

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.603.210>

Cultural, Morphological and Pathogenic Variability of Different Isolates of *Sclerotium rolfsii* Obtained from Rice-Tomato-Rice Cropping System of Undulating Red and Lateritic Zone of West Bengal, India

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ABSTRACT

An experiment was carried out to study the cultural, morphological, and pathogenic variability of different isolates of *Sclerotium rolfsii* infecting tomato crop under the undulating red and lateritic zone of West Bengal. Cultural and morphological variability of isolates were studied based on their mycelial growth rate, colony colour, mycelial dispersion and appearance and sclerotia formation, colour, weight and number of sclerotia, arrangement and maturity days of sclerotia using potato dextrose agar medium. Pathogenic variability was studied based on pathogenicity test and host range study by soil infestation method. All the ten isolates of *S. rolfsii* expressed significant differences with respect to cultural, morphological, and pathogenic characters. In pathogenicity test, all the isolates showed their pathogenic temperament to tomato plant where as in host range study, all test crop species showed susceptible except wheat (*Triticum vulgare*) showed some extent of resistance. Three isolates were found to be very fast growing (diam. > 9 cm), three were fast growing (8-8.9 cm), three moderately fast growing (5-6 cm) and one was observed slow growing (< 3 cm). Four isolates were white, one each of extra white and cottony white, two isolates were light white and another two were dull white. The colour of sclerotia ranged from brown to dark brown, shape ranged from spherical to oval and also irregular, sclerotial weight ranged from 3.7 to 8.6 mg. Six isolates were observed scattered, two peripheral and two were reported be central in Sclerotia arrangement. Sclerotia took a range of 9 to 15 days after inoculation for maturity. A range of 154.0 to 395.0 sclerotia production per plate was reported from the tested isolates.

Keywords

Sclerotium rolfsii,
Tomato, Cultural,
Morphological,
and Pathogenic
Variability.

Article Info

Accepted:
24 February 2017
Available Online:
10 March 2017

Introduction

Sclerotium rolfsii, casual agent of collar rots disease of Tomato (*Solanum lycopersicum* L.), is a soil borne plant pathogen. It is highly polyphagous, non-target, soil inhabitant, moisture loving, sclerotia and basidia producing, ubiquitous facultative parasitic basidiomycetes fungi which produces oxalic acid, polygalacturonase and cellulose as pathogenic weapons. The fungus was first

reported by Rolfs (1892) as a cause of tomato blight from Florida in U.S.A. Saccardo (1911) named the fungus as *S. rolfsii* sp. nov. The fungus is distributed in tropical and subtropical regions of the world where high temperatures prevail. Cooper (1961) has termed *Sclerotium rolfsii* as an omnipathogenic fungus, as it possesses the ability to attack a large number of monocot and dicot plant

species, belonging to about 100 families. According to Tu (1978) and Wydra (1996), *Sclerotium rolfii* is more severe on legumes, solanaceous crops, cucurbits and other vegetables grown in rotation with beans. This is one of the most destructive and common pathogen of tomato crop causing collar-rot disease in undulating red and lateritic zone of West Bengal. There are several reports of *S. rolfii* where significant variations in morphological behavior were observed (Sharma *et al.*, 2002). Variability among *S. rolfii* populations from different geographical regions was demonstrated by several workers (Harlton *et al.*, 1995, Okabe *et al.*, 1998, Sarma *et al.*, 2002). To understand the ecology, pathogenicity, epidemiology and evolutionary potential aspects of the *S. rolfii*, it is essential to study about the cultural, morphological and pathogenic variability of the *S. rolfii* present in undulating red and lateritic zone of West Bengal. Therefore, the present study was undertaken with a view to study the morphological, cultural and pathogenic variability's among isolates of *S. rolfii* collected from tomato plants of different geographic locations.

Materials and Methods

A roving survey was carried out during 2014-15 in undulating red and lateritic zone of West Bengal which consist Birbhum, Bankura, Purulia Pachim Medinapur and some part of Burdwan district where tomato crops are grown exclusively by the farmers and collar rot infected plant were collected. These diseased materials were kept in sterilized polythene bags and brought to the laboratory for the purpose of isolation of the pathogen.

