Mycelial Compatible Groups of the *Sclerotium rolfsii* Isolates and Comparison of Morphological and Pathogenic Characters*

İpek YAŞAR[1] Figen MERT[1]

[1]Çanakkale Onsekiz Mart University, Agricultural Faculty, Plant Protection Dept. Çanakkale, Turkey
Corresponding author: F. Mert, E-mail address: fturk@comu.edu.tr

Received: 18 November 2018 Accepted for publication: 30 December 2019

ABSTRACT

Sclerotium rolfsii Sacc. is a polifag soil-borne fungal pathogen. This research aims to find out the mycelial compatible groups (MCG) of *S. rolfsii*, the differences in pathogenicity and morphological characters. All isolates collected from diseased green beans in Çanakkale during September, 2015. Twenty seven isolates in total were paired with each other in potato dextrose agar (PDA) to determine mycelial compatible groups (MCG). Pairings were scored as mycelial compatible if sclerotia formed in the interaction zone, however pairings were scored as mycelial incompatible if a barrier occurred. Three MCGs were determined. There were statistical differences in means of mycelial growth, the number and diameter of sclerotia produced. The correlation analysis exhibited that there was a negative correlation between sclerotium number-mycelial growth and sclerotium number and sclerotium diameter; however there have been positive correlation between mycelial growth and sclerotium diameters. All isolates were found to be highly virulent on tested bean seedlings. This research shows that there is phenotypic variation among isolates of *S. rolfsii*.

Keywords: *Sclerotium rolfsii*, mycelial compatible groups, sclerotium, virulence

ÖZ

Sclerotium rolfsii İzolatlarının Miselyal Uyum Gruplarının Saptanması ve Morfolojik ve Patojenik Olarak Karşılaştırılması

Sclerotium rolfsii Sacc. polifag toprak kökenli fungal etmendir. Bu çalışmanın amacı *S. rolfsii* izolatları içinde miselyal uyum gruplarının (MUG), patojenisitelerinin ve morfolojik farklılıklarının saptanmasıdır. Tüm izolatlar Çanakkale'de fasulye üretim alanlarında, Eylül, 2015 tarihinde toplanmıştır. İzolatlar arasında MUG gruplarının saptanabilmesi için toplam 27 izolat patates dekstroz agar (PDA) ortamında eşleştirilmiştir. PDA üzerinde interaksiyon noktasında birleşme olur ve sklerot oluşumu gözlenirse etmen uyumlu, eğer çarpışma noktasında bariyer oluşursa uyumsuz olarak tanımlanmıştır. Eşleştirme sonucunda 3 MUG saptanmıştır. Misel gelişimi, sklerot sayısı ile sklerot büyüklüğü açısından izolatlar arasında istatistiki olarak farklılıklar olduğu saptanmıştır. Yapılan korelasyon analizi sonucunda sklerot sayısı - misel büyümesi ve sklerot sayısı - sklerot çapı arasında negatif; misel büyümesi ile sklerot çapı arasında pozitif bir ilişki bulunmuştur. Tüm izolatların fasulyede yüksek oranda virulent olduğu saptanmıştır. Bu çalışmada *S. rolfsii* izolatları arasında fenotipik farklılıkların olduğu saptanmıştır.

Anahtar Kelimeler: *Sclerotium rolfsii*, miselyal uyum grupları, sklerot, patojenisite

* This study is master science thesis. This study was presented at AGROSYM 2016 congress (2016) and its summary was published in the proceedings book.

INTRODUCTION

Sclerotium rolfsii Sacc. (Telemorf: *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr.) is a well-known soil inhabiting fungus that causes severe diseases on more than 500 plant species in tropical and subtropical regions (Aycock, 1966; Punja, 1985). The disease causes significant loss in many crops however; the symptom production is variable on different crops. The disease is usually seen in patches in the field and the spread to adjacent plants in row can be rapid under favorable conditions. The pathogen can cause serious losses in many crops. For example, the Southearn blight caused by *S. rolfsii* in peanut production in United States (US) resulted 25% yield losses. Annual economic losses caused by *S. rolfsii* in peanuts were estimated to be around 36.8 million dollars in Georgia between 1988-1994 (Franke *et al.*, 1998). The fungus is also an important fungal disease in Turkey and distributed especially in vegetable and peanut cultivation areas (Biçici *et al*., 1994; Erkılıç *et al*., 2006).

