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STUDIES ON SCLEROTIAL CHARACTERISTICS OF *SCLEROTIUM ROLFSII* ISOLATES OF GROUNDNUT ON DIFFERENT MEDIA

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Abstract

The groundnut plants showing the typical symptoms of stem rot (*Sclerotium rolfsii*) were collected from diverse geographic locations of Maharashtra and after confirmation of Koch's postulates seven isolates of *S. rolfsii* were reisolated. The sclerotial characters of seven isolates were studied on fourteen solid media.

The sclerotial bodies of each isolate showed variation in position, test weight and number per cm^2 on different fourteen medium. The numbers of sclerotial bodies per cm^2 were varies from 6.07 to 1.51 cm^2. While, the test weight of sclerotia was varies from 261.33 to 53mg. All these sclerotial characters revealed the existence of variability among the isolates of *S. rolfsii* of groundnut.

Key words : *Sclerotium rolfsii*, groundnut, growth media, sclerotia.

Introduction

Groundnut (*Arachis hypogea* L.) is a major edible oil seed crop of tropical and subtropical region of the world. The low productivity in groundnut is attributed by many production constraints. Among these, biotic factors particularly diseases play a major role in limiting the yield of groundnut. Among several diseases, stem rot caused by *Sclerotium rolfsii* is the most damaging to the groundnut crop in field and causing great losses. *Sclerotium rolfsii* Sacc. is a soil inhabitant, non-target, polyphagous and an ubiquitous facultative parasite. It has wide host range infecting cultivated crops. This fungus is distributed throughout the world.

Variation is a rule in most of the root infecting fungi. The variation may arise following change in crop cultivation, genetic modification of hosts, physical or chemical modification of the soil, environment or accidental introduction of new genetic material into a region or local gene pool. It may also be a way of survival of the pathogen under adverse conditions. The extreme variation in morphological characteristics of *Sclerotium rolfsii* has been observed in worldwide collection of this pathogen from different hosts and also from the same hosts (Cooper, 1961; Kim, 1974 and Punja, 1985).

The cultures of *S. rolfsii* originating from various plant species and different geographical regions present wide variation in growth rate, morphological characteristics, mycelial compatibility and also exhibited genetic variability. However, the cultures of S. rolfsii can be identified by the size, color and structure of their sclerotia. The objective of present study was to compare the sclerotial characteristics of indigenous fungal strains and to study differences among different fungal strains isolated from various locations of Maharashtra.

Materials and Methods

The groundnut plants showing the typical symptoms of stem rot (*Sclerotium rolfsii*) were collected from diverse geographic locations of Maharashtra and isolation was done on PDA. The isolates of the fungus were confirmed as *S. rolfsii* by comparing with the characters described by Mundkur (1934). The pathogenicity tests

Abbreviations used : BAM - Basal agar media, CA - Carrot agar, CMA - Corn meal agar, CDA - Czapek'sdox agar, EA - Elliot's agar, HLEA - Host leaf extract agar, MEA - Malt extract agar, OMEA - Oat meal extract agar, PDA - Potato dextrose agar, RA - Richards's agar, RBA - Rose Bengal agar, SA - Sabouroud's agar, TA - Tochinai's agar, YEA - Yeast extract agar

of seven isolates were carried out by sick soil technique. After Koch's postulates all the seven isolates were reisolated and compared with the original cultures.

The sclerotial characters of *S. rolfsii* isolates were studied on fourteen solid media. The 20 ml each medium was poured in 90 mm petriplates. The colony culture disc (5mm diameter) of an actively growing fungal isolates (3-4 days old) were cut with the help of cork borer and placed by facing mycelial side downwards in the center on petriplate containing medium under aseptic conditions and incubated at 28±2°C. Each isolate was replicated thrice. The various sclerotial characteristics of individual isolates of *S. rolfsii* on different media were recorded.

