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Variabilities of *Sclerotium rolfsii* Sacc. Inciting Stem Rot Disease of Groundnut (*Arachis hypogea* L.) in North Bengal Districts of West Bengal

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A B S T R A C T

Stem rot disease (causal organism- *Sclerotium rolfsii* Sacc) is important disease of groundnut causing severe loss in production. Morphological and cultural characters of *Sclerotium rolfsii* was studied on PDA solid media in the present study. Twenty five isolates of *Sclerotium rolfsii* collected from different locations of North-Bengal districts of West Bengal from rice-based cropping system, infecting groundnut in cross inoculation, were studied on potato dextrose agar(PDA) for their cultural and morphological variability. Significant variability with reference to mycelial and sclerotial characters across isolates of *Sclerotium rolfsii*, isolated from different locations was observed. It was found that the growth rate was varied from 0.772mm/hr to 1.12 mm/hr. and size of the sclerotia was found to be in the range between 2.9mm to 7.5mm. Colony colour varied from buff white to pure white, with smooth to coarse texture and regular to irregular margin. Production of sclerotial bodies started from 5th day onwards. Sclerotial shapes were ellipsoidal to spherical with light brown to dark brown colour.

Introduction

Groundnut (*Arachis hypogea* L.) is one of the important oilseed crop grown in India, China and the United States of America and is rich in energy and contains minerals, nutrients, vitamins and antioxidants that support health. Kernel is a good source of dietary protein; they contain amino acids of fine quality that are important for growth and development. Groundnut is India's major oilseed crop, accounting for 29% of the world's area and 36% of production. During the year 2016-17, India's total area under groundnut cultivation was 5.86 million hectares and total production with productivity of 1060 kg / ha was 9.25 million metric tonnes (Anon., 2017). The major Indian groundnut growing states are Andhra Pradesh, Karnataka, Gujarat, Tamil Nadu and Maharashtra, which together account for about 80% of the production area and 81% of the production(Deepthi and Eswara Reddy, 2013). With 0.71 million tonnes produced in an area of 0.46 million hectares with a productivity of 1543 kg / ha, West Bengal ranks 9th (Anon., 2017). In

West Bengal, characterised by climate (tropical temperate) and annual precipitation (800-2000 mm), the region of North Bengal has recently gained momentum for the cultivation of groundnuts in the rice-based cropping system. Groundnut is cultivated in West Bengal as a kharif, rabi and summer crop in all three seasons. Midnapore (West), Purulia, Bankura and Birbhum are the potential districts of rainfed kharif groundnut, which accounts for 30 percent of the state 's total groundnut production area. In the districts of Midnapore (East), 24 parganas (North), Hoogly, Nadia and Murshidabad, the next 70 percent are in the rabi or summer season and dispersed (Basu and Singh, 2004, Singh, 2011).

Presently cultivated area of groundnut in West Bengal is 46 thousand ha with a production of 71 thousand tonnes and productivity of 1543kg/ha in 2014-15. Paschim Medinipur is ranked 1st out of 21 districts in West Bengal based on groundnut production. Groundnut production in Paschim Medinipur amounted to 27,141 tonnes as of 2015, representing 14.05 percent of the production of West Bengal groundnut production. It accounts for 85.95 percent of the top 6 districts are Alipurduar, Jalpaiguri, Hoogly, Puruliya, Birbhum, and Murshidabad (Anon., 2016). At various stages of development, the groundnut crop is affected by several diseases. One of the significant diseases among these diseases is stem rot from groundnut caused by *Sclerotium rolfsii*. Typical symptoms of this disease include branch yellowing and wilting, white mycelial growth in the collar region, mustard seed development such as sclerotia (Punja, 1988). About 100 families, including green bean, groundnut, lima been, onion, cabbage, pepper, tomato, sweet potato and water melon, the fungus *Sclerotium rolfsii* has a wide host range of more than 500 species (Aycock, 1966, Punja, 1988, Jogia *et al.,* 2016). Stem rot disease is a possible threat to the development of groundnut and is of significant economic importance for groundnut which cultivated under irrigated conditions. At any stage of crop growth found susceptible and yield losses of over 25 percent have been recorded, the disease causes severe damage to the crop (Mayee and Datur, 1988, Asghari and Mayee, 1991). There are numerous *Sclerotium rolfsii* studies that have major morphological behaviour variations (Punja, 1985, Punja, 1988, Sharma *et al.,* 2002). Variability is an organism's property to modify its characters from one generation to another. This pathogen is a host of unique and important obstacles to the production of groundnuts in tropics and sub-tropics requires a knowledge basis on fungal characteristics and culture and pathogenic variability. That's why, there is need to study and characterize the fungus in culture, isolated from different districts of North-Bengal districts of West Bengal as Groundnut is grown on a large scale. The information on cultural, morphological and pathological variability among the groundnut isolates of *Sclerotium rolfsii* from North Bengal is limiting. Existence of *Sclerotium rolfsii* in groundnut already reported from West Bengal (Maiti and Choudhari, 1975, Maiti and Sen, 1985, Biswas and Sen, 2000, Baskey *et al.,* 2020) but no information is so far available about the new alarming disease under West Bengal condition.