The fungus shows characteristic white, radiating abundant mycelial growth on potato dextrose agar (PDA). Large numbers of sclerotial initials occurs on the mycelial mat. Initially sclerotia exhibited white color turning to dark brown. Sclerotia were superficial on the mycelial mat, spherical or ellipsoidal and measured 0.5 mm to 2.5 mm in diameter (Punja and Rahe, 1992). The fungus overwinters as mycelium and/or sclerotia in infected tissues or plant debris however; sclerotia serve as the principal over wintering structure and primary inoculum for disease (Punja, 1985).

There is genetic variation within a fungal population. Although sexual reproduction plays a key role in heterogenousity, genetic diversity can also occurs through heterokaryon formations. Heterokaryon formation between the two fungal strains is common in Ascomycota and Basidiomycota and defined as ‘sexual’ or ‘vegetative’ compatibility (Leslie, 1993). Vegetative compatibility of the hyphae of two isolates combines specific *vic* (vegetative incompatibility) or *het* (heterokaryon incompatibility) genes and allows the formation of stable heterokaryons (Saup, 2000). Vegetative incompatibility results rapid death of the cells of fused hyphae.

Isolates located in the same vegetative compatible groups (VCG) allows the sharing of genetic information during parasexual cycle (Leslie, 1993). The mycelial compatibility refers to the formation of a colony, which united the two isolates of the same species; this phenomenon has not been observed between incompatible isolates. Also mycelial compatibility groups (MCG) are often used to detect differences in the populations of *Sclerotinia sclerotiorum* and some other fungi. The isolates of *S. sclerotiorum* in the same MCG are considered to have similar genetic background (Kohn *et al.*, 1991; Xie *et al.*, 2014).

The purpose of this study was to collect *S. rolfsii* isolates from diseased bean plants, to find out the mycelial compatible groups within the population and to understand the variability in sclerotium formation and diameter of sclerotia.

MATERIALS and METHODS

Sample Collection and Isolation of the Pathogen

Infected bean plants from bean growing areas in Çanakkale, Turkey were the main material in this study; therefore thirty fields were surveyed for the disease. Diseased plants showed crown rot and foliar chlorosis. Plants collapsed as disease progressed. In the presence of abundant moisture, white mycelium developed on the infected tissue along with light to dark brown sclerotia (3.0 to 4.8 mm in diameter). The bean plants showing disease symptoms were collected during September 2015.

Infected bean plants is first cut in the size of 7−8 mm and washed in tap water. Newly infected lesions or a single sclerotium were selected for isolation. The pieces were first surface-sterilized in sodium hypochloride solution (%1) for 1−2 min. then rinsed several times before putting in petri dishes (9 cm) containing potato dexrose agar (PDA, Merck, Germany). Then petri dishes were incubated at 25°C for 3−4 days. A disk was taken from the tip of freshly growing colonies of *S. rolfsii* using a cork borer then transferred onto a new PDA.

Determination of Mycelial Compatibility Groups

A total of 27 isolates were collected in order to determine mycelial compatible groups within the population. All isolates were paired in all combinations (Punja and Grogan, 1983). Isolates were also paired with themselves. Two disks from each isolates were put 3−4 cm apart onto PDA containing red food dye (Super Cook, UK) and incubated in a dark incubator at 27°C (Kohn *et al*., 1991). If colonies of two isolates formed a barrier with red line, then assumed incompatible. If there was a red line and sclerotium formation at the contact of the two colonies, then we noted that two isolates are compatible with each other (Punja and Grogan, 1983).

Morphological Characterization of *S. rolfsii* Isolates

In order to determine whether there were differences in colony growth among the isolates, mycelial disks were taken from the freshly growing fungal culture and transferred onto PDA medium and allowed to grow on the 25°C incubator. The colony diameter was measured using a ruler two days after incubation. In this study, three replicates used for each isolate (Punja and Damiani 1996).

After measurement of the colonies, the petri dishes were transferred into an incubator once again for two weeks in order to induce sclerotium formation. The lids of the petri dishes were opened after two weeks incubation and let to dry for two more weeks. The number of sclerotia in each Petri was noted and the diameter of 20 sclerotia selected randomly were measured at 10 × 10 magnification in a microscope (Olympus) equipped with ocular micrometer (Punja and Damiani 1996).

