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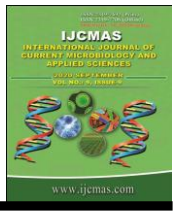

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Morphological Characterization of Kurnool Strains of Chickpea Collar Rot Casual Agent *Sclerotium rolfsii* Sacc.

M. Saileela[1], M. Lal Ahamed[1], J. V. Ramana[1*] and S. Khayum Ahamed[2]

[1]*Department of Molecular Biology and Biotechnology, APGC, Lam, Guntur, India*
[2]*Department of Plant Pathology, AICRP on Chickpea, RARS, Nandyal, India*

**Corresponding author*

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A B S T R A C T

Sclerotium rolfsii causing collar rot is a major disease affecting crop stand and yield in chickpea. A comprehensive study was performed to report the variability in *Sclerotium rolfsii* isolates present in a major chick pea growing area, Kurnool District of Andhra Pradesh. A roving survey was conducted to collect 50 samples and was used for isolation of disease causing pathogen. 18 isolates of *Sclerotium rolfsii* were isolated to study the variability in morphological and cultural characteristics in the present study. The variation noted for mycelial traits and sclerotial characters were very huge. The trait, total colony diameter, values after 72 hours of incubation ranged from 42 mm to 72 mm and was exploited to classify the isolates as slow, medium and fast. The colour of the mycelia colony was pure white to dull white while, colony morphology or topography varied from compact to fluffy in some isolates. Further, the trait, sclerotia initiation, varied significantly from 5 to 10 days indicating the aggressiveness of the isolates in causing the disease. Sclerotia colour was light brown to dark brown and black in KCSR 16 and the shape was round, spherical to oval and irregular in few. The test weight (100 sclerotia) was ranged from 40 to 320mg and the number varied from 25 to 124 indicating presence of sufficient variability among the isolates for characterization. This characterization of *S.rolfsii* isolates is necessary to devise effective management strategies to control the disease.

Introduction

Chickpea (*Cicer arietinum* L.; 2n=14) is a major legume of the world. It is an annual crop and had the capability to fix atmospheric nitrogen in the soil. It is a crop of subsistence farming and completes its life cycle within 90 days and provides economic benefits to the farming community. The desi and kabuli types are highly cultivated in India and the yields of these genotypes are highly fluctuating over the years. The reason for yield fluctuations is mainly due to the various biotic and abiotic stresses. Among the biotic stresses, diseases caused by the pathogens affecting root like wilts and root rots are of special significance as they affect the crop stand in the initial stages of crop growth and reduce the yield. *Sclerotium rolfsii* is one of the major pathogen of soil borne nature and

causes collar rot in chickpea. This disease is getting lot of importance at present because of its effect on the seedlings mortality in early stages of plant growth *i.e.*, 54.7-95% in conducive environment (Aycock *et al.*, 1966 and Kotasthane *et al.*, 1976) and yield loss of up to 10–30% in India (Maurya *et al.*, 2008). In Andhra Pradesh, the incidence of this disease is reported to be increasing year after year *i.e.*, 6.31% - 12.21% (Nagamani *et al.*, 2015).

The management strategies for this disease are with limited success as the pathogen has wide host range of 500 species, presence of variations among the isolates, growth habit in favourable environments, saprophytic nature and production of dormant structures like sclerotia during unfavourable environmental conditions. The existence of geographical variability among *S. rolfsii* populations was clearly presented by the authors like Harlton *et al.*, (1995), Nalim *et al.*, (1995), Okabe *et al.*, (1998), Punja and Sun (2001), Sarma *et al.*, (2002) and Shukla and Pandey (2007) but the presence of variation within the geographical regions is not well studied. This forms a crucial step in designing strategies to manage the disease as this helps in documentation of changes happen in the population (Sarma *et al.*, 2002) and also to understand the population structure in designing efficient management strategies to reduce disease outbreak (Epsita Swain *et al.*, 2018). Development of efficient breeding strategies to incorporate the resistance to these isolates in the cultivated types will help to overcome the problem and effective for a longer period of time. But isolates variability documentation in the cultivated regions of chickpea is very crucial for making proper management strategies. We are making concrete efforts to document the variability in chickpea growing areas of Kurnool district by collecting the samples. In the present study, efforts were made to document morphological variability among the *Sclerotium rolfsii* isolates of Kurnool district of Andhra Pradesh.

Materials and Methods

A roving survey was conducted to record occurrence and distribution of collar rot disease incidence in the fields of chickpea in Kurnool district of Andhra Pradesh during *rabi* 2019-2020.

Isolation of pathogen

The selected plant samples in the roving survey were used for the isolation of the pathogen. The pathogen was isolated using tissue segment method as suggested by Rangaswami and Mahadevan (1999). The infected collar region of plant was cut into small pieces of about 0.5 cm through sterilized scalpel blade and were surface sterilized with 1% sodium hypochlorite for 30sec followed by washing with sterile distilled water for three times. They were kept on blotting paper to avoid excess water and were later transferred onto petriplates containing PDA medium and incubated at 23±1°C. These cultures were kept under observation from time to time to note the growth of fungus. Axenic cultures of the fungus was obtained by single hyphal tip method and maintained on PDA. The fungus was identified based on mycelial and sclerotial characters (Barnett and Hunter, 1972).

