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Morphological and cultural variability among the *Sclerotium rolfsii* isolates

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Abstract
Sclerotium rolfsii is a polyphagus soil inhabitant and ubiquitous facultative saprophyte. This pathogen is known to cause disease in more than 500 crops. Studies of variability within the population in a geographical region are important because these also document the changes occurring in the population. Hence different pathogenic isolates of *S. rolfsii* were obtained from different regions and crops of southern Karnataka. The morphological studies on the pathogen showed variation among different isolates. The colony diameter of all the isolates varied from 4.10 to 8.00 cm after 72 h of incubation, sclerotial number per plate ranged from 261.7 to 1048.7. However, the sclerotial colour ranged from light to dark brown, and their size varied from 1.10 to 2.10 mm with spherical to round shape. The test weight of sclerotial bodies ranged between 40.40 to 71.00 mg. Among the liquid media, ragi flour broth showed maximum dry mycelial weight (352.00 mg) whereas, sclerotial production was more in malt extract broth (247.70).

Keywords: *Sclerotium rolfsii*, Variability, Foot rot, Morphological variations

Introduction
Sclerotium rolfsii Sacc. is a well known ubiquitous soil inhabiting and most destructive soil borne fungus; this fungus, has been initially described by Rolfs (1892) [7] on tomato. The *Sclerotium rolfsii* is widely distributed and causes severe damage to more than 500 crops (Aycock, 1966) [1]. *Sclerotium rolfsii* Sacc. is predominantly distributed throughout tropical and subtropical regions where, the temperature reaches higher levels during the rainy season. *Sclerotium rolfsii* Sacc. having a saprophytic activity in soil and can survive in soil for many years by producing sclerotial bodies (Webber, 1931) [10]. These sclerotia constitute the primary inoculum of the pathogen and mainly for dispersal and survival of the fungus under adverse environmental conditions. The isolates of *Sclerotium rolfsii* cause variety of symptoms on different hosts *viz*., collar rot in chickpea, southern blight of sugar beet, foot rot of finger millet, leaf spot in *Lotus meliloti*, bud rot of *Colocasia variagata and* fruit rot in *Citrullus vulgaris* etc. (Sarma *et al*., 2002) [8].
The fungus, *Sclerotium rolfsii* having more than 500 host plants which are grown across the globe and the *Sclerotium rolfsii*, causing dreadful diseases in majority of the host plants which are grown across the semi arid tropics including Asian countries. The isolates of *Sclerotium rolfsii* are having different modes of survival under adverse climatic conditions, among them, the dormant mycelia forms into dark coloured, circular or globe shaped sclerotia, which are playing an important role in survival of the fungus under adverse climatic conditions (Webber, 1931) [10]. The sclerotia of the fungus requires minimum amount of moisture to germinate and to infect the plant through mycelium strand and the germinated mycelia enters the plant mainly through root or stem portion. Moreover, the fungus also invading the plant system as a facultative parasite (Webber, 1931) [10]. The symptomatology of the fungus, *Sclerotium rolfsii* is varying according to the host plant they infect (Sarma *et al*., 2002) [8], type and age of the plant are two major favouring factors for *Sclerotium rolfsii* fungal isolates infection (Higgins, 1927) [4].
There were several aspects on *Sclerotium rolfsii* including its cultural, morphological, molecular variability among the isolates was well documented by several workers (Harlton *et al*., 1995, Okabe *et al*., 1998) [2, 5]. The research documents mainly on the *Sclerotium rolfsii* isolates causing individual disease in single host plant are available. Hence, there is no much work has been focused to explicit the variability of the isolates causing different diseases in various crop host. In the view of the identified lacuna, the present study was focused to explicit the occurrence of the variability among the isolates of *Sclerotium rolfsii* from different crop hosts. The purpose of the present study was to understand the variability in cultural morphology, sclerotium formation, their shape, size and test weight of the sclerotial bodies.

This study also concentrates to know the better media for the growth and sclerotial production of some isolates of *S. rolfsii* collected from different hosts and geographic locations from Karnataka.

Material and methods

Collection of infected samples and isolation of the fungus

The symptoms showing foot rot or collar rot portion of host plants were collected from twelve different crops and regions. The crops includes finger millet from Bangalore, Mandya and Tumkur (3 isolates); chickpea from Bangalore and Hiriyur (2 isolates); groundnut from Bangalore (1 isolate); tomato, onion and Cyperus from Hiriyur (3 isolates); wheat from Mandya (1 isolate); soybean and field bean from Dharwad (2 isolates). The infected plant parts were subjected for standard tissue isolation.

