	3.450	13.25
SS3	Abo-Homous	Beige	9	23.75	3.335	14.75
SS4	Abo-Homous	Beige	9	44.75	3.270	23.25
SS5	Abo-Homous	Beige	9	25.50	4.327	17.75
SS6	Abo-Homous	White	9	25.75	2.707	10.75
SS7	Abo-Homous	Beige	9	19.25	4.980	22.75
SS8	Abo-Homous	Beige	9	36.25	5.485	14.75
SS9	EL-Delengat	Beige	9	31.25	3.157	17.25
SS10	EL-Delengat	White	9	22.00	4.900	17.75
SS11	EL-Delengat	Beige	9	34.00	2.837	11.50
SS12	EL-Delengat	Beige	9	37.50	3.640	10.00
SS13	EL-Delengat	White	9	23.00	2.980	15.50
SS14	EL-Delengat	White	9	31.75	3.050	11.25
SS15	EL-Delengat	Beige	4.87	32.75	4.015	12.75
SS16	EL-Delengat	Beige	9	22.75	4.605	21.50
SS17	EL-Delengat	White	4.25	8.00	5.657	80.25
SS18	EL-Delengat	White	9	12.75	4.562	32.75
SS19	EL-Delengat	White	9	14.00	4.112	20.50
SS20	EL-Delengat	White	7.62	12.00	4.015	26.75
SS21	EL-Delengat	Beige	9	31.25	3.825	15.00
SS22	Etai EL-Baroud	White	9	13.00	5.390	26.25
SS23	Etai EL-Baroud	White	9	22.75	4.560	24.25
SS24	Etai EL-Baroud	Brown	8	18.25	4.855	23.50
SS25	Etai EL-Baroud	Brown	9	29.25	3.662	17.00
SS26	Etai EL-Baroud	Brown	9	25.75	4.287	18.25
SS27	Etai EL-Baroud	Brown	9	20.75	4.517	23.25
SS28	Etai EL-Baroud	Beige	9	31.75	3.857	16.50
SS29	Etai EL-Baroud	Brown	9	21.50	5.060	18.75
SS30	Etai EL-Baroud	White	7.12	18.25	4.165	21.00
SS31	EL-Mahmoudia	Brown	8	20.75	4.325	22.50
SS32	EL-Mahmoudia	Beige	5.2	24.50	5.080	21.50
SS33	EL-Mahmoudia	Beige	9	23.25	4.715	17.75
SS34	EL-Mahmoudia	Brown	9	26.50	3.672	16.25
SS35	EL-Mahmoudia	Beige	9	31.25	4.017	13.00

L.S.D at 0.05 for: radial growth = 0.357
L.S.D at 0.05 for: number of sclerotia = 10.981
L.S.D at 0.05 for: size of sclerotia = 0.776 L.S.D at 0.05 for: weight of sclerotium = 3.57

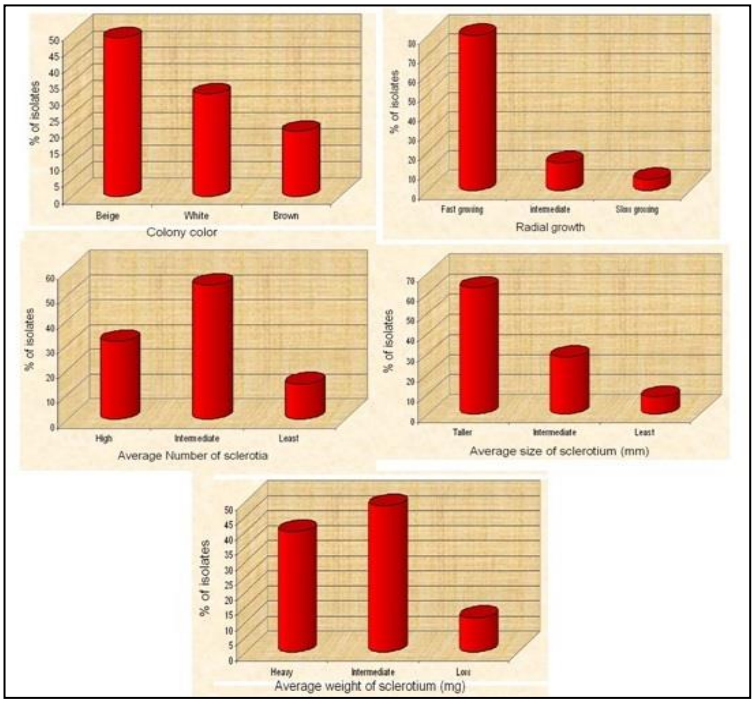

Fig. 3: Frequency of phenotype of *Sclerotinia sclerotiorum* isolates recovered from beans, showed stem and pod rot, collected from different fields in EL-Behera governorate.