Pathogenicity Tests

In order to find out whether the isolates collected from the diseased bean tissues were virulent, we conducted this part of the experiment. Randomly selected total seven isolates were used in this experiment. Oat seeds coated with mycelial mass were used as inoculum. One hundred grams of seeds were moistened in 50 ml water and autoclaved in 1 L flasks for 60 min at 121°C. The sterilized seeds were then infested using 10 mycelial discs, 5 mm in diameter, from the growing edge of a *S. rolfsii* colony on PDA dishes and incubated at 25 ± 1°C in the dark for 2 weeks. Three pots containing five seedlings for each isolate were used in the experiment.

Bean seedlings cv Gino were inoculated at two-leaf stage. For inoculation, two selected oat seeds heavily and homogenously colonized with mycelium of an isolate were buried at a depth of 0.5 cm and a distance of 0.5−1 cm from the plant stem. Plants in pots with non-infested seeds served as controls. Plants were incubated in a walk-in growth chamber adjusted to 28 ± 1°C with a 14-h photoperiod of fluorescent light and 60−90% relative humidity for 18 days. Disease reaction was characterized as disease incidence (DI), established as the number of dead plants at the end of the experiment, 7 days after inoculation (dai). *S. rolfsii* isolates were classified in terms of virulence based on the DI that they induced: highly virulent (DI ‡66%), moderately virulent (66 < DI ‡33%), mildly virulent (33 < DI > 0%) and non-pathogenic (DI = 0%) (Remesal *et al*., 2012).

Statistical Analysis

All data obtained from the trials were analyzed using SAS statistical software package V8 Technical Analysis of Variance (ANOVA) (SAS Inst., 1999). LSD (Least Significant Differences) was used for each feature in order to identify the differences between the means. Correlation test (Pearson’s correlation test) was conducted to determine the existing relationships among the experimental features such as mycelial diameter, the number and diameter of sclerotium.

RESULTS and DISCUSSION

Mycelial Compatibility Groups

A total of 27 isolates were collected from the diseased bean plants. Three MCGs were identified among the isolates of *S. rolfsii* tested in the study. Mycelia compatibility among isolates of a MCG was characterized by their interaction zone as described in materials and methods section. There was a red line barrier and thinned mycelia at

interaction zone if two isolates were incompatible; however mycelia of two isolates grew and sclerotia were formed at interaction zone when two compatible isolates were paired (Figure 1).

MCGs were designated in numerical order from 1 to 3. All 27 isolates were compatible with themselves, showing a reaction similar to that described for compatible. The selfing and pairing experiments were replicated twice and both experiments produced identical results.

There were three MUGs within the population. MCG1 contained 15 isolates and was the most prevalent among the population representing 55.5% of the isolates. MCG2 and MCG3 contained 11 (40.74%) and one isolates, respectively. MUG3 was self-compatible, however it was not compatible with any other isolates tested (Table 1).

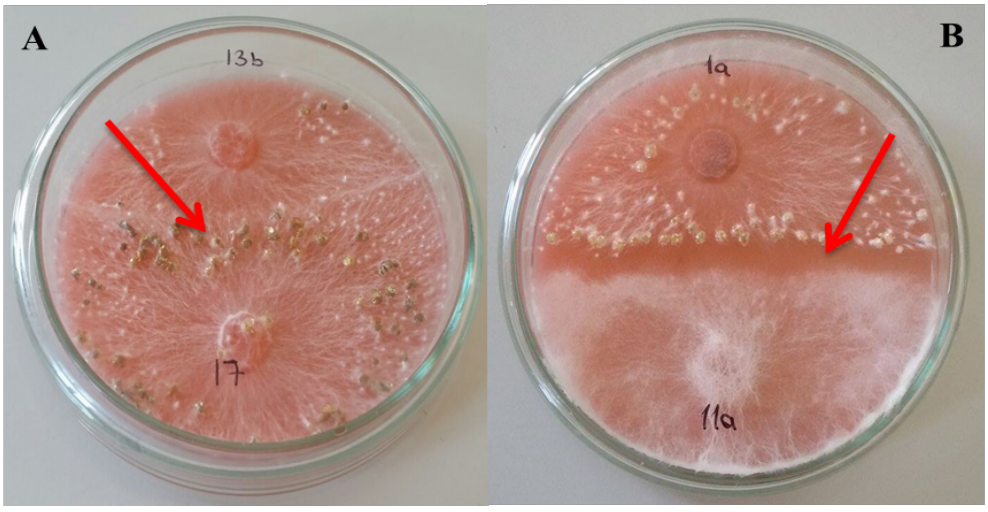

Figure 1. Mycelial interactions between isolates of *Sclerotium rolfsii*, (A) compatible reactions (same MCG) and (B) incompatible reactions. Arrows indicate interaction zone of pared isolates

Determination of Colony Diameter, Sclerotia Number and Sclerotial Diameter

The isolates of *S. rolfsii* produced the typical silky-white mycelium and the brown or dark brown sclerotia on PDA medium. Considerable variation was observed in growth rate, the number and size of sclerotia.