Results and Discussion

The sclerotial bodies of seven isolates of *S. rolfsii* on different fourteen media revealed that, there was significant difference with respect to position of sclerotial bodies, number of sclerotial bodies per cm^2 and test weight of hundred sclerotia.

a) Position of sclerotial bodies

From the results presented in table 1, it was observed that each isolate showed variation in position of sclerotial bodies on different fourteen medium.

On BAM, the position of sclerotial bodies of isolates Sr-1 and Sr-4 were irregular, Sr-2 and Sr-6 were uniform, Sr-3, Sr-5 and Sr-7 were at near edges of petriplates. On CA, the sclerotial bodies of isolates Sr-1, Sr-2 and Sr-5 were uniformly distributed, Sr-3 were near growth circle periphery, Sr-4 and Sr-7 were irregular and Sr-6 were near edges of petriplates. On CMA, the sclerotial bodies of isolates Sr-1 were near edges of petriplate while, the sclerotial bodies of isolates Sr-7 were at edges of petriplates. The isolates Sr-2, Sr-3, Sr-5 and Sr-6 were recorded the irregular distribution of sclerotial bodies while, Sr-4 were recorded uniform distribution. On CDA, the sclerotial bodies of isolates Sr-1, Sr-2 and Sr-6 were irregularly distributed, Sr-3 were uniformly distributed. While, isolate Sr-4 and Sr-7 produced sclerotia at near edges of petriplates and Sr-5 were at circle periphery of petriplate.

On Elliot's agar all isolates produced sclerotia at irregular intervals in petriplate. On HLEA, the sclerotia of isolates Sr-1 were at edges and on the top of petriplates, Sr-2 and Sr-6 were uniform, Sr-3 were near circle periphery of growth. While, the isolate Sr-4 produced sclerotia at near edges of petriplate and Sr-7 were at edges of Petriplates. The isolate Sr-5 produced sclerotia irregularly distributed in petriplates. On MEA, the isolates Sr-1 and Sr-4 produced sclerotia at centre of growth, Sr-2 and Sr-5 were at circle periphery, and Sr-3, Sr-6 and Sr-7 were uniformly distributed. On OMEA, the sclerotia of isolates Sr-1 were irregularly distributed but covering all petriplate. While, sclerotia of Sr-2 were at edges and at the top of petriplates. The sclerotia of Sr-3 and Sr-6 were uniformly distributed, Sr-4 was at centre of growth, Sr-5 was irregularly distributed and Sr-7 were densely populated sclerotia.

On PDA, the sclerotia of isolates Sr-1 and Sr-6 were uniformly distributed, Sr-2 were at edges and at the top of petriplates, Sr-3 and Sr-7 were centre of growth, Sr-4 were near edges of petriplate, Sr-5 were covered all petriplates. On RA, the sclerotia of isolates Sr-1, Sr-3 and Sr-7 were at near edges, Sr-2 were near circle periphery, Sr-4 and Sr-5 were irregularly distributed and Sr-6 were at edges of petriplates. On RBA, the sclerotia of Sr-1 were at near edges, Sr-2 were at circle periphery, Sr-3, Sr-4 and Sr-5 were irregularly distributed, Sr-6 were at edges of petriplates and Sr-7 were near circle periphery of growth.

On SA, the sclerotia of Sr-1 and Sr-5 were uniformly distributed, Sr-2 were near circle periphery, Sr-3 were near edges, Sr-4 were centre, Sr-6 were at edges and Sr-7 were irregularly distributed. On TA, the sclerotia of Sr-1, Sr-5 and Sr-7 were irregularly distributed, Sr-2 and Sr-3 were uniformly distributed, Sr-4 was near edges and Sr-6 was at circle periphery of growth. On YEA, the sclerotia of Sr-1 were near circle periphery, Sr-2 were near edges, Sr-3 and Sr-7 were uniformly distributed, Sr-4 and Sr-6 were irregularly distributed and Sr-5 were covering all petriplate.

b) Test weight of sclerotial bodies

The results presented in table 2 showed that all the seven isolates showed significant variation in sclerotial test weight on different media. The highest sclerotial test weight was recorded in isolate Sr-2 (261.33mg) on OMEA followed by Sr7 on OMEA. While, lowest sclerotial test weight was recorded in isolates Sr-2 (53.00 mg on CDA) and Sr-5 (53.00mg on CDA). All the isolates were recorded the highest sclerotial test weight on OMEA and lowest on CDA, followed by CMA.