Infected plant parts from the field were cut into several bits of 5 mm including the advancing margins of infection. The segments were surface disinfected in 0.1% sodium hypochlorite solution for 1 min and rinsed separately three times changes in sterile water to remove the traces of chemicals present on the infected portion and such bits taken out separately, dried on sterile tissue paper and transferred to sterile Petri plates containing sterilized solidified potato dextrose agar (PDA) and incubated for 7 days at 27±°C.

The initiated growth from the incubated plates was observed and separated on to other Petri dishes contain PDA and again such plates were incubated for 7 days at 27±°C and maintained them in slants, which were stored in refrigerator at 4°C and they were sub-cultured periodically once in a month for further studies

Morphological variations

The variability studies on *S. rolfsii* isolates from different host plants were studied on PDA medium. The characters on PDA and influence of liquid media on growth of *S. rolfsii* isolate. Twenty ml of molten potato dextrose agar (PDA) was poured into sterilized individual 90 mm Petri dishes. The periphery portion of individual isolate growth was taken out with the help of cork borer (approximately 5 mm in diameter) and such discs were placed at the centre of the dishes; three replications per isolate was maintained and such plates incubated at 28°C for 30 days. Dishes were observed daily for the presence of the characteristic mycelium and sclerotia. Radial growth (colony diameter, cm), colony morphology, and sclerotial characters like sclerotial initiation, total number per plate, size, shape, colour and test weight were evaluated.

The colony diameter was measured every day to a maximum of 3 days by taking two measurements at right angles. Mycelial colour, distribution of mycelium in Petri dishes, type of growth and appearance of concentric circles were also recorded on 7 days of inoculation. Diameter of 10 sclerotia/plate was measured and for test weight, 100 sclerotial weight were recorded. Morphological variation of the colonies on PDA was observed after 7 days of incubation and the sclerotial characters was taken after 30 days of incubation. The data from the replicated plates were collected and data was analyzed statistically.

Growth of *Sclerotium rolfsii* on different liquid media

The influence of liquid media on *Sclerotium rolfsii* (SrMR isolate) was studied on ten liquid media *viz.*, oat meal broth, Elliot's broth, Tochinai's broth, malt extract broth, yeast extract broth, ragi flour broth, Richards's broth, potato dextrose broth, host leaf extract broth, Czapek's Dox broth. Twenty ml of each the above broth was taken in 100ml conical flasks and sterilized at 121ºC and 1.1 kg/cm² for 20 minutes. Each treatment was replicated thrice. Five mm discs of seven day old culture from individual isolate was used for inoculation. Such flasks were then incubated at 27±1°C for 10 days. After incubation period, the grown mycelial mat was filtered through Whatman No. 42 filter paper and the mycelial mat was dried to a constant weight in an electrical oven at 60°C, cooled in desiccators and weighed immediately on an analytical electrical balance. The weight of dry mycelium was recorded and the number of sclerotial bodies was counted in each of the flasks for each isolate. The data was analyzed statistically.

Results and Discussion

Sclerotium rolfsii from infected foot/collar/stem portions of different crops was isolated and designated as per the host plant and place of their collection. The isolated fungus was identified as *Sclerotium rolfsii* based on the cultural and morphological characters as given by Punja and Rahe (1992) [6] and Sarma *et al.*, (2002) [8].

Morphological variations

The variability among the isolates of *S. rolfsii* was studied with respect to morphological characters like radial growth, colony colour, mycelial characteristics, shape and number of sclerotial bodies per plate and test weight of sclerotial bodies were recorded. Variation in colony diameter (Table 1) on potato dextrose agar was found significant after 72 h of incubation. Maximum colony diameter (8.0 cm) was noticed in Mandya isolates from finger millet and wheat followed by soybean (7.63 cm) and Cyperus (7.55 cm) isolates. However, the minimum colony diameter (4.10 cm) was noticed in tomato isolate of Hiriyur followed by Bangalore chickpea isolate (6.76 cm). There was significant interaction effect between isolates and incubation interval.

Based on the mean colony diameter among the different isolates of *S. rolfsii* were subjected for categorization into different groups at higher incubation period (72 h) was done. Among 12 isolates, 2 (SrMR and SrWT) of them were fast growers with 8.0 cm growth, 8 isolates (SrBR, SrTR, SrHC, SrHO, SrBG, SrHR, SrDS and SrDF) were medium fast growing with 7.0-7.9 cm growth. However, Two isolates (SrBC and SrHT) were slow growers recording <7.0 cm growth at the end of 72h of incubation, irrespective of the geographic location of these isolates.