Colony diameters of the isolates were differed statistically among the isolates (Table 1). Thirteen isolates showed maximum mycelial growth on PDA after 2 days of incubation period varied between 53.7 to 64.7 mm. Five isolates exhibited the least mycelial growth differing between 31.6−36.3 mm. The average mycelial growth of all 27 isolates was 51.9 mm. The mycelial growth exhibited variation among the replication so growth speed of the isolates may not predict variations truly among isolates.

The mean number of sclerotia produced per petri dishes varied greatly among the isolates. It ranged from 26.7 to 479 averaging 169.91 per dish. The isolates within MCG1 produced consistently similar number of sclerotia. The number of sclerotia produced by the isolates within MCG2 differed from 147.3 to 479.7 and these differences were statistically different. The isolate Sr10a was the unique isolate in MCG3 and produced 277 sclerotia per dish; although the number of sclerotia produced by the isolate similar to the isolates belonging to MUG2, it is statistically different from the MCG1.

The diameter of sclerotia produced on PDA medium was also statistically different. Although the isolates belonged to MCG2 consistently produced similar size of sclerotia, the isolates within MCG1 varied greatly.

A correlation analysis was performed among the data obtained from the experiment (Table 2). The correlation analysis exhibited that the isolates growing faster on culture media produced less but larger sclerotia. Sclerotia diameter of isolates also correlated negatively with the number of sclerotia produced on PDA dishes.

Table 1. The mycelial compatible groups (MCGs), the radial growth, the number and diameter of sclerotia produced by the isolates of *Sclerotium rolfsii*

Isolates	MCG	CD[1] (mm)	NS[2]	DS[3] (mm)
Sr1a	MCG1	52.0 b-f[4]	28.3 d	1.6 a-c
Sr1a-1	MCG1	58.7 a-d	32.3 d	1.8 a-c
Sr1b-1	MCG1	63.3 ab	44 d	1.6 a-c
Sr1c-2	MCG1	55 a-e	39.3 d	0.8 d
Sr1d	MCG1	56.7 a-e	42.3 d	0.8 d
Sr2a	MCG1	53.7 a-e	68.7 d	0.8 d
Sr2d	MCG1	64.7 a	67 d	0.8 d
Sr2e	MCG1	59 a-c	29.7 d	0.8 d
Sr10b	MCG1	63.3 ab	63.3 d	1.5 bc
Sr11b-2	MCG1	61 ab	41.7 d	1.7 a-c
Sr12	MCG1	58.7 a-d	26.7 d	1.8 a-c
Sr13a	MCG1	52 b-f	49.7 d	1.6 a-c
Sr13b	MCG1	61 ab	35.3 d	1.9 a
Sr17	MCG1	54.7 a-e	54.7 d	1.8 ab
Sr24	MCG1	59.3 a-c	55 d	1.5 a-c
Sr1b-2	MCG2	46.7 e-ı	345 a-c	0.8 d
Sr1c-1	MCG2	47 e-ı	479.7 a	0.8 d
Sr11a	MCG2	36.3 ı-j	436.3 ab	0.8 d
Sr11b-1	MCG2	47.3 d-ı	338 a-c	0.8 d
Sr11c	MCG2	37.7 h-j	327.3 a-c	0.8 d
Sr11d	MCG2	42 f-j	147.3 cd	0.8 d
Sr11d-1	MCG2	49 c-h	412.7 ab	0.8 d
Sr11e	MCG2	39.3 g-j	383.3 ab	0.8 d
Sr16a	MCG2	49.3 c-g	154.3 cd	0.8 d
Sr16b	MCG2	46.7 e-ı	296.3 a-c	0.9 d
Sr18	MCG2	31.6 j	312.3 a-c	0.8 d
Sr10a	MCG3	54.7 a-e	277 bc	0.9 d
Mean		51.9	169.91	1.28
Rep		58.68**	7067.5	0.02
Isolates		238.03***	73803.4***	0.59***
Standard error		12.74	4021.6	0.014