The variation in the test weight of 100 sclerotial bodies was ranged as fallows on different media. The isolate Sr1 (253.67-55.67mg), Sr2 (261.33-53.00mg), Sr3 (250.67-55.33mg), Sr4 (249.33-56.00mg), Sr5 (245.00-53.00mg), Sr6 (240.67-54.00mg) and Sr7 (259.00-54.00mg). This may be due to strainal variation in *S. rolfsii*. Similar type of study was conducted by many workers and reported variation in size of sclerotia of *S. rolfsii viz.,* 0.60-1.44 mm (Manjappa, 1979), 0.50-0.80

Table 1 : Variability in position of sclerotial bodies of *S. rolfsii* isolates on different media.

Media	Position of sclerotial bodies of *S. rolfsii* isolates						
	Sr1	**Sr2**	**Sr3**	**Sr4**	**Sr5**	**Sr6**	**Sr7**
BAM	Irregular	Uniform	Near edges	Irregular	Near edges	uniform	Near edges
CA	Uniform	Uniform	Near circle periphery	Irregular	Uniform	Near edges	Irregular
CMA	Near Edges	Irregular	Irregular	Uniform	Irregular	Irregular	At Edges
CDA	Irregular	Irregular	Uniform	Near edges	At circle periphery	Irregular	Near edges
EA	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular
HLEA	Edges & at top of petriplate	Uniform	Near circle periphery	Near edges	Irregular	Uniform	At edges
MEA	Centre	At circle periphery	Uniform	Centre	At circle periphery	Uniform	Uniform
OMEA	Covered all petriplate irregularly	Edges & at top of petriplate	Uniform	Centre	Irregular	Uniform	Densely populated
PDA	Uniform	Edges & at top of petriplate	Centre	Near edges	Covered all petriplate	Uniform	Centre
RA	Near edges	Near circle periphery	Near edges	Irregular	Irregular	At edges	Near edges
RBA	Near edges	At circle periphery	Irregular	Irregular	Irregular	At edges	Near circle periphery
SA	Uniform	Near circle periphery	Near edges	Centre	Uniform	At edges	Irregular
TA	Irregular	Uniform	Uniform	Near edges	Irregular	At circle periphery	Irregular
YEA	Near circle periphery	Near edges	Uniform	Irregular	Covered all petriplate	Irregular	Uniform

mm (Wolf, 1914), 0.28-2.40 mm (Rayes, 1937), 0.40-2.50 mm (Singh and Srivastava, 1953) and 1.08-2.23 mm (Palaiah, 2002), 1.20 to 2.40 mm (Prabhu, 2003). This variation in size may be attributed to different strains of the *S. rolfsii*.

Abeygunawardena and Wood (1957) studied the sclerotial production of *S. rolfsii* on nine different media, which included both synthetic and nonsynthetic media. The maximum weight of sclerotia was observed in the case of Weindling's medium. Czapek's-Dox medium did not support the production of sclerotia. Many workers showed variation in sclerotial characteristics on different media (Wheeler and Sharan, 1965; Henis *et al*., 1973; Shapira *et al*., 1984; Aycock, 1966).

c) Number of Sclerotial bodies per cm^2

The data presented in table 3 does not follow normal distributions, it varies from 1.51cm^2 to 6.07 cm^2 hence data is transferred to $\sqrt{x+0.5}$ transformations to form normality. All the seven isolates showed significant variation in number of sclerotial bodies per cm^2 on different media. The maximum sclerotial bodies per cm^2 were recorded in isolate Sr-2 (6.07 cm^2) on OMEA. While, minimum numbers of sclerotial bodies per cm^2 were recorded in isolates Sr-4 (1.51cm^2) on BAM.