Materials and Methods

Isolation of the pathogen

A fixed and roving surveys were conducted during 2016-2018 in groundnut growing areas in North Bengal districts of West Bengal and infected plant (Table 1) with stem rot pathogen, *Sclerotium rolfsii* were collected. *Sclerotium rolfsii*, the pathogen, was isolated on potato dextrose agar (PDA) medium from

the stems of infected plants by tissue segment method (Rangaswami and Mahadevan, 1999). Small pieces of tissue with some healthy tissue were cut with sterile scalpel from the infected collar region of about 0.5 to 1 cm. The bits were sterilized with 1% sodium hypochlorite solution on the surface for 30 seconds. The tissue sections were subsequently washed to remove excess sodium hypochlorite through repeated washing by sterile distilled water was done and then the parts were transferred to PDA media in Petri dishes. At 28 ± 1 ° C, the plates were incubated and periodically observed for fungus growth. Throughout the present investigation, pure pathogen culture was obtained by a single hyphal tip method and preserved on PDA slants. Based on its mycelial and sclerotial characters, the pathogen was known as *Sclerotium rolfsii* (Barnett and Hunter, 1972). All of the isolates showed pathogenic to ground nut in the green house during cross inoculation.

Morphological and Cultural Variability

Different isolates of *Sclerotium rolfsii* isolated from eight districts of West Bengal were studied using potato dextrose agar (PDA) solid media for their cultural, morphological characteristics, growth rate, sclerotia formation, color and texture, etc. All *Sclerotium rolfsii* isolates were grown in Petri dishes on PDA. The 4 mm diameter mycelial disc of each isolate was inoculated in the center of the plate and repeated three times. At 28±1^{0}C for 15 days, the inoculated plates were incubated. Every colony's radial growth was measured. Visual observations of sclerotial formation were reported. Mycelial-based morphological characters such as mycelial development, pattern, colony color, appearance and sclerotial parameters such as sclerotial color, weight and form, number of sclerotia, sclerotial arrangement on media surface were recorded for each isolate at 24 hour interval up to 15 days of incubation.

Results and Discussion

Based on mycelia growth pattern, the isolates of *Sclerotium rolfsii* were categorized in to two groups. These are Surface and surface + aerial mycelia growth. Observation was recorded on isolates among these 64 % isolates having surface mycelium and 36% isolates form their mycelia in surface + aerial (Table.2).However, 36% isolates showed thin strand and suppressive growth appearance whereas, 56% isolates was puffy cottony upright growth appearance and also 8% isolates was of aggregated dense cottony growth pattern (Table 2).Pigmentations of mycelium also varies with the isolates, 32% isolates showed buff white, 60% pure white and 4% dull white, whereas, no variation was observed in case of the parameter texture of mycelium(Table. 2). Sab *et al.,* (2014) studied thirteen isolates of *Sclerotium rolfsii* on their morphological diversity, these were collected from different host and geographical locations of southern Karnatak, India and reported that the isolates varied in morphological characters like colony appearance, growth rate, and characters of sclerotia, categorized the isolates into three groups viz., fast, medium and slow growing based on radial mycelia growth rate. Similarly, Divya Bharathi and Benagi, 2018, also studied on ten isolates for their cultural and morphological variability from northern Karnatak and reported fast growing, medium, and slow growing *Sclerotium rolfsii* based on radial growth rate of the isolates.

