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# Cultural and Morphological Characteristics of Different Isolates of *Sclerotium rolfsii* Sacc. in Potato Dextrose Agar Medium

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**Abstract**

Ten different isolates of *Sclerotium rolfsii* Sacc. were isolated from different sources and their cultural characteristics like mycelial growth, patterns of sclerotial distribution, day's of appearance of sclerotials initials as well as there maturity, size and shape of sclerotia and other morphological characters of fungal hyphae were observed. Mycelial aversion of all different isolates in all possible combination revealed that there are eight clearly distinguishable strains. The result of the present study also confirms ample variation among different isolates of *S. rolfsii.*

**Key words : *Sclerotium rolfsii* Sacc., Cultural and morphological characteristics, Mycelial aversion, Sclerotia.**

The fungus *Sclerotium rolfsii* Sacc. is a pathogen of more than 500 different hosts. In nature ample variation exists in respect with different parameters (like growth and reproduction) within a same species of a pathogen. To study these parameters in respect of the pathogen *S. rolfsii,* the present study was undertaken *in vitro* on semi-synthetic medium potato dextrose agar (PDA).

## Methods

Different isolates of *S. rolfsii* were isolated from different sources as listed in Table 1. Samples were collected from different host plants of different area while one isolate was also collected from field soil. Further sub-culturing was carried out by taking 6 mm discs of the isolates from periphery of 4-day old culture throughout the experimental period. Cultural and morphological characteristics like rate of mycelial radial growth (mm or cm/day), growth pattern, day of appearance of sclerotial initials, day of maturity of sclerotia, size of sclerotia, pattern of sclerotia development, distribution on petri plates, color, growth pattern of mycelium and measurement of fungal mycelium were observed.

### *Study of Mycelial Aversion*

This experiment was carried out to see the compatibility among different isolates to fuse as indicated by non-aversion or different degree of aversion. For the purpose 5 mm discs of the isolates were cut from periphery of 5-day old cultures of the isolates grown on PDA in petridishes by means of sterilized disc cutter and 2 to 3 mycelial discs in different combinations of isolates were placed in sterilized petridishes containing 20 ml sterilized PDA at two or three opposite points near the periphery. Three replications were taken for each combination. The petriplates were kept in an incubator at 28 ± 1C for 10 days after which the reaction of three different isolates at the point of contact (free mixing of hyphae or any aversion) was noted. The degree of aversion among different isolates was measured according to the following scales : 0 = No aversion ; 1 = Slight aversion (antagonism zone up to 1 mm) ; 2 = Moderate aversion (antagonism zone up to 1.1 to 2.0 mm) ; 3 = High aversion (antagonism zone up to 2.1 to 3.0 mm) ; 4 = Strong aversion (antagonism zone more than 3.0 mm).

## Results and Discussion

### *Morphological Characteristics of the Colony*

The general morphological features of the isolates grown on PDA showed considerable variations which are given below.

*Isolate 1.* Sparse white cottony growth with evenly pattern of sclerotial distribution was observed.

**Table 1.** Isolates of *Sclerotium rolfsii* isolated from different sources.

| Isolate no. | Source |
|---|---|
| 1. | Soil |
| 2. | Chili (*Capsicum annum* L.) |
| 3. | Elephant Foot yam (*Amorphophallus* sp.) |
| 4. | Tuberose *(Polyanthus tuberosa)* |
| 5. | Brinjal *(Solanum melongena)* |
| 6. | Tomato *(Lycopersicon esculentum)* |
| 7. | Barley (*Horderum vulgarae* L.) |
| 8. | Cumin *(Cuminum cyminum)* |
| 9. | Potato *(Solanum tuberosum)* |
| 10. | Groundnut (*Arachis hypogaea* L.) |

Sclerotial initials starts appearing from day 6 onwards which becomes matured within 10 to 11 days.

*Isolate 2.* Moderate white wooly growth of mycelium with evenly distributed sclerotia all around the petriplates but more on the periphery. Sclerotial initials starts appearing from day 11 onwards. Days of maturity of sclerotia differ from 13 to 20 days.

