RESEARCH ARTICLE

Isolation and Morphological Characterization of Sclerotium rolfsii-Inciting Stem rot Disease in Groundnut (*Arachis hypogaea* L .)

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Abstract

Sclerotium rolfsii is a highly threatening pathogen,affecting more than 500 hosts worldwide. In the present study, ten different isolates of *Sclerotium rolfsii* were isolated from groundnut growing regions of Tamil Nadu, and their morphological characteristics were observed on potato dextrose agar (PDA) medium. The virulent isolate SR-8 produced pure white with thin mycelia growth, very fast growing mycelium, dark brown colored sclerotia, and the highest number of sclerotia /plate (538). The pathogenicity test was conducted on pot-cultured, grown groundnut plants. The virulent isolate SR-8, *S. rolfsii*, is infected at various stages of its growth and development in groundnut plants. Lipid peroxidation and pathogenicity are crucial to identify the virulent nature of the pathogen. The pathogenicity of the virulent isolate SR-8 that produced the highest disease incidence was 89.29 % and had a high amount of lipid peroxidation of 0.9589 μmol/g. The least virulent isolates produced the minimum amount of lipid peroxidation and disease incidence. All the ten isolates exhibited different cultural and morphological characteristics, lipid peroxidation rates, and various levels of disease incidence on groundnut.

Keywords: *Groundnut;Sclerotium rolfsii; Disease incidence; Pathogenicity; Virulent*

Introduction

Groundnut (*Arachis hypogaea* L.) is a major oilseed crop commercially grown in India, China, Nigeria, Senegal, Sudan, Burma, and the United States. Groundnuts are high in energy (567 calories per 100 g) and consist of various health-promoting elements, minerals, antioxidants, and vitamins. The kernels are high in dietary protein and include high-quality amino acids necessary for growth and development. They are high in monounsaturated fatty acids, especially oleic acid. Many diseases harm the groundnut crop at various phases of development. One of the most devastating diseases in groundnut is stem rot, which is caused by *Sclerotium rolfsii.* Yellowing and withering of branches, the presence of white mycelial growth in the collar region, and the formation of mustard seeds like sclerotia are all common indications of this disease. Stem rot disease is a potential threat to groundnut production and has significant economic implications for irrigated groundnut. At any stage of crop growth, the disease causes serious harm to the crop, with yield losses of over 25% documented (Mayee and Datar, 1988). Morphological and phenotypic characteristics of *S. rolfsii* may be connected to virulence level, which is significant in pathogen isolate selection for disease resistance evaluation in crop breeding programs. In research relating to sclerotia morphological features and mycelial development rates, variability among *S. rolfsii* isolates and allied species such as *S. sclerotiorum* that infects different crops has been examined (Paul *et al*., 2017). Rasu *et al*. (2013) described seventeen *S. rolfsii* isolates that were evaluated for cultural morphological differences; most had compact colonies, while a few had fluffy colonies. They were divided into three categories based on their development rates: slow-growing, fast-growing, and intermediate. The quantity of sclerotia and dry weight of 100 sclerotia also differed significantly among the isolates. Most of the isolates had dark to light brown sclerotia. Lipid peroxidation has been linked to various biological processes in fungus, including cell growth and differentiation. During differentiation, filamentous fungus shows a significant rise in lipid peroxidation. During its differentiation state, *S. rolfsii* induces an considerable increase in

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lipid peroxidation and the amount of lipid peroxidation plays a crucial role in the virulent mechanism of *S. rolfsii* (Georgiou, 1997). As a result, an attempt was made in this work to look into the variation of cultural, morphological, and pathogenicity characteristics of *S. rolfsii*-induced groundnut stem rot.

MATERIALS AND METHODS

Isolation of the stem rot pathogen of groundnut

Isolation of the stem rot pathogen was done through a tissue segmentation method (Kumar *et al.*, 2014). With the help of a sterile scalpel knife, infected parts of the groundnut stem are cut into 1–1.5 cm pieces, and their surfaces are sterilized with 0.1% mercury chloride for 1 minute. It should be washed with sterile distilled water three times and then placed at an equal distance on the Petri plates containing the PDA. Otherwise, dark melanized sclerotia are extracted from the infected region and transferred aseptically with sterile forceps to a Petri plate containing PDA, where they are incubated for five days at 28± 2°C at room temperature and then transferred to agar slants for future research.

Morphological characterization of *Sclerotium rolfsii*

S. rolfsii is confirmed by the presence of mycelial and sclerotial characteristics reported by Barnett and Hunter (1972). Morphological characters like mycelia growth and, colony color, mycelia weight were recorded, and also sclerotial characters like sclerotial color, weight, and shape, the number of sclerotia production per plate, and their arrangement on the media surface should be recorded (Latha and Rajeswari, 2019).