Cultural and morphological variability

Eighteen isolates of *S. rolfsii* (KCSR 1-18) collected from different Mandals of Kurnool District, Andhra Pradesh were studied for their cultural and morphological characters on PDA. The mycelial disc of 4mm diameter of each isolate was placed in the centre of the plate and replicated twice. The inoculated

plates were incubated at 23±1°C for 20 days. They were studied for eight morphological characters based on mycelial (mycelia growth, colony colour, mycelial dispersion and appearance) and sclerotial (sclerotial colour, weight and shape, number of sclerotia and their arrangement on surface of media) parameters recorded from 3rd to 20th day of incubation, respectively, for each isolate (Bansal *et al.,* 1990) along with total growth and growth rate. Radial growth of each colony in two directions at right angles was measured. Visual observations on sclerotial formation were recorded.

Assessment of aggressiveness of isolates by pot culture method

The pathogen was further purified and multiplied on jowar grains to carry out aggression test. Jowar grains were boiled in fresh water for 30 minutes and drained excess water allowed to cool down by adding 5 per cent sucrose. 200g of jowar seeds are transferred into 500ml flasks and autoclaved. The flasks were allowed to cool at room temperature and inoculated with 5 mm culture discs of 5 days old *S. rolfsii* grown on PDA. Four discs per flask were added and the flasks were incubated for one week at 28 ± 1°C.

A pot culture experiment in the glass house was conducted to know the variation in aggressiveness of the eighteen isolates of *S. rolfsii* and to the pathogenic potential in terms of time taken for disease expression of each isolate of *S. rolfsii* and per cent of disease incidence on chickpea susceptible variety, L 550.

Soil inoculation method was adopted to test the aggressiveness among *S. rolfsii* isolates (Haware and Nene, 1980). The plastic pots of size 45x30 cm each containing 3kgs of autoclaved soil were used for raising the host plants. The two week old mass cultured *S. rolfsii* isolates was mixed with the soil in pots @ 50 g Kg^{-1} soil. The seeds of chickpea (var. L 550) were sown in the pathogen inoculated soil @ 10 seeds per pot. The observations were recorded up to 20 DAI by maintaining two replications in each treatment. Per cent disease incidence was calculated by using the following formula.

$$\text{PDI} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

Results and Discussion

The present study with 18 isolates of *Sclerotium rolfsii* collected from the chickpea growing villages of Kurnool district of Andhra Pradesh indicated the existence of considerable variation in terms of morphological and cultural traits of the mycelia and sclerotia.

Morphological variability in mycelial parameters

The data pertaining to the mycelia growth and growth rate of different isolates is presented in the Table 1 and Plate 1. The isolates showed great variability for total growth from 40 mm (KSCR 16) to 72 mm (KCSR 1) after 72 hrs/ 3DAI. The average total growth of the isolates was 56.22 mm. Ten isolates showed more than average total growth while eight isolates recorded lower than the average value of total growth. Each isolate growth rate varied from 15 mm/day (KCSR 16) to 25 mm/day (KCSR 11) and the average growth rate was 19.22 mm/ day. Nine isolates showed more than 19.22 mm/day growth while others showed less than 19.22 mm/day growth. This growth rate is used for the differentiating the isolates as slow, medium and fast growing types. The isolates with growth rate from 15 to 18 mm/day are grouped as moderate types (KCSR 5, KCSR6, KCSR7, KCSR12, KCSR13, KCSR15 and KCSR 16). The

isolates with growth rate of 19 to 22 mm/day are classified as fast growing isolates (KCSR 1, KCSR 2, KCSR 3, KCSR 4, KCSR 8, KCSR 10, KCSR 14, KCSR 17 and KCSR 18) while, growth rate of 23 to 26 mm/day are characterized as very fast growing types (KCSR9 and KCSR11). The highest and lowest growth rates per day are observed in KCSR 11(25) and KCSR 16(15.2), respectively This data clearly indicated the existence of fast growing isolates in Kurnool district of Andhra Pradesh.

The mycelial colony characters (colony colour and appearance) of *S. rolfsii* isolates on PDA are presented in Table 2. The mycelial colour was varied from pure/extra white (KCSR 2, KCSR 6, and KCSR 7) to dull/ cottony white (KCSR 15 and KCSR 16) and white in other 13 isolates. The appearance of the isolates, KCSR 10, KCSR 11 and KCSR 14, was flower like while the isolates, KCSR 1, KCSR 5, KCSR 8, KCSR 15 and KCSR 16, showed cottony growth on the media. The isolates, KCSR 6 and KCSR 7, appearance was dense mat like and the isolates, KCSR 4 and KCSR 9, showed wavy like pattern on the growth media. Among the isolates, KCSR 16 showed upright growth and KCSR 17 appearance was sparse on the media indicating these isolates different in its appearance and growth of the mycelia per day. Some isolates were similar in their morphological traits and growth pattern which