[1] The numbers represent the colony diameters (CD) of the isolates on PDA medium 2 days post-transfer; [2]Mean values of the number of sclerotia (NS) produced per plate; [3] Mean values of the diameters of sclerotia(DS); [**] Mean values within a column followed by the same letter are not significantly different at the $p < 0.05$ level. ***Significant at $p < 0.001$

It was reported that mycelial compatibility may help to get insight into the genetic relatedness of isolates for some fungi or to identify intraspecific variation within field populations of plant pathogens. When two isolates confronted with each other a barrage zone between the colonies places these isolates in a different MCGs. Kohn *et al*. (1991) suggested that isolates in the same MCGs may be clonal and generally show a higher degree of genetic relatedness than isolates from different MCGs. Based on our findings 3 MCGs were identified out of 27 isolates of *S. rolfsii* collected from the diseased bean plants in Çanakkale. In line with our results. Punja and Grogan (1983) reported that 25 MCGs were detected from the *S. rolfsii* population consisted of 72 isolates. Similar results were obtained from another report by Harlton *et al*. (1995). They collected 119 *S. sclerotiorum* isolates from diseased plants worldwide and found 49 MCGs. MCGs were detected from diverse crop species in many other countries suggesting that *S. rolfsii* populations differ in morphological and genetically basis. Even though only 27 isolates used in this experiment, which were collected from a limited area. We found three MCGs.

Table 2. Coefficient of correlation (r) for colony diameter. sclerotium number and sclerotium diameter in the 27 *Sclerotium rolfsii* isolates (P ≤ 0.001***)

	Colony diameter	Sclerotium number
Sclerotium number	-0.68512***	
Sclerotium diameter	0.72399***	-0.84891***

In our present research, we aimed to compare the size of sclerotium among the isolates. We found that isolates differed in means of sclerotium size measuring 0.8−1.9 mm in diameter. There are different records relating to sclerotium size of *S. rolfsii*. Punja and Damiani (1996) recorded that when *S. rolfsii* isolates were incubated at 20°C sclerotia size ranged between of 0.2−1 mm. Similar research conducted by Kown and Park (2002); in order to work the fungus morphological characteristics. They incubated the isolates at 30°C. They reported that the fungus produced sclerotia measuring between 1−3.2 mm.

The number of sclerotia produced by isolates was also statistically different referring to 26.7−479.7 sclerotia per plate in average. These results confirm and extend the data obtained in previous studies on *S. rolfsii* isolates from other crops and geographic locations (Punja and Damiani, 1996; Kown and Park, 2002; Kokub *et al*., 2007). We also made a correlation analysis among the characters searched. The isolates produced more sclerotia produced smaller sized sclerotia or *vice versa*. The isolates grew faster on PDA tended to produce fewer amount but larger sclerotia.

Virulence of *S. rolfsii* Isolates

All isolates tested were pathogenic and highly virulent on bean seedlings (Table 3). The initial symptom was pale and wilted leaves then complete collapse and death of plants. Most of plants inoculated with the isolates of *S. rolfsii* collapsed completely by the end of a week.

The mean disease incidence (DI) of dead bean plants ranged from 66.67% to 100%. Among the isolates. the highest DI (100%) disease was observed in all isolates belonged to MCG1 and MCG3. however the lowest DI was in the isolate Sr18 (MCG2). Although DI was less in the isolates belonging to MCG2. the isolates were still very virulent. There was no disease incidence in non-inoculated control bean plants.

Table 3. Disease incidense (DI) of the isolates of *S. rolfsii* belonging to different MCGs 7 days after inoculation (dai)

Isolate no	MCGS	DI (%)[a]	Reaction
Sr2d	MCG1	100b	Highly virulent
Sr13a	MCG1	100	Highly virulent
Sr17	MCG1	100	Highly virulent
Sr11b-1	MCG2	94.44	Highly virulent
Sr16b	MCG2	94.44	Highly virulent
Sr18	MCG2	66.67	Highly virulent
Sr10a	MCG3	100	Highly virulent
Control	-	0	-

[a] Highly virulent (DI ‡66%), moderately virulent(66 < DI ‡33%), mildly virulent (33 < DI > 0%) and non-pathogenic (DI = 0%).

All isolates used in this research were highly virulent on green bean cultivar. Most of the inoculated plants collapsed within a week of inoculation with the isolates of *S. rolfsii*. We did not searched the virulence on different crop plants; however some other research suggest that there is a variation in the population of the fungus in means of virulence at different crop plants (Yaqub and Shahzad, 2005; Remesal *et al*., 2012).