Pathogenicity of *Sclerotium rolfsii*

The pathogenicity of different isolates of *S. rolfsii* was tested in pot cultured grown groundnut plants (Billah, 2017). The 30cm diameter of the earthen pots was filled with 5 kg of sterilized garden soil, which was sterilizedunder 1.4 $kg.cm^{-2}$ pressure for two hours on two successive days. The pots were inoculated with 5 per cent of a pathogen inoculum prepared in a sand maize medium. The groundnut seeds were sown in pots randomly. Each treatment was replicated three times. The pots were maintained under screen house conditions with adequate moisture under optimum growing conditions for groundnut and continuous observation of disease symptoms up to 25 DAS. The per cent disease incidence was calculated and found which isolate provided the maximum stem rot incidence, and such an isolate was considered a virulent pathogen. The virulent pathogen was reisolated and confirmed as *S. rolfsii* (Chandra Sekhar *et al*., 2017a).

Assessment of lipid peroxidation for S. *rolfsii*

Thelipid peroxidation was estimated for all ten isolates of *S. rolfsii* on 7 DAI. The amount of thiobarbituric acid reactive substances (TBARS) was determined by the thiobarbituric acid (TBA) reaction method (Heath and Packer, 1968). In the test tube, 0.5g mycelium of the S. *rolfsii* was taken, then 0.1 M NaCl, 0.5mL25%(v/v) HCl and 0.5mL1%(w/v) TBA (in 0.05M NaOH) were added into the test tube to estimate the lipid peroxidation. The lipid peroxidation was calculated as malondialdehydes (MDA) produced from the putrefaction of lipid hydroperoxides during the heating stage of the test (Georgiou, 1997). The test tube was kept at 100^0 C for 15 min in the water bath, the red-purple color was formed. After that, the entire content was centrifuged in 8000 rpm for 10 mins. The aliquot was read at 532 nm in a UV visible spectrophotometer. The value of distracting absorption at 600nm was subtracted from the 532nm comprehension. Finally, the lipid peroxidation was estimated as µmol/g by using the formula

$$\text{Lipidperoxidation} = (OD532-OD600)/155\times1/0.5\times1000 \ \mu mol/g$$

RESULTS AND DISCUSSION

Isolation of *Sclerotium rolfsii*

Sclerotium rolfsii is isolated from symptoms collected from the entire survey area using the tissue segmentation method, or sclerotia is picked from the symptom and cultured on a Petri plate containing PDA medium (Table.1). Rashmi *et al.* (2017) reported that *S. rolfsii* infected symptoms collected from the field and that the pathogen was isolated by (tissue segmentation method) a small piece of the diseased portion placed in a PDA medium containing a Petri plate. And also, Chandra Sekhar *et al.* (2017b) reported that *S. rolfsii* was isolated from the small tissue of an infected stem or roots on a PDA medium amended Petri plate. Paparu *et al*. (2020) reported that *S. rolfsii* was isolated from the Southern Blight infected roots of the common bean by the tissue segmentation method.

Morphological characteristics of *Sclerotium rolfsii*

Among the ten isolates of *S. rolfsii* (SR-1 to SR-10), the highly virulent isolate SR-8 produced the purest white with thin mycelia growth, very fast growing mycelium, dark brown colored sclerotia, and the highest number of sclerotia (538) produced per plate. But in the case of the least virulent isolate,SR-10 exhibited dull white with thin mycelial growth, very slow growing mycelium, light brown, and produced the least number of sclerotia 60 per plate (Table .2; Table.3; Fig.1). Pandi *et al*.(2017) stated that the sclerotial productions and color of eight different isolates of *S. rolfsii,* found that the number of sclerotia varied from 274 to 360 sclerotia /plate. The size and color of sclerotia varied in different isolates, mainly from light brown to reddish brown at maturity. Savita *et al.* (2016) reported the sclerotial characteristics of *S. rolfsii.* Mustard seeds like sclerotia were produced, which were deep brown or brownish black, shiny, hard, spherical and irregular in shape. Mahato and Biswas (2017) state that isolates of S. *rolfsii* varied in their mycelia dispersion and appearance in Petri plates and showed dispersed growth all over the plate in an aggregated fashion and their appearance was loose to dense and cottony with sparse or fluffy mycelium. Praveen and Kannan (2021) reported that twenty isolates of *S. rolfsii* grown on Potato Dextrose Agar medium containing Petri plates exhibited variation in the colony characteristics such as fluffy white mycelium, profuse cottony white mycelium, dense cottony white mycelium, dull white profuse mycelium, and white cottony mycelium. Among them, the virulent isolate Sr6 showed profuse cottony white mycelium.Sutha Raja Kumar (2021) reported that nine isolates of *S. rolfsii* (SR-1 to SR5) varied in their ability to produce sclerotia in PDA medium. On the 15th day of inoculation, the maximum number of sclerotia (205 nos.) per nine mm culture disc was obtained from SR5, which was also the most virulent isolate. The minimum number of sclerotia of 82 was recorded by SR9, the least virulent isolate. On PDA, different isolates of *S. rolfsii* produced different colored sclerotia. The isolate SR5, SR4 and SR7 produced brown color, SR2 and SR8 produced chocolate color, SR3, SR6 and SR9 produce dark brown color and SR1 produced light brown colored sclerotia.