Isolation of pathogen

Sclerotium rolfii was isolated from the stems of infected tomato plants by tissue segment

method (Rangaswami and Mahadevan, 1999) on potato dextrose agar (PDA) medium. The plant specimens were washed with tap water, small pieces of tissue of about 0.5 to 1 cm from infected collar region with some healthy tissue were cut with sterile scalpel. The pieces were surface sterilized with 0.01% mercuric chloride solution for about 30 seconds and rinsed in sterilized water for three times subsequently to eliminate all the traces of mercuric chloride and then dried between folds of sterilized filter paper. After that the surface sterilized pieces were transferred onto PDA medium in Petri dishes. Plates were incubated at $27 \pm 1^\circ\text{C}$ and observed periodically for growth of the fungus. The colony of the fungal pathogens grew from the infected pieces were isolated. The fungal isolates were purified following hyphal tip technique (Tuite, 1969), identified based on its mycelia and sclerotial characters (Barnett and Hunter, 1972). Repeated culture has been done from tip of the single hypha to obtain pure culture of the identified *Sclerotium rolfii* and the pure culture was stored in the PDA slants at 10°C for further use.

Pathogenic Variability

Pathogenicity test

The pathogenicity of different isolates, collected from different regions was carried out through pot culture experiment by soil infestation method in shade condition. Inocula of the different selected isolates of *S. rolfii* were prepared on autoclaved moist wheat grains in 500 ml Erlenmeyer flask. Sterilized soil was taken in plastic pots of size 45cm x 30cm. One month old healthy tomato seedling (var- Punjab Chuhara) was transplanted singly in each pots and after few days when seedling get well established, the inocula of different isolates were incorporated in collar region at the rate of 20 g/kg soil in five selected pots separately. Pots without inoculation were

served as control for each case. Soil moisture was maintained properly by adding non-sterile filtered tap water throughout the period. The inoculated plants were observed daily. As soon as the disease symptoms were evident, the pathogen was again re-isolated to confirm the infectivity of the isolated pathogen. The plants showing typical wilting symptoms were recorded subsequently.

On the basis of wilt symptom observed of plant, isolates were graded different category. Grads were as minus (-) for no symptom and plus (+) for wilt symptom in inoculated pot. Appearance of symptom was again divided into four groups viz., up to 25 % wilt ranked as single plus (+), 25.1 to 50% ranked as double plus (++) , 50.1 to 75% were ranked triple plus (+++) and more than 75% were ranked tetra plus sign (++++) after 15 days of inoculation and reacted as slow pathogenic, moderate pathogenic, pathogenic and highly pathogenic isolates respectively.

Host range study

The host range of *Sclerotium rolfsii*, causal agents of tomato collar rot disease was studied by artificial inoculation to different plant species in pot culture experiments in *in vitro* condition. Sterilized soil was taken in plastic pots of size 45cm x 30cm. Thirty days old apparently healthy seedlings of Brinjal, Lady's finger; Chilli and Maize were transplanted in such pots. Whereas, groundnut seeds, potato tubers, elephant foot yam corms, Bitter gourd, Ridge gourd, Bean, Zinger, Onion, Gram and Berseem were sown/planted separately in five numbers of plastic pots and after 25 days of emergence of seedlings said amount of inoculums was incorporated in each pots. Pots without inoculum were served as control for each case. Soil moisture was maintained properly by adding non-sterile filtered tap water throughout the period. The plants showing typical wilting symptoms

were recorded subsequently. The pots of each host were examined regularly upto 15 days and symptoms in the plants were noted and compared with the un-inoculated plants of control pots. Finally after 15 days, the degree of infection was categorized as given in Table-1 according to the collar region lessening of the plants (Chaurasia *et al.*, 2014).

Cultural and Morphological Variability

Variability in cultural and morphological character i.e. mycelial growth rate, colony color, appearance and sclerotial color, shape, arrangement, weight, maturity and number of sclerotia production of different isolates were studied by using Potato Dextrose Agar (PDA) medium. The mycelia disc of 4 mm diameter of each isolate was taken from the actively growing cultures and centrally placed on 90 mm Petri plates containing sterilized PDA medium and inoculated at 26 ± 1 °C for 15 days. Each isolate was replicated three times. Day to day cultural and morphological characters was recorded upto 15 day of inoculation.