All the isolates showed significant variation in number of sclerotial bodies per cm^2 *viz.,* Sr-1 (5.60-1.80cm^2), Sr2 (6.07-2.62cm^2), Sr3 (5.82-2.18cm^2), Sr4 (5.72-1.51cm^2), Sr5 (5.33-2.86cm^2), Sr6 (5.84-2.50cm^2) and Sr7 (5.94-2.84cm^2) on different media. Also, all the isolates showed variation in maximum numbers of sclerotia per cm^2 on different media *viz*., Sr-1(5.60cm^2 on MEA), Sr-2 (6.07cm^2 on OME), Sr-3(5.82cm^2 on HLEA), Sr-4(5.72cm^2 on RA), Sr-5(5.33cm^2 on RA), Sr-6(5.84cm^2 on SA) and Sr-7(5.94cm^2 on PDA). While, the isolates Sr-1(1.80cm^2), Sr-2(2.62cm^2), Sr-3(2.18cm^2), Sr-5(2.86cm^2) and Sr-7(2.84cm^2) were produced minimum numbers of sclerotia per cm^2 on EA, and Sr-4(1.51cm^2) and Sr-6(2.50cm^2) were on BAM.

Sulladmath *et al*. (1977) reported that, maximum numbers of sclerotia were produced by the isolate from pigeonpea followed by sunflower, wheat and groundnut. Similarly

Table 2 : Variability in test weight of sclerotial bodies (mg) of *S. rolfsii* isolates on different media.

S. no.	Media	Test weight of sclerotial bodies (mg) of *S. rolfsii* isolates						
		Sr1	Sr2	Sr3	Sr4	Sr5	Sr6	Sr7
1	BAM	124.00	108.00	119.67	120.00	123.00	112.00	114.33
2	CA	113.00	114.33	118.67	112.67	107.33	110.33	108.33
3	CMA	83.67	82.00	82.67	81.00	81.33	80.33	84.67
4	CDA	55.67	53.00	55.33	56.00	53.00	54.00	54.00
5	EA	115.00	120.00	117.00	118.00	111.00	125.33	133.00
6	HLEA	143.33	142.33	124.00	131.33	136.00	143.67	136.00
7	MEA	134.33	132.33	110.33	134.33	110.33	105.67	144.00
8	OMEA	253.67	261.33	250.67	249.33	245.00	240.67	259.00
9	PDA	187.33	157.67	126.33	139.00	135.00	144.67	160.33
10	RA	150.67	147.33	170.33	135.00	147.00	149.00	180.67
11	RBA	139.33	131.67	137.67	124.67	102.00	87.67	107.67
12	SA	144.67	139.00	165.00	135.33	143.33	132.67	145.00
13	TA	110.33	123.33	165.33	161.00	165.67	138.33	118.67
14	YEA	175.67	155.67	148.00	165.00	141.67	151.00	159.33
SE±	6.47	7.45	9.79	7.95	8.12	6.68	8.53	
CD	18.75	21.59	28.36	23.04	23.55	19.36	24.71	

Table 3 : Variability in number of sclerotial bodies per cm² of *S. rolfsii* isolates on different media.