Pathogenecity of *Sclerotium rolfsii*

During pathogenicity, the virulent isolate SR-8 caused 89.27% of the disease. In the case of least virulent isolate exhibited 18.72 % (Table 1; Fig. 2; Fig. 3). The comparable findings also reported by Yrlânia *et al*. (2015) proved the virulence of S. *rolfsii* on groundnut plants. The first symptoms were tested 48 h after inoculation, which evolved to stem bottleneck and plant wilting with the presence of white mycelium under greenhouse conditions. Chandra Sekhar *et al.* (2017c) reported the pathogenicity reactions for all the 10 isolates of *S. rolfsii.* The isolate Sr9 exhibited 100% disease incidence, followed by 90.67% disease incidence in the isolate Sr7. The lowest disease incidence (46.33%) was recorded in the Sr6 isolate under pot culture experiments. The disease intensity of different *S. rolfsii* isolates upon artificial inoculation exhibited the virulence nature of isolates to determine the disease intensity in their host. Similarly,Praveen and Kannan, (2021) reported that the pathogenicity test was conducted for twenty isolates (Sr-1 to Sr-20) of *S. rolfsii* isolated from the major groundnut growing areas of Tamil Nadu. The isolate Sr6 exhibited the maximum disease incidence (42.05 %) under pot culture conditions, followed by the isolate Sr8, which exhibited the disease incidence of 40.51 per cent, and the least disease incidence (16.65%) was recorded in Sr13. The variation in stem rot incidence could be well attributed to the difference in the virulence of the *S. rolfsii* isolates prevalent in the respective areas. The difference in virulence obtained in this present study agrees with the reports of many earlier workers (Muthukumar and Suthinraj, 2019; Ünal *et al.,* 2019). Yan *et al.* (2021) noticed isolates with high aggressiveness with a disease incidence of more than 66.7 % and weak aggressiveness showing disease incidence of less than 33.30% when conducting pathogenicity tests under pot cultured grown groundnut.

Lipid peroxidation of S. *rolfsii*

The lipid peroxidation is positive proportionality to the virulentof *S. rolfsii.*The lipid peroxidation was estimated from all the ten isolates of *S. rolfsii.* Among the ten isolates, isolate 8 produced a high amount of lipid peroxidation of 0.9589 µmol/g and produced more mustard-shaped resting sclerotia (538) shown in table .4; Fig.4.The least virulent isolate had the minimum amount of lipid peroxidation. Similarly, Georgiou (1997) reported high lipid peroxides levels of 66.1±5.3 and 64.1±6.8 mmol MDA mol^{-1} of total phospholipids produced at 6 and 10 DAI (sclerotial initiation and sclerotial development stage) of S. *rolfsii* respectively.

CONCLUSION

In this study, all ten isolates of S. *rolfsii* were differentiated by morphological, cultural, and pathogenicity characteristics. The characters of isolate SR-8 varied from the other isolates. Which morphological and cultural characteristics led to a SR-8 virulent. Similarly, the opposite characteristics determine the least virulent of isolate SR-10. From the study of lipid peroxidation, the virulent isolate showed the highest amount of lipid peroxidation rate. The lipid peroxidation and pathogenicity is a key to identify the virulent nature of the pathogen. Based on the findings, the virulent isolate may be sent to molecular studies to better understand the relationship between S. *rolfsii* cultural, morphological, and molecular characteristics.

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Originality and plagiarism

Authors should ensure that they have written and submit only entirely original works, and if they have used the work and/or words of others, that this has been appropriately cited

Consent for publication

All the authors agreed to publish the content.

Competing interests

The authors declare that they have no competing interests

Data availability of

All the data of this manuscript are included in the Manusript.