Results and Discussion

Pathogenic Variability

Pathogenicity test

The result of pathogenicity test of different isolates of *S. rolfsii* which were collected from different region of undulating red and lateritic zone of West Bengal is presented in Table -2. All isolates reviled some degree of pathogenic reaction to tomato seedling. Among the ten isolates three isolates i.e. SRPU-1, SRPU-2 and SRPM-1 showed highly pathogenic reaction, three isolates i.e. SRBN-2, SRBW-1 and SRPM-2 showed pathogenic reaction, three isolates i.e. SRBN-1, SRBR-2 and SRBW-2 showed moderate

pathogenic reaction and one isolate SRBR-1 showed slow pathogenic reaction with the Tomato seedlings.

Host range study

The results of host range study is presented in Table -3. Results of the host range study clearly showed that all selected crop species are susceptible to the tomato collar rot causing pathogen *Sclerotium rolfsii*. Among the fifteen crop species Brinjal (*Solanum melongena* L.), Potato (*Solanum tuberosum* L.), Chilli (*Capsicum annum*), Zinger (*Zingerber officinalis*), Onion (*Allium cepa*. L), Gram (*Cicer arietinum*) and Berseem (*Trifolium alexandrinum*) were found to be most susceptible for the disease, as maximum degree of infection have been recorded in them and these plants were completely collapsed within 15 days after inoculation. Out of them, crops like Groundnut (*Arachis hypogaea*), Lady's finger (*Abelmoschus esculentus*), Bitter gourd (*Momordica charantia* L.), Ridge gourd (*Luffa acutangula* L), Bean (*Dolichos lablab*), Maize (*Zea mays*) and Elephant foot yam (*Amorphophallus paeoniifolius*) were showed Moderate degree of susceptibility where as Wheat (*Triticum vulgare*) was showed poor degree of susceptibility against the tomato collar rot causing pathogen.

Cultural and Morphological Variability

Different cultural and morphological characters of ten isolates of *S.rolfsii* were studied based on mycelial and sclerotial parameters. The result of cultural and morphological characters study presented in table -4 and table -5. The mycelial growth rate on PDA medium was very fast in isolate SRPU-1 isolated from Raghunathpur-1 block of Purulia district, SRPU-2 isolated from Hura block of Purulia district and SRPM-1

isolated from Binpur block of Paschim Medinapur district. The isolate SRBR-1 which was isolated from Labpur block of Birbhum district showed Slow growth reaction. Three isolates i.e. SRBN-2 isolated from Gangajalghati block of Bankura district, SRBW-1 isolated from Raniganj block of Burdwan district and SRPM-2 isolated from Garbeta block of Paschim Medinapur district showed fast mycelial growth reaction on PDA medium. The isolates like SRBN-1 isolated from Chatana block of Bankura district, SRBR-2 isolated from Bolpur block of Birbhum district and SRBW-2 isolated from Pandebeswar block of Burdwan district showed Moderate type growth reaction on PDA medium.

All isolates produced light white to dull white mycelial colony on PDA medium. All isolates of *S.rolfsii* differed in their mycelia dispersion and appearance in Petri plates. All isolates showed dispersed growth all over the plate to aggregated fashion and their appearance was loose to dense cottony with sparse or fluffy mycelium. Isolates SRBR-1, SRBW-2 and SRBW-1 were produced aggregated dispersion of mycelium on PDA media (Table 5). Similar, reports were given by Rakholiya *et al.*, (2011) and Kumar *et al.*, (2014) studied cultural and morphological variability of *S.rolfsii* and reported considerable variability in mycelial and sclerotial dimensions.

Among the morphological characters sclerotial shape were varies from oval to irregular. The five numbers of isolates i.e. SRPU-1, SRPU-2, SRBR-1, SRBW-1 and SRPM-2 were produce spherical sclerotia and three numbers of isolates i.e. SRBN-1, SRBN-2 and SRBW-2 produce oval sclerotia where as isolates SRBR-2 and SRPM-1 were produced Irregular sclerotia. Sclerotia were light brown to dark brown in colour.