*Isolate 3.* Heavy white wooly growth with sclerotia distributed on peripheral side. Days of appearance of sclerotial initials was day 13, while its maturity generally starts from day 20 onwards.

*Isolate 4.* Heavy white cottony growth of mycelium followed by evenly distribution of sclerotia was observed. Days of appearance of sclerotial initials vary from 4 to 7 days. Sclerotia got matured within 20 days.

*Isolate 5.* Dense white cottony growth with maximum numbers of sclerotia concentrated towards periphery ; few sclerotia were also observed near center of petriplate. Sclerotial initials were appeared within 4 to 6 days and got matured within 8 days.

*Isolate 6.* Wooly white growth of mycelium with maximum numbers of sclerotia concentrated towards periphery, some were also observed near center of the petriplate. Within 4 to 5 days sclerotial initials appears which got mature within 6 to 7 days.

*Isotate 7.* White, cottony growth, sclerotia evenly distributed throughout the petriplate, sclerotial initials appears within 4 to 5 days and starts maturing within 6 to 7 days.

*Isolate 8.* White cottony growth not too dense with numerous sclerotia evenly distributed throughout the petriplate. Sclerotia appear within 4 days and got matured within 5 to 6 days.

*Isolate 9.* White, cottony growth, numerous sclerotia throughout the petriplate but more towards periphery, sclerotial initials appear within 4 days and got matured within 6 to 7 days.

*Isolate 10*. Heavy white cottony growth, numerous sclerotia throughout the petriplate but more towards periphery, sclerotial initials appear within 5 to 6 days and got matured within 6 to 7 days.

### *Quantitative Characteristics of Different Isolates of S. rolfsii on PDA*

All different isolates of *S. rolfsii* were grown on PDA medium showed wide range of variation in respect with mean mycelial diameter ; mean dry myce-

**Table 2.** Quantitative characteristics of different isolates of *S. rolfsii* on PDA.

| Isolates | Mean mycelial diameter growth in hours 24 | 48 | 72 | 96 | 120 | 144 | Mean dry mycelial wt (mg) | Mean numbers of sclerotia | Hyphal thickness (micron) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2.26 | 5.56 | 9 | | | | 373.0 | 67.0 | 2.55 to 17.5 |
| 2 | 1.5 | 3.56 | 5.7 | 7.53 | 9 | | 461.0 | 19.6 | 2.55 to 17.5 |
| 3 | 1.5 | 2.86 | 4.4 | 6.23 | 7.46 | 9 | 354.6 | 23.0 | 2.55 to 17.5 |
| 4 | 1.2 | 2.6 | 4.4 | 7.5 | 9 | | 257.6 | 48.6 | 2.55 to17.5 |
| 5 | 1.26 | 3.93 | 6.93 | 9 | | | 403.3 | 47.6 | 2.55 to 17.5 |
| 6 | 1.63 | 4.56 | 7.3 | 9 | | | 404.3 | 76.3 | 2.55 to 17.5 |
| 7 | 2.1 | 5.2 | 9 | | | | 389.3 | 154 | 2.55 to 17.5 |
| 8 | 1.56 | 4.36 | 7.6 | 9 | | | 420.3 | 39.3 | 2.55 to 17.5 |
| 9 | 2.1 | 5.5 | 9 | | | | 355.6 | 73.3 | 2.55 to 17.5 |
| 10 | 1.53 | 5.06 | 7.73 | 9 | | | 473.3 | 45.6 | 2.55 to 17.5 |
| | CD (.05) | CD (.01) | SE | | | | SE = 20.8465 | SE = 8.6961 | |
| T | 0.33589 | 0.44674 | 0.16792 | | | | CD (0.05) = 43.48 | CD (0.01) = 24.74 | |
| H | 0.18398 | 0.24469 | 0.09197 | | | | CD (0.01) = 59.31 | CD (.05) = 18.13 | |
| T h | 0.58178 | 0.77377 | 0.29085 | | | | | | |