S. no.	Media	Number of sclerotial bodies per cm² of *S. rolfsii* isolates						
		Sr1	Sr2	Sr3	Sr4	Sr5	Sr6	Sr7
1	BAM	1.81(1.50)	3.17(1.90)	2.30(1.66)	1.51(1.40)	3.08(1.88)	2.50(1.73)	2.85(1.82)
2	CA	3.08(1.88)	3.48(1.98)	3.44(1.98)	3.28(1.94)	4.42(2.21)	4.67(2.27)	5.71(2.49)
3	CMA	2.85(1.82)	2.84(1.82)	2.87(1.81)	3.39(1.97)	3.29(1.94)	3.07(1.88)	3.46(1.98)
4	CDA	3.25(1.92)	2.71(1.78)	3.28(1.93)	3.63(2.03)	4.11(2.14)	3.72(2.05)	3.65(2.03)
5	EA	1.80(1.51)	2.62(1.76)	2.18(1.63)	2.74(1.79)	2.86(1.83)	2.72(1.79)	2.84(1.82)
6	HLEA	4.49(2.22)	5.26(2.39)	5.82(2.50)	3.74(2.05)	4.03(2.12)	4.68(2.27)	4.57(2.25)
7	MEA	5.60(2.46)	4.37(2.19)	4.54(2.24)	3.61(2.02)	3.94(2.10)	5.02(2.34)	4.58(2.25)
8	OMEA	5.56 (2.45)	6.07(2.56)	5.44(2.43)	4.29(2.18)	4.76(2.29)	5.12(2.36)	5.73(2.49)
9	PDA	4.88(2.31)	4.33(2.18)	4.49(2.21)	5.55(2.45)	4.35(2.20)	4.90(2.31)	5.94(2.53)
10	RA	4.90(2.32)	4.16(2.15)	4.55(2.23)	5.72(2.49)	5.33(2.41)	5.13(2.37)	3.52(2.00)
11	RBA	4.49(2.23)	5.13(2.36)	5.43(2.42)	4.02(2.12)	3.81(2.07)	5.78(2.50)	5.63(2.47)
12	SA	4.39(2.20)	2.86(2.08)	3.48(1.97)	3.99(2.11)	5.22(2.39)	5.84(2.51)	4.56(2.24)
13	TA	5.28(2.39)	4.44(2.21)	5.04(2.41)	3.69(2.04)	4.58(2.25)	3.65(2.03)	4.63(2.26)
14	YEA	4.66(2.26)	4.87(2.30)	5.10(2.35)	5.48(2.44)	4.72(2.28)	5.15(2.37)	3.72(2.05)
SE±	0.11	0.12	0.13	0.06	0.06	0.07	0.06	
CD	0.33	0.35	0.38	0.17	0.18	0.20	0.18	

Note: Figures in the parenthesis are square root transformation values.

significant variation with respect to the number of sclerotia produced per cm² was also reported by Manjappa (1979), Palaiah (2002) and Prabhu (2003).

Abeygunawardena and Wood (1957) studied sclerotial production of *S. rolfsii* on nine different media, which included both synthetic and nonsynthetic media. Among liquid media, the sclerotial production were maximum in carrot extract and Weindling's medium. Among solid media, the sclerotial production were maximum on potato extract medium. Czapek's-Dox medium did not support the production of sclerotia.

Geographical variability among *S. rolfsii* populations was demonstrated by earlier workers (Harlton *et al*., 1995; Okabe *et al*., 1998). In India, Sharma *et al.* (2002)

studied variability among 26 isolates of *S. rolfsii* collected from various hosts/soil samples and localities. Studies of variability within the population in a geographical region are important because these also document the changes occurring in the population. The differences in sclerotial characteristics among isolates could be a useful parameter for differentiating isolates. Almeida *et al.* (2001) reported considerable variability among *Sclerotium rolfsii* isolates from Brazil in terms of number, size and location of sclerotia on the medium surface. While, Sarma *et al.* (2002) recorded remarkable variation in colony colour, diameter, sclerotia production (size and colour) among 26 isolates of *Sclerotium rolfsii* obtained from different host and geographical regions. Shukla and Pandey (2007) reported diversity in colony morphology, mycelial growth rate, sclerotial formation, size and colour of sclerotia of 32 isolates of *Sclerotium rolfsii* isolated from different hosts, soil samples and locations of Central India. While, Adondonen (2000) reported variation in growth rate, sclerotial number and time required for first appearance of sclerotia in *Sclerotium rolfsii* isolates collected from different villages in the Oueme valley.

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