Table.1 Degree of infection of plants

Lesioning on basal part of plants	Degree of infection	Symbol
Healthy plant (no lesioning)	No infection	0
Initial lesioning	Poor infection	1 +
Heavy basal lesioning	Moderate infection	2 +
Collapsed plant	Highly infection	3 +

Table.2 Pathogenic variability *Sclerotium rolfsii* of isolates

S. No.	Isolate	Location	G.P.S Location	Pathogenicity
1.	SRPU-1*	Purulia, Raghunathpur-1	N 23 ⁰ 31'9.74" E 86 ⁰ 40'56.41"	++++
2.	SRPU-2	Purulia, Hura	N 23 ⁰ 17'27.41" E 86 ⁰ 37'28.74"	++++
3.	SRBN-1	Bankura, Chatana	N 23 ⁰ 18'22.17" E 86 ⁰ 58'0.74"	++
4.	SRBN-2	Bankura, Gangajalghati	N 23 ⁰ 25'17.78" E 87 ⁰ 6'39.67"	+++
5.	SRBR-1	Birbhum, Labpur	N 23 ⁰ 49'18.96" E 87 ⁰ 47'28.22"	+
6.	SRBR-2	Birbhum, Bolpur	N 23 ⁰ 39'56.14" E 87 ⁰ 37'57.21"	++
7.	SRBW-1	Burdwan, Raniganj	N 23 ⁰ 38'55.31" E 87 ⁰ 5'31.69"	+++
8.	SRBW-2	Burdwan, Pandebeswar	N 23 ⁰ 42'36.34" E 87 ⁰ 16'25.78"	++
9.	SRPM-1	Paschim Medinapur, Binpur	N 22 ⁰ 34'43.59" E 87 ⁰ 0'16.55"	++++
10	SRPM-2	Paschim Medinapur, Garbeta	N 22 ⁰ 52'18.67" E 87 ⁰ 21'39.48"	+++

*SR: *Sclerotium rolfsii*, PU: Purulia, BN: Bankura, BR: Birbhum, BW: Burdwaman, PM: Paschim Medinapur.

Table.3 Host range of *Sclerotium rolfsii* under artificial inoculation

S.No	Host	Degree of infection
1	Brinjal (<i>Solanum melongena</i> L.)	3+
2	Potato (<i>Solanum tuberosum</i> L.)	3+
3	Groundnut (<i>Arachis hypogaea</i>)	2+
4	Lady's finger (<i>Abelmoschus esculentus</i>)	2+
5	Chilli (<i>Capsicum annum</i>)	3+
6	Bitter gourd (<i>Momordica charantia</i> L.)	2+
7	Ridge gourd (<i>Luffa acutangula</i> L)	2+
8	Bean (<i>Dolichos lablab</i>)	2+
9	Zinger (<i>Zingerber officinalis</i>)	3+
10	Onion (<i>Allium cepa</i> . L)	3+
11	Maize(<i>Zea mays</i>)	2+
12	Gram (<i>Cicer arietinum</i>)	3+
13	Berseem (<i>Trifolium alexandrium</i>)	3+
14	Wheat (<i>Triticum vulgare</i>)	1+
15	Elephant foot yam (<i>Amorphophallus paeoniifolius</i>)	2+

Table.4 Growth rate of different isolates of *Sclerotium rolfsii* on PDA media

S. No.	Isolate	Location	Growth rate (cm)				Reaction
			24 hr	48 hr	72 hr	96 hr	
1	SRPU-1	Purulia, Raghunathpur-1	2.6	5.6	7.6	9	Very Fast
2	SRPU-2	Purulia, Hura	2.6	5.4	7.5	9	Very Fast
3	SRBN-1	Bankura, Chatana	1.2	3.6	4.2	5.4	Moderate
4	SRBN-2	Bankura, Gangajalghati	1.7	4.2	6.8	8.7	Fast
5	SRBR-1	Birbhum, Labpur	0.5	1.2	2	2.7	Slow
6	SRBR-2	Birbhum, Bolpur	1	2.8	4.6	5.8	Moderate
7	SRBW-1	Burdwan, Raniganj	1.5	3.8	6.4	8.6	Fast
8	SRBW-2	Burdwan, Pandebeswar	1.3	3.6	4.5	5.7	Moderate
9	SRPM-1	Paschim Medinapur, Binpur	2.8	5.8	8	9	Very Fast
10	SRPM-2	Paschim Medinapur, Garbeta	1.6	4	6	8.2	Fast
	Sem \pm		0.11	0.12	0.22	0.16	
	Cd at 1%		0.43	0.50	6.71	0.66	

Table.5 Mycelial and Sclerotial Characters of Isolates of *S.rolfsii* on PDA Media