Radial mycelial growth of twenty five isolates of among these 36% isolates having surface mycelium and 64 % isolates form their mycelia in surface + aerial (Table.2).was measured at 24 hr interval and growth rate was calculated accordingly. The growth rate was varied from 0.772mm/hr to 1.12 mm/hr.

Based on mycelial growth rate; isolates were classified into three groups i.e. slow, medium and fast. The result showed that 24% isolates exhibited slow growth rate (SR-9, SR-10, SR-14, SR-15, SR-20 and SR-21), 8% isolates belong to the category of medium growth rate (SR-1 and SR-19) and 68% isolates exhibited fast growth (SR-2, SR-3, SR-4, SR-5, SR-6, SR-7, SR-8, SR-11, SR-12, SR-13, SR-16, SR-17, SR-18, SR-22, SR-23, SR-24 and SR-25)(Table 2).Thus, from the present findings, it may be concluded that majority of the isolates of *Sclerotium rolfsii* are of fast growth rate type. The variability among different isolates of *Sclerotium rolfsii* based on radial growth rate has already been reported by various researchers. Sahana et. al., (2017) studied the morphological and cultural diversity of different isolates of *Sclerotium rolfsii* collected from different tomato plants and from different locations of India and reported that the isolates varied in morphological characters like colony growth, growth rate, colony appearance and sclerotia and categorized the isolates into three groups viz., fast, medium and slow growing based on radial mycelia growth rate. Similarly, Manu (2012) also reported fast growing, medium, and slow growing *Sclerotium rolfsii* based on radial growth rate of the isolates. In a study, Sab *et al.,* (2014) reported that thirteen isolates of *Sclerotium rolfsii* of millets showed considerable variability in size of the sclerotia, however, the morphological variability among the isolates from each others, it varied diameter of colony ranged from 1.35cm (SrMR) to 2.72 cm (SrHC and SrBSn) at 24 h, 4.42cm (SrMR) to 6.77 cm (SrHC and SrBSn) at 48 h and 8.83cm (SrDF) to 9cm at 72 h. The colony colour ranged from pure white to dull white and the topography was fluffy to flat form, this study also revealed.

Morphological variations of the isolates were studied based on the phenotypic appearance. The observations of twenty five isolates of *Sclerotium rolfsii* grown on PDA medium were recorded on the basis of several sclerotial features, Sclerotial parameters viz., time taken for initiation of sclerotia, the number, weight of sclerotia, size of sclerotia, sclerotial location, sclerotial position and secretion of sclerotia, found significant variance among isolates(Table 3).

Time taken for initiation of sclerotia

The isolates of *Sclerotium rolfsii* were distributed into two clusters (Early and Late) on the basis of time taken for initiation of sclerotia. The time taken for initiation of sclerotia was varied from 124.59 hr to 133.39 hr. Result showed that 28% isolates belongs to early group (SR-1, SR-2, SR-3, SR-6, SR-11, SR-12 and SR-13) and 72% as late (SR-4, SR-5, SR-7, SR-8, SR-9, SR-10, SR-14, 15, 16, SR-17, SR-18, SR-19, SR-20, SR-21, SR-22, SR-23, SR-24 and SR-25) (Table.3). This was in accordance with the observation of Vidya Bharathi and Benagi, (2018), also studied on ten isolates of *Sclerotium rolfsii* for their cultural and morphological variability from northern Karnatak and reported as early, medium, and slow based on time taken for initiation.

Number of Sclerotia

The isolates of *S. rolfsii* were distributed into three clusters (low, moderate, and high) on the basis of quantity of sclerotia production. Result showed 32% isolates belongs to low number of sclerotial forming group (SR-9, SR-14, SR-15, SR-16, SR-19, SR-20, SR-21 and SR-22) and 44% as medium (SR-4, SR-5, SR-6, SR-7, SR-8, SR-10, SR-17, SR-18, SR-23, SR-24 and SR-25) and 24% isolates as high number of sclerotial forming group(SR-1, SR-2, SR-3, SR-11, SR-12, and SR- 13) (Table 3). This result was in accordance with the observation of Ayed *et al.,* (2018)

reported that based on production of sclerotia by the isolates of *Sclerotium rolfsii*, isolates grouped as higher, medium and lowest number of sclerotia producer isolates. Similarly, Sab *et al.,* (2014) reported that thirteen isolates of *Sclerotium rolfsii* of millets showed considerable variability in size of the sclerotial (length),however, Sclerotial number among the isolates varied from 81(SrBSn) to 459 (SrBR).

Location of sclerotia

Based on location of sclerotial formation, twenty five isolates of *Sclerotium rolfsii* were divided into two groups (peripheral and scattered sclerotia). In the first group, 21 number of isolates formed peripheral sclerotia (84%) on the surface of the petriplates. Second group consist of four isolates forming scattered sclerotia (16%) (Table 3). Whereas, no variation was observed in sclerotial secretion and texture in none of the the twenty five isolates. This was in line with the observation of Manu, 2012 reported that based on sclerotial location i.e. where the sclerotia were actually formed, by the isolates of *Sclerotium rolfsii*, isolates grouped as peripheral and scattered sclerotia producing isolates.

Table.1 Location of *Sclerotium rolfsii* isolates collected from different agro-climatic region of West Bengal

Sl No.	Isolates No.	Host	Location
1	SR-1	Groundnut (*Arachis hypogaea* L.)	Alipurduar
2	SR-2	Groundnut (*Arachis hypogaea* L.)	Alipurduar
3	SR-3	Groundnut (*Arachis hypogaea* L.)	Jalpaiguri
4	SR-4	Groundnut (*Arachis hypogaea* L.)	Jalpaiguri
5	SR-5	Groundnut (*Arachis hypogaea* L.)	Coochbehar
6	SR-6	Groundnut (*Arachis hypogaea* L.)	Coochbehar
7	SR-7	Groundnut (*Arachis hypogaea* L.)	Darjeeling
8	SR-8	Groundnut (*Arachis hypogaea* L.)	Darjeeling
9	SR-9	Groundnut (*Arachis hypogaea* L.)	Uttar Dinajpur
10	SR-10	Groundnut (*Arachis hypogaea* L.)	Uttar Dinajpur
11	SR-11	Groundnut (*Arachis hypogaea* L.)	Dakkhin Dinajpur
12	SR-12	Groundnut (*Arachis hypogaea* L.)	Dakkhin Dinajpur
13	SR-13	Groundnut (*Arachis hypogaea* L.)	Malda
14	SR-14	*Swertia chirayita* Roxb.	Kalimpong
15	SR-15	*Valeriana jatamansi* Jones	Kalimpong
16	SR-16	*Coptis teeta Wall.*	Kalimpong
17	SR-17	*Berginia ciliata (Haw.)* Sternb.	Kalimpong
18	SR-18	*Aloe vera (L.) Burm.f.*	Kalimpong
19	SR-19	*Malaxis acuminata D.Don*	Kalimpong
20	SR-20	*Centella asiatica (L.) Urban*	Kalimpong
21	SR-21	*Ocimum sanctum L.*	Kalimpong
22	SR-22	*Asperagus racemosus Willd*	Kalimpong
23	SR-23	*Stevia rebaudiana (Bertoni) Bertoni*	Coochbehar
24	SR-24	*Cymbopogon citratus, Stapf*	Kalimpong
25	SR-25	*Thysanolaena maxima (Roxb.) O. Ktze*	Kalimpong

Table.2 Morphological variation of different isolates of *Sclerotium rolfsii* on the basis of mycelial characters on PDA

Isolate	Growth Rate/h (mm)*	Mycelial growth patter	Pigmentation of mycelium	Texture	Appearance
SR-1	0.957	Surface	Buff white	smooth	Thin strand suppressed
SR-2	1.048	Surface	Pure white	smooth	Thin strands, suppressed
SR-3	1.024	Surface	Buff white	smooth	Puffy at centre, suppressed
SR-4	1.120	Surface	Buff white	smooth	Thin strand suppressed
SR-5	1.059	Surface	Buff white	smooth	Cottony suppressed growth
SR-6	1.097	Surface + Aerial	Pure white	smooth	Puffy cottony growth
SR-7	1.074	Surface + Aerial	Pure white	smooth	Puffy cottony upright growth
SR-8	1.063	Surface + Aerial	Pure white	smooth	Puffy cottony growth
SR-9	0.732	Surface + Aerial	Buff white	smooth	Cottony growth, dense at margin
SR-10	0.722	Surface	Pure white	smooth	thin strand suppressed
SR-11	1.291	Surface	Buff white	smooth	thin strand suppressed
SR-12	1.130	Surface	Buff white	smooth	thin strand suppressed
SR-13	1.091	Surface	Buff white	smooth	thin strand suppressed
SR-14	0.813	Surface + Aerial	Pure white	smooth	Puffy cottony upright growth
SR-15	0.856	Surface + Aerial	Pure white	smooth	Puffy cottony upright growth
SR-16	1.060	Surface + Aerial	Pure white	smooth	Puffy cottony upright growth
SR-17	1.083	Surface + Aerial	Pure white	smooth	Puffy cottony upright growth
SR-18	1.067	Surface + Aerial	Pure white	smooth	Puffy cottony upright growth
SR-19	0.912	Surface + Aerial	Pure white	smooth	Puffy cottony upright growth
SR-20	0.771	Surface + Aerial	Dull white	smooth	Aggregated dense cottony growth
SR-21	0.812	Surface + Aerial	Pure white	smooth	Puffy cottony upright growth
SR-22	1.030	Surface + Aerial	Pure white	smooth	Puffy cottony upright growth
SR-23	1.068	Surface + Aerial	Pure white	smooth	Aggregated dense cottony growth
SR-24	1.055	Surface + Aerial	Pure white	smooth	Aggregated dense cottony growth
SR-25	1.110	Surface + Aerial	Pure white	smooth	Puffy cottony upright growth
S.Em±	**0.0053**				
CD at 5%	**0.0152**				

*Average of three replication

Table.3 Morphological variation of different isolates of *Sclerotium rolfsii* on the basis of sclerotial characters on PDA

Isolate	Time taken for initiation of sclerotia formation (Hours)*	Number of sclerotia /Plate (15 DAI)*	Dry weight of 100 sclerotial bodies (mg)*	Size of Sclerotia (mm)*	Maturity (DAI)*
SR-1	128.59	314.67	44.50	0.46	08
SR-2	126.87	304.00	44.31	0.43	11
SR-3	124.59	324.00	43.35	0.46	10
SR-4	133.39	284.00	56.33	0.52	12
SR-5	133.22	274.00	35.32	0.54	12
SR-6	129.61	254.00	70.37	0.56	13
SR-7	132.47	261.00	71.29	0.63	14
SR-8	131.95	281.00	78.33	0.72	15
SR-9	133.39	214.00	73.34	0.84	15
SR-10	132.47	271.00	71.67	0.78	14
SR-11	126.82	340.00	45.67	0.41	09
SR-12	125.45	317.00	44.79	0.44	08
SR-13	127.12	308.00	45.57	0.45	11
SR-14	132.46	221.00	72.19	0.64	14
SR-15	132.76	233.00	74.38	0.73	15
SR-16	133.12	231.00	73.78	0.77	15
SR-17	130.27	266.00	61.19	0.53	12
SR-18	131.29	273.00	58.27	0.52	12
SR-19	132.15	227.00	72.98	0.81	14
SR-20	132.49	235.00	77.12	0.78	14
SR-21	133.21	219.00	75.29	0.69	15
SR-22	131.99	247.00	71.39	0.68	15
SR-23	132.12	269.00	55.79	0.55	13
SR-24	132.45	259.00	62.45	0.51	12
SR-25	130.27	279.00	63.47	0.54	12
S.Em±	**0.05**	**0.13**	**0.035**	**0.004**	
CD at 1%	**0.15**	**0.38**	**0.099**	**0.010**	

*Average of three replication

Size of the sclerotia

In the present study, size of the sclerotial (length) was found to be in the range between 2.9mm to 7.5mm. On the basis of sclerotial size *S. rolfsii* isolates were categorized in to three groups (small, medium and big). Among these, 56% isolates (SR-1, SR-2, SR-3, SR-4, SR-5, SR-6, SR-11, SR-12, SR-13, SR-17, SR-18, SR-23, SR-24 and SR-25) showed small, 24% medium (SR-7, SR-14, SR-15, SR-16, SR-21 and SR-22) and 20 % (SR-8, SR-9, SR-10, SR-19 and SR-20) produced big size sclerotia on PDA media. (Table 3). Sab *et al.*, (2014) reported that thirteen isolates of *Sclerotium rolfsii* of millets showed considerable variability in size of the sclerotial (length), however, sclerotial diameter varied from 1.05 mm (SrBR) to 2.11 mm (SrMW).

Sclerotial weight

Dry weight of sclerotia ranged from 35.32 to 77.12 mg (on randomly selected 100 sclerotia of 15 days old). The sclerotial dry weight indicated a wide level of variability among *S. rolfsii* isolates. Based on sclerotial dry weight isolates were divided into three groups viz low, moderate, and high. Data revealed that 28% isolates (SR-1, SR-2, SR-3, SR-5, SR-11, SR-12 and SR-13) showed low sclerotial weight, 24% (SR-4, SR-17, SR-18, SR-23, SR-24 and SR-25) moderate and 48% isolates (SR-6, SR-7, SR-8, SR-9, SR-10, SR-14, SR-15, SR-16, SR-19, SR-20, SR-21 and SR-22) exhibited as high weight of sclerotia. (Table 3). Differentiation of *S. rolfsii* isolates based on colony colour on growth media, mycelial growth pattern, growth rate, differences in sclerotial number, size and weight, have been reported in previous studies (Okabe et al,1998; Basamma,2008; Rakholiya and Jadeja, 2011; Prasad *et al.,* 2012; Kumar *et al.,* 2014 Sahana *et al.,* 2017; Ayed *et al.,* 2018). Sab *et al.,* (2014) reported that there were obvious variability in sclerotia dry weight and number among the different *Sclerotium rolfsii* isolates of millets and test weight of sclerotia was higher in SrBSn (136 mg).

Time taken for maturity of sclerotia

The isolates of *Sclerotium rolfsii* were grouped into three clusters (Early, medium and Late) on the basis of time taken for maturity of sclerotia. The time taken for maturity of sclerotia was varied from 8days to 15days. Result showed that 16% isolates belongs to early group (SR-1, SR-3, SR-11 and SR-12),36% as medium (SR-2, SR-4, SR-5, SR-6, SR-13, SR-17, SR-18, SR-24 and SR-25) and 44% as late (SR-7, SR-8, SR-9, SR-10, SR-14, SR-15, SR-16, SR-19, SR-20, SR-21 and SR-22) (Table 3). This was in accordance with the observation of Sab et. al., (2014) reported that thirteen isolates of *Sclerotium rolfsii* of millets showed considerable variability in time taken for maturity of sclerotia, however, Sclerotial initiation also varied significantly among different isolates from 5 day to 13 day. These results are in agreement with the findings of Kakade *et al.,* (2017) who observed the heterogeneity among 13 isolates of *Sclerotium rolfsii* on Potato dextrose agar, where colony diameter ranged from 51.70 to 80.30 mm. Mycelium color ranged from cottony white to smooth or wavy margins of buff white. The color ranged from brown to dark brown with a spherical to sub-spherical form of the sclerotial bodies. Manu (2012) and Savita *et al.,* (2016) have made similar observations.

In conclusion the pathogen of Stem rot disease (*Sclerotium rolfsii* Sacc) is causing severe loss in production due to it's wide host range and variability in morphological and cultural. Twenty five isolates of *Sclerotium rolfsii* collected from different locations of North-Bengal districts of West Bengal from rice-based cropping system, infecting groundnut in cross inoculation, were studied on potato dextrose agar(PDA) showed wide significant variation in their cultural and morphological characters. It was found that the growth rate was varied from 0.772mm/hr to 1.12 mm/hr. and size of the sclerotia was found to be in the range between 2.9mm to 7.5mm. Production of sclerotial bodies started from 5th day onwards, Colony colour varied from buff white to pure white, Sclerotial shapes were ellipsoidal to spherical with light brown to dark brown colour. From the study, it may be concluded that for the management of this pathogen needs location specific good agricultural practices.

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