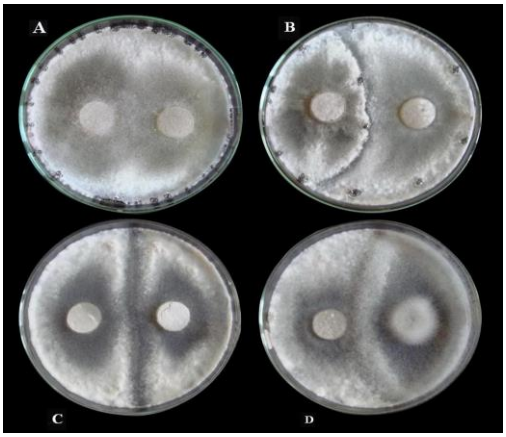

Fig. 4: Mycelial compatibility reaction types in *Sclerotinia sclerotiorum*.
(A) Compatible reaction. (B) Line-gap reaction. (C) Gap reaction. (D) Barrage reaction.

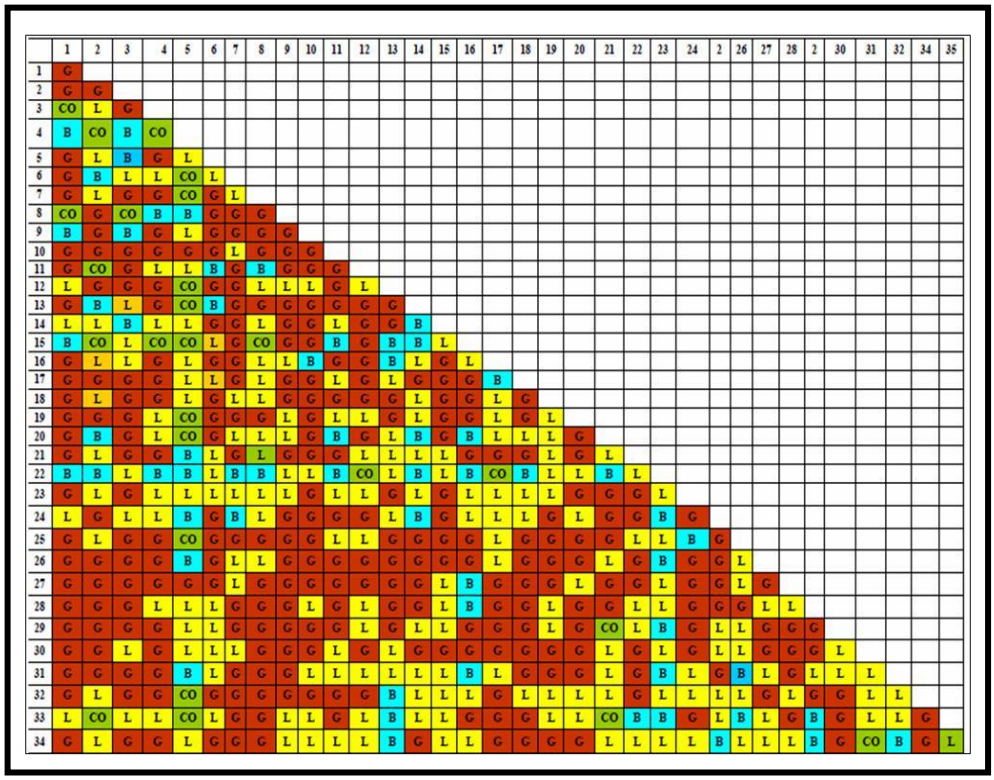

Fig. 5: Mycelial compatibility reactions among 35 isolates of *Sclerotinia sclerotiorum* associated with stem and pod rot of Beans.

- Mycelial compatibility reactions among isolates were scored after 7 days of incubation at 25± 2°C.

CO = Compatible reaction.
B = Barrage reaction.
L = Line – Gap reaction.
G = Gap reaction

Discussion

Considerable variations in all the morphological characteristics of *S. sclerotiorum* were observed in the present study. Based on radial growth, the isolates were classified into three groups, very fast, intermediate and slow growing. Also, on the basis of number of sclerotia, isolates were classified into high, intermediate and low number of sclerotial formation. As for size of sclerotia is concerned, isolates were classified into three groups: big, intermediate and small size of sclerotia. These results were in agreement with Punja & Damiani (1996), Zarani & Christensin (1997), Abdia Akram *et al*. (2008) and Ziqunli *et al*., (2008) as they recorded differences in growth rates among different isolates of *S. sclerotiorum* obtained from various host species.

Also, based on sclerotial diameter several workers recorded variation in size of sclerotial among different isolates of the fungus (Dhingra & Sinclair, 1973; Mirza *et al*., 1985 and Abida Akram, *et al*., 2008). According to the weight of sclerotia, the isolates were categorized in three groups: isolates having heavy sclerotia (sclerotial weight more than 20 mg), isolates having intermediate sclerotia (sclerotial weight ≥12 mg < 20 mg) and isolates with low weight of sclerotia (sclerotial weight less than12 mg), this was in harmony with Abida Akram *et al*. (2008). Number of sclerotia of *S. sclerotiorum*, as inocula and the primary long-term survival structures in soils, is associated with the extent of damage caused by this disease in fields. However, up till now, little attention has been given to the factors affecting the sclerotia formation.