S. No.	Isolate	Mycelial Characters			Sclerotial Characters					
		Growth Rate	Colony color	Appearance	No./plate (15 DAI)	Color	Shape	Wt (mg)	Arrangement	Maturity (DAI)
1.	SRPU-1	Very Fast	Light White	Cottony, puffy at edges, dense at margins	214.00	Light Brown	Spherical	6.7	Scattered	15
2.	SRPU-2	Very Fast	Dull White	Sparse, thin strands	320.50	Dark Brown	Spherical	8.5	Scattered	11
3.	SRBN-1	Moderate	Light White	Puffy at centre, upright growth habit	195.00	Dull Brown	Oval	5.7	Peripheral	12
4.	SRBN-2	Fast	White	Cottony, sparse	265.00	Dark Brown	Oval	6.6	Central	12
5.	SRBR-1	Slow	Extra White	Upright growth habit, cottony & puffy aggregated	154.00	Light Brown	Spherical	3.7	Peripheral	15
6.	SRBR-2	Moderate	White	Suppressed, thin strands	185.00	Light Brown	Irregular	5.4	Scattered	14
7.	SRBW-1	Fast	Dull White	Aggregated, dense cottony	212.00	Dark Brown	Spherical	6.8	Central	9
8.	SRBW-2	Moderate	Cottony White	Upright growth habit, cottony & puffy aggregated	178.00	Dull Brown	Oval	4.3	Scattered	12
9.	SRPM-1	Very Fast	White	Suppressed, thin strands	395.00	Dark Brown	Irregular	8.6	Scattered	10
10	SRPM-2	Fast	White	Suppressed, thin strands	285.00	Dark Brown	Spherical	7.9	Scattered	13

The isolates SRPU-1, SRBR-1 and SRBR-2 were produced light brown color sclerotia where as isolates SRPU-2, SRBN-2, SRBW-1, SRPM-1 and SRPM-2 produced Dark Brown sclerotia and SRBN-1, SRBW-2 were produced Dull Brown sclerotia. Six no of isolate i.e. SRPU-1, SRPU-2, SRBR-2, SRBW-2, SRPM-1 and SRPM-2 were shown Scattered sclerotial appearance where as SRBN-1 and SRBR-1 showed Peripheral and SRBN-2 and SRBW-1 showed Central sclerotial appearance on PDA medium. Sclerotial weight was range from 3.7 mg to 8.6 mg. highest sclerotial weight 8.6 mg was recorded in SRPM-1 and lowest 3.7 mg recorded in SRBR-1 isolate. The maximum number, 395.00 no/plate of sclerotia production was recorded in SRPM-1 isolate and lowest 154.00 no./ plate was recorded in SRBR-1 isolate. Sclerotial maturity time was range from 9 to 15 days after inoculation. Fasted maturity 9 DAI recorded in SRBW-1 isolate and slowest maturity 15 DAI recorded in SRPU-1 and SRBR-1 isolates. The cultural and morphological characters of *S.rolfsii* isolates tested were highly variable. Variations in sclerotial colour, shape and size and their ability to infect plants have been reported by different scientists on various hosts and media (Sharma *et al.*, 2002; Palaiah and Adiver 2006; Kumar *et al.*, 2014). Geographical variability among *S.rolfsii* populations was demonstrated by earlier workers (Harlton *et al.*, 1995; Okabe *et al.*, 1998). Studies of variability within the population in a geographical region are important because these also document the changes occurring in the population. The significant variation in culture characteristics, mycelial morphology and pathogenicity amongst test isolates indicated that *S.rolfsii* can best be characterized by a combination of culture characteristics, morphology and virulence on host plants. The differences in sclerotial forming capacity among isolates could be a useful parameter for characterizing

isolates, due to the fact that number of sclerotia formed among fungal isolates was significant (Horsfall, 1932).

In conclusion, the tomato collar rot causing pathogen *S. rolfsii* possesses wide diverse cultural, morphological and pathogenic variability which help it for wide range adaptability and survivability in diverse ecological situation and it make very difficult to manage during crop production.

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How to cite this article:

Asish Mahato and Mohan Kumar Biswas. 2017. Cultural, Morphological and Pathogenic Variability of different Isolates of *Sclerotium rolfsii* Obtained from Rice – Tomato –Rice Cropping System of Undulating Red and Lateritic Zone of West Bengal, India. *Int.J.Curr.Microbiol.App.Sci*. 6(3): 1843-1851. doi: <https://doi.org/10.20546/ijcmas.2017.603.210>