The colony appearance on the solid media (Table-2), all the isolates exhibited fluffy colony growth on PDA except Mandya ragi, Hiriyur onion and Mandya wheat isolates, these isolates showed flat growth of colony, but concentric circle in colonies were present in case of three isolates *viz.*, Bangalore chickpea, Bangalore groundnut and Dharwad field bean isolates.

Differences in sclerotial bodies number and there initiation on culture media were significantly differ (Table 2). The time required for sclerotial initiation was 4th day onwards in majority of isolates. However, five days in case of two isolates (Tumkur ragi and Hiriyur onion isolates) and six days in Hiriyur tomato and Dharwad field bean isolates and Bangalore chickpea and Bangalore groundnut isolates, took 7 and 9 days respectively for sclerotial initiation. Among all the isolates, the formation of sclerotia was significantly differing with their place of origin. The peripheral region of Petri dish was mostly preferred by most of the isolates included in the study, but, isolates like Hiriyur chickpea, Bangalore

groundnut, Mandya wheat and Dharwad soybean, sclerotia production was uniformly distributed all over the plate. However, the Mandya ragi isolate produced its sclerotia at peripheral and also at the centre of the Petri dish.

With respect to shape of sclerotia (Table 2), Mandya ragi isolate showed spherical shape, Dharwad soybean and Mandya wheat isolates showed irregular shape and remaining isolates showed round shape. With regard to sclerotial colour, four types of colours (Table 2) were observed. Among the isolates Bangalore ragi, Mandya wheat, Hiriyur chickpea and tomato isolates showed brown coloured sclerotia. Dharwad soybean isolate showed light brown coloured sclerotia and the isolates Mandya ragi, Tumkur ragi, Hiriyur onion, Hiriyur Cyperus and Dharwad field bean showed dark brown coloured sclerotia whereas, Bangalore chickpea and Bangalore groundnut isolates showed chocolate brown coloured sclerotia.

The variation in size of sclerotial bodies of different isolates under study was found significant (Table 2). Bangalore groundnut isolate produced bigger sclerotial bodies (2.10 mm) followed by Dharwad soybean isolate (1.90 mm) and Hiriyur onion isolate (1.85 mm), but there was no significant difference between these isolates. Ragi isolates produce different sclerotial diameter with respect to the regions *i.e.*, Bangalore isolate produce bigger sclerotia (1.40 mm) whereas, Tumkur isolate produced smaller sclerotia (1.10 mm). Chickpea isolates of Hiriyur and Bangalore and Hiriyur Cyperus isolate produced similar sized sclerotia (1.10 mm) whereas, Mandya wheat and Dharwad field bean isolates produced 1.20 and 1.30 mm sclerotia respectively. Sulladmath *et al*. (1977) [9] and Sarma *et al.*, (2002) [8] reported the variation among the isolates of *Sclerotium rolfsii*, suggesting that, the variation among isolates depends on soil type, host crop and the environmental factors. However in the present study also as the isolates were collected from different hosts across diverse climatic regions of Karnataka the differences were obvious.

The variations among isolates were attributed mainly due to the nutritional status of culture medium (Henis *et al.*, 1965) [3]. However as the present studies were carried out on single medium (PDA), the variations in their cultural characters explicitly states that, the isolates from different agroclimatic regions and crop plants were acclimatized for their natural habitat (Higgins, 1927; Henis *et al.*, 1965) [4, 3].

Table 1: Colony characters of different isolates of *Sclerotium rolfsii*

Sl no.	Crop	Place of collection	Designation	Mean colony diameter* (cm)	Mycelial colour	Type of growth	Concentric circle	Distribution of mycelial growth
1	Ragi	Bangalore	SrBR	4.10	Pure white	Fluffy	Absent	Irregular
2	Chickpea	Bangalore	SrBC	3.73	Pure white	Fluffy	Present	Thin
3	Groundnut	Bangalore	SrBG	3.93	Pure white	Fluffy	Present	Thick
4	Ragi	Mandya	SrMR	4.67	Dull white	Flat	Absent	Irregular
5	Wheat	Mandya	SrMW	4.63	Dull white	Flat	Absent	Thin
6	Chickpea	Hiriyur	SrHC	4.07	Pure white	Fluffy	Absent	Thick
7	Onion	Hiriyur	SrHO	3.67	Cottony white	Flat	Absent	Irregular
8	Tomato	Hiriyur	SrHT	1.83	Pure white	Fluffy	Absent	Irregular
9	Cyperus	Hiriyur	SrHR	4.03	Pinkish white	Fluffy	Absent	Thick
10	Soybean	Dharwad	SrDS	4.17	Dull white	Fluffy	Absent	Irregular
11	Fieldbean	Dharwad	SrDF	4.10	Pure white	Fluffy	Present	Thin
12	Ragi	Tumkur	SrTR	4.03	Pure white	Fluffy	Absent	Thick