Vegetative compatibility is the ability of hyphae of two strains of fungi to fuse and form a stable heterokaryon. In order for the strains to form a stable heterokaryon, they must share identical alleles at a particular site of loci. Strains that differ at any of these loci will not be able to form a stable heterokaryon and will result in an incompatible reaction typified by death of the heterokaryotic cells (Leslie, 1993). Among asexual fungi (including *S. sclerotiorum*), vegetative compatibility groups (VCGs) represent genetically isolated subpopulations, and members of the same VCG are generally more similar than members of different VCGs (Dobinson *et al*., 1998; Gordon and Okamoto, 1992; Jacobson & Gordon, 1991; Kohn, *et al*., 1991 and Stenlid, 1985). There is high genetic diversity in *S. sclerotiorum* populations, as determined by mycelia compatibility groups (MCGs) and high variation in six pathogenicity-related factors between isolates and MGCs, in respective of their region of origin (Li *et al*., 2008 and Litholdo Júnior *et al*., 2011). Based on mycelia compatibility, four types of mycelia compatibility reactions were observed among the 35 isolates tested. They are referred as gap, lin-gap, barrage, and compatible reaction types, and these results agreed with that of Deng *et al.* (2002).

Consequently the results of the present study revealed a wide variation among isolates of *S. sclerotiorum.* Since the sexual stage of *S. sclerotiorum* may be rare in nature and its role in the life cycle of the fungus is unknown, genetic exchange in mycelia of *S. sclerotiorum* isolates are largely thought to be limited to mycelia compatibility (Nalim, *et al*., 1995). However, consistent production of the teleomorph stage in isolates of *S. sclerotiorum* on PDA medium may strengthen the claim that genetic exchange may occur through normal genetic recombination, *i.e.* meiosis. The absence of teleomorph stage in most of the isolates may be because they have lost the ability to produce ascospores during the course of evolution or they require specific conditions. However, According to Nalim, *et al*., (1995) nuclear exchange through anastomosis of hyphae may be responsible for normal genetic recombination in this fungus. The high rate of antagonistic reaction in the mycelia compatibility test further shows the extent of the diversity among these isolates of *S. sclerotiorum*. The death of mycelia at the interaction zone is attributed to the heterokaryotic condition of the nuclei (Punja 1985), but the involvement of toxin(s) cannot be ruled out (Punja, 1985).

Conclusively, populations of *S. sclerotiorum* from four localities in EL-Behera governorate were a heterogeneous mix of mycelial compatibility groups (MCGs). This collaborates reports of *S. sclerotiorum* MCGs population diversity 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), soybean in Argentina (Durman, *et al.,* 2001) and Canada (Hambleton, *et al*., 2002) and oilseed rape in the north of Iran (Barari, *et al*., 2011). The population occurrence and variation of *S. sclerotiorum*, based on MCGs, appears similar irrespective to host crop and field location. This is the first report demonstrating such morphological variation within population of *S. sclerotiorum* in EL-Behera governorate. The understanding of variation and characteristics of the basal and pod rot pathogen *S. sclerotiorum* is vital approach for a sustainable and long lasting management.

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المجاميع الميسليوميه المتوافقه والاختلافات المظهريه بين عزلات الفطر *سكليروتينيا سكليروتيورم* المسبب لمرض عفن الساق والقرن في الفاصوليا

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تم دراسه الأختلافات المظهريه بين 35 عزله من *سكليروتينيا سكليروتيورم* المسبب لمرض عفن الساق والقرن للفاصوليا والتى تم عزلها من أماكن مختلفه بمحافظه البحيره.

أظهرت الدراسة أن العزلات اختلفت فيما بينها في شكل المستعمرات الفطريه, معدل النمو القطري, عدد الأجسام كما تم دراسه تفاعل التوافق الميسليومي بين العزلات.

وقد لوحظ اربعه أنواع من تفاعلات التوافق الميسليومي وكانت محصله عدد التفاعلات بين العزلات هى 594. وقد وجد من ال 594 تفاعل اكثر من النصف فى حاله عدم توافق مابين العزلات المختبره. وبناء علي المجاميع الميسليوميه المتوافقه تم تحديد المجاميع غير المتوافقه فيما بين العزلات المختبره, فوجد من ال م569 تفاعل: 307 تفاعل متباعد, 205تفاعل شبه متباعد بينما57 تفاعل متراكب . بينما أظهرت النتائج أن 25 تفاعل فقط كانت متوافقه من مجموع التفاعلات الناتجه.