**Table 3.** Mycelial aversion of different isolates in all possible combinations (aversion zone cm). Where (+) indicates aversion and (-) indicates no aversion

| Isolates | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | 0.4 | 0.6 | 0.6 | 0.5 | 0.5 | 0.2 | 0.5 | 0.4 | 0.5 |
| 2 | + | - | 0.4 | 0.3 | 0.4 | 0.2 | 0.2 | 0.2 | 0.1 | 0.4 |
| 3 | + | + | - | 0.0 | 0.4 | 0.0 | 0.6 | 0.2 | 0.5 | 0.5 |
| 4 | + | + | - | - | 0.6 | 0.0 | 0.3 | 0.1 | 0.3 | 0.3 |
| 5 | + | + | + | + | - | 0.3 | 0.6 | 0.5 | 0.3 | 0.6 |
| 6 | + | + | - | - | + | - | 0.5 | 0.6 | 0.5 | 0.6 |
| 7 | + | + | + | + | + | + | - | 0.6 | 0.5 | 0.6 |
| 8 | + | + | + | + | + | + | + | - | 0.7 | 0.4 |
| 9 | + | + | + | + | + | + | + | + | - | + |
| 10 | + | + | + | + | + | + | + | + | + | - |

lial weight produced, mean numbers of sclerotia (Table 2). Isolates 1, 7 and 9 require 72 hours of incubation to cover the petriplate of 9 cm diameter while Isolate 3 requires 144 hours of incubation to cover the same.

Mean dry mycelial weight of Isolate 10 was highest (473.3 mg) while Isolate 4 had lowest dry mycelial weight (257.6 mg). Mean numbers of sclerotia was highest in case of Isolate 7 and found to be lowest in Isolate 2. No variation in respect of hyphal thickness (2.55 to 17.5 micron) was observed among different isolates.

*Interaction Among Different Isolates of S. rolfsii*

Results of the mycelial compatibility revealed that apart from Isolate 3, Isolate 4, and Isolate 6 all other isolates shows mild to strong aversion zone when compared for all possible combinations (Table 3), the phenomenon described earlier by Nakata (1). Where he stated that *S. rolfsii* is a group species comprising numerous biological forms, which can be distinguished by the phenomenon of aversion. Aversion was reported to occur between different strains of the fungus but not between two cultures of the same strain. When two colonies of the same strain are started at opposite side of an agar plate, the colonies grow together and sclerotia are produced usually along a line where the colonies meet. When two colonies of different strains are started and the colonies meet, the sclerotia are produced in two parallel bands leaving a narrow but distinct strip between two lines of sclerotia. But it may be also noted that the isolates belonging to same mycelial compatibility group may or may not show identical morphological and physiological character as observed by Harlton et al. (2) when same mycelial compatible group (MCG) showed different ITS-RFLP partterns.

From the results it can be revealed that different isolates isolated from different sources differ from each other in few to many characters. No two isolates were exactly similar with each other in all respects. It can be further supported by the evidence of many workers who have also reported variability in this fungus. Harlton et al. (2) observed that within a mycelial compatible group, isolates were from same host and geographic area. Okabe et al. (3) had also observed variants in southern blight fungal isolates of Japan. Ansari and Agnihotri (4) observed morphological, pathological and physiological variation among the isolates of soybean. Based on the mycelial compatibility, they found 12 races of the pathogen. Further, Sarma et al. (5) studied variability among Indian isolates of the fungus. The isolates varied in colony, morphology, mycelial growth rate, sclerotium formation, teleomorph production, sclerotial size and color. Out of the 26 isolate, only four produced the teleomorph stage. They also collected 121 isolates of *S. rolfsii* from 15 localities and seven plant species (groundnut, sunflower, soybean, beet, carrot, valeriana, and lupinus) throughout South Africa and compared them. Thirteen MCG'S were identified, some containing isolates of same host plant or geographic area, suggesting a possible relationship between MCG and host plant or locality.

Thus it can be concluded that different isolates differ from each other in respect their cultural and morphological characteristics which further confirms variability of the pathogen *S. rolfsii* Sacc.

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