* = Mean of 24, 48, and 72 h after incubation

Table 2: Characters of sclerotia of *Sclerotium rolfsii* isolates

Sl no.	Isolates	Sclerotial Initiation (DAI)	Distribution	No. Per plate	Shape	Colour	Diameter (mm)	Test weight (mg)*
1	SrBR	4	All over the plate	471.30	Round	Brown	1.40	52.30
2	SrBC	7	Periphery	542.00	Round	Chocolate brown	1.10	40.40
3	SrBG	9	Irregular	261.70	Irregular	Chocolate brown	2.10	68.20
4	SrMR	4	Periphery and at centre	1048.70	Spherical	Dark brown	1.30	47.30
5	SrMW	4	All over the plate	638.70	Irregular	Brown	1.30	46.30
6	SrHC	4	All over the plate	948.30	Round	Brown	1.10	41.70
7	SrHO	5	Periphery and at centre	375.30	Round	Dark brown	1.70	61.50
8	SrHT	6	Centre	274.00	Round	Brown	1.85	71.00
9	SrHR	4	Irregular	402.00	Round	Dark brown	1.10	52.70
10	SrDS	4	All over the plate	668.30	Irregular	Light brown	1.90	63.20
11	SrDF	6	Centre	433.00	Round	Dark brown	1.20	51.30
12	SrTR	5	Periphery	535.70	Round	Dark brown	1.10	46.10

*= Weight of Hundred sclerotial bodies, DAI= Days after incubation

Growth of *Sclerotium rolfsii* on different liquid media

The average mycelial weight of the fungus was taken after 10 days of incubation. Maximum dry mycelial weight of fungus was obtained in finger millet flour broth (352 mg) and potato dextrose broth (327.90 mg) (Table 3). These were on par with one another and significantly superior over the rest of the liquid media tested, followed by host leaf extract (250.10 mg) and oat meal agar (227.10 mg). The synthetic media produce least mycelial weight compared to semi synthetic media. Among the synthetic media tested, the maximum mycelial weight of the fungus was obtained in Czapek's Dox broth (130.00 mg) which was followed by Tochinai's broth (53.60 mg) and Elliot's broth (47.00 mg) which were on par with each other and in Richards's broth the growth of the pathogen was not observed.

With respect to sclerotial production (Table 3) semi synthetic media proved better than that of synthetic media. Among the semi synthetics, malt extract broth (247.70) produced more

number of sclerotial bodies than others which was followed by ragi flour broth (235.70) and potato dextrose broth (227.30). Among the synthetic media Richards's broth does not produce any sclerotial bodies whereas, Tochinai's broth (148.70) produce more sclerotial bodies followed by Czapek's Dox broth (87.00) and Elliot's broth (51.00). Sclerotial body colour also varied in different liquid media. In most of the broth sclerotial colour is dark brown except Elliot's broth as it produced light brown coloured sclerotial body and potato dextrose broth produce light to dark coloured sclerotial body. Henis *et al.* (1965) [3] and Sulladmath *et al*. (1977) [9] have reported the sclerotial number variation in different media, suggesting that, the sclerotial number depends on nutritional factors of the media. However in the present study also variation was observed due to the nutritional factors of the media.

Table 3: Growth of SrMR isolate of *Sclerotium rolfsii* on different liquid media

Sl no.	Media	Dry mycelial weight (mg)	No. of sclerotial bodies	Sclerotial body colour
1	Oat meal broth	227.10	220.00	Dark brown
2	Elliot's broth	47.00	51.00	Light brown
3	Tochinai's broth	53.60	148.70	Dark brown
4	Malt extract broth	190.50	247.70	Dark brown
5	Yeast extract broth	111.30	133.00	Dark brown
6	Ragi flour broth	352.00	235.70	Dark brown
7	Richards's broth	0.00	0.00	-
8	Potato dextrose broth	327.90	227.30	Brown
9	Host leaf extract broth	250.10	208.30	Dark brown
10	Czapek's Dox broth	130.00	87.00	Dark brown
	SEm±	16.54	24.06	
	C.D at 1%	34.74	50.55	

By these results it was concluded that variations was there in different Sclerotium isolates and these variations depends on the host plant, geographical location of the isolate and the media composition used for testing the variations.

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