Phenotypic variability with variations in mycelial growth rates and the numbers and sizes of sclerotia were observed among *S. rolfsii* isolates in the present study. Establishing the MCG population structure and virulence variability among *S. rolfsii* isolates may help in the management of the diseases. Further research on morphological and molecular studies is essential for characterizing *S. rolfsii* isolates from different crop plants and geographical locations in order to see insight of the pathogen's population diversity.

LITERATURE CITED

Aycock, R. 1966. Stem Rot and Other Diseases Caused by *Sclerotium rolfsii* or The Status of Rolf's Fungus After 70 Years. North Carolina State University Agricultural Experiment Station. Tech. Bull. 174.

Biçici, M., Çınar, Ö. And Erkiliç, A. 1994. The control of stem rot caused by *Sclerotium rolfsii* Sacc on peanut by cultural, chemical, physical and biological methods. Tr J. Agric. For. 18: 423-435.

Erkılıç A., Güven, B. and Akgün, S.D. 2006. Effects of Some Plant Activators and Plant Materials on Stem Rot Disease of Peanut and Pepper Caused by Sclerotium rolfsii. The journal of Turkish Phytopathol, 34; 15-28.

Franke M., Brenneman, T., Stevenson, K. and Padgett. G. 1998. Sensitivity of İsolates of *Sclerotium rolfsii* from Peanut in Georgia to Selected Fungicides. Plant Dis. 82: 578-583.

Harlton, C.E., Levesque, C.A. and Punja, Z.K. 1995. Genetic Diversity in *Sclerotium* (*Athelia*) *rolfsii* and Related Species. Phytopathology. 85: 1269-1281.

Kohn, L.M., Stasoviski, E,. Carbone, I., Royers, J. and Anderson, J.B. 1991. Mycelial Incompatibility and Molecular Markers Identify Genetic Variability in Field Populations of *Sclerotinia sclerotiorum*. Phytopathology. 81: 480-485.

Kokub D., Azam, F., Hassan, A., Ansar, M., Asad, M.J. and Khanum, A. 2007. Comparative Growth. Morphological and Molecular Characterization of Indigenous *Sclerotium rolfsii* Strains Isolated from Different Locations of Pakistan. Pak. J. Bot.. 39(5): 1849-1866.

Kown, J.H. and Park, C.S. 2002. Stem Rot of Tomato Caused by *Sclerotium rolfsii* in Korea. Mycobiology. 30(4): 244-246.

Leslie, J.F. 1993. Fungal Vegetative Compatibility. Annu Rev Phytopathol. 31: 127-150.

Punja, Z.K. and Grogan, R. G. 1983. Hyphal Interactions and Antagonism among Field Isolates and Single-Basidiospore Strains of *Athelia (Sclerotium) rolfsii*. Phytopathology. 73: 1279-1284.

Punja, Z.K. 1985. The Biology. Ecology. and Control of *Sclerotium rolfsii*. Annu Rev of Phytopathol. 23: 97-127.

Punja, Z. K. and J. E. Rahe. 1992. *Sclerotium. In: Singleton. LL. Mihail JD. Rush CM. eds. Methods for research on soilborne phytopathogenic fungi.* St. Paul: APS Press. 166–170 pp.

Punja, Z.K. and Damiani, A. 1996. Comparative Growth. Morphology. and Physiology of three *Sclerotium* species. Mycologia. 88: 694-706.

Remesal, E. R., Jordán-Ramírez, R.M., Jiménez-Díaz, J. and NavasCortés, A. 2012. Mycelial Compatibility Groups and Pathogenic Diversity in *Sclerotium rolfsii* Populations From Sugar Beet Crops in Mediterranean-Type Climate Regions. Plant Pathol. 61: 739-753.

Saupe, S.J., 2000. Molecular Genetics of Heterokaryon Incompatibility in Filamentous Ascomycetes. Microbiol Mol Biol Rev. 64: 489-502.

Xie, C., Huang, C.H., and Vallad, G. E. 2014. Mycelial compatibility and pathogenic diversity among *Sclerotium rolfsii* isolates in the southern United States. Plant Dis. 98:1685-1694.

Yaqub, F. and Shahzad, S. 2005. Pathogencity of *Sclerotium rolfsii* on Different Crops and Effect of inoculum Density on Colonization Of Mungbean and Sunflower Roots. Pak. J. Bot.. 37(1): 175-180.