Authors' contributions

Ayyandurai Marimuthu:I did all the experiments under the guidance of co authors

Akila Ramasamy :Chief advisor of conducting all the experiments and technical guidance

Manonmani Karunagaran:Major contributor for manuscript correction

Mini M.L:Analysis the data and was a major contributor in writing the manuscript

Consent for publication

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Table 1.Pathogenicity of different isolates of Sclerotiumrolfsii on groundnut in pot

culture condition

S. No.	Name of Isolates	Disease incidence (%)*
1.	SR-1	53.41f
		(47.27)
2.	SR-2	33.41g
		(36.82)
3.	SR-3	71.37c
		(60.28)
4.	SR-4	55.83e
		(49.49)
5.	SR-5	86.52b
		(63.84)
6.	SR-6	43.33g
		(40.57)
7.	SR-7	25.98i
		(31.29)
8.	SR-8	88.34a
		(70.88)
9.	SR-9	69.49d
		(55.91)
10.	SR-10	18.72j
		(26.39)
11	Control	0.00k
		(0.00)
CD(P=0.05)		2.18

*Mean of three replications

Values in the parentheses are arc sine transformed values

Table 2.Mycelial characteristics of *Sclerotium rolfsii*

S. No.	Name of Isolates	Growth pattern of S. *rolfsii* mycelium	Colony colour	Growth rate	Mycelial Fresh weight (g)*	Mycelial Dry weight (g)*
1.	SR-1	Thin mycelial strand with sparse growth	Dull white	Moderate	3.29h	0.41h
2.	SR-2	Cottony mycelial growth	Dull white	Fast	4.56d	0.52e
3.	SR-3	Cottony mycelial growth with aggregated centre	Dull white	Moderate	4.10f	0.62c
4.	SR-4	Thin mycelial growth	Pure white	Moderate	3.97f	0.48f
5.	SR-5	Cottony mycelial growth	Pure white	Moderate	4.86c	0.75b
6.	SR-6	Very thin mycelial strand	Dull white	Moderate	5.23b	0.35i
7.	SR-7	Sparse and thin mycelial growth	Pure white	Fast	4.38e	0.58d
8.	SR-8	Cottony mycelial growth	Dull white	Very fast	5.78a	0.83a
9.	SR-9	Very thin mycelial growth	Pure white	Very slow	3.67g	0.44g
10.	SR-10	Thin mycelial growth	Dull white	Very slow	2.38i	0.27j
CD (P = 0.05)					0.139	0.025

*Values are the means of three replicates; means in a column followed by the same letters are not significantly different according to Dungun's multiple range test at P= 0.05.

Table 3.Sclerotial characteristics of *Sclerotium rolfsii*

S.No.	Name of Isolates	Sclerotia shape	Colour of sclerotia	Sclerotial formation (No. of days)	Weight of 100 sclerotia (mg)*
1.	SR-1	Spherical and small	Dark brown	10e	42.33a
2.	SR-2	Round and more in upper plate	Light brown	12d	38.67b
3.	SR-3	Globose and scatterd on the plate	Dark brown	14c	33.67c
4.	SR-4	Round and small	Light brown	16a	41.33a
5.	SR-5	Globose	Dark brown	10e	19.33f
6.	SR-6	Round and medium	Dark brown	15b	26.67e
7.	SR-7	Round and very small	Light brown	12d	32.33c
8.	SR-8	Round and small aggregate in margin of the plate	Dark brown	15b	37.67b
9.	SR-9	Round and small present on upper side	Dark brown	10e	29.33d
10.	SR-10	Globose and aggregate on upper plate	Light brown	8f	17.24g
CD(P= 0.05)				0.71	1.415

*Values are the means of three replicates; means in a column followed by the same letters are not significantly different according to Dungun's multiple range test at P= 0.05.

Table .4 Estimation of Lipid peroxidation of S. *rolfsii*

S. No	Name of Isolates	Lipid peroxidation (μ mole/gram)*	No of sclerotia production per/ plate*
1.	SR-1	0.6364e	374d
2.	SR-2	0.5504f	486b
3.	SR-3	0.4214g	278f
4.	SR-4	0.5375f	108h
5.	SR-5	0.8729b	329e
6.	SR-6	0.5547f	404c
7.	SR-7	0.7095c	362d
8.	SR-8	0.9589a	538a
9.	SR-9	0.6665d	480b
10.	SR-10	0.3956h	60g
	CD (P = 0.05)	0.025	18.821

*Means followed by the same letter differ non-significantly at $P \leq 0.05$ according to DMRT; values are mean of three replications

Fig.1Mycelial and Sclerotial stage of (SR-8) *Sclerotium rolfsii*

1. Mycelial growth
2. Initiation of sclerotial formation
3. Immature white sclerotia
4. Partially matured light brown sclerotia
5. Matured brown sclerotia

Fig.2 Pathogenicity under potculture condition

Fig. 3 Virulent Isolate *Sclerotium rolfsii* SR-8

Lipid peroxidation of *Sclerotium rolfsii*

Fig. 4 Lipid peroxidation of S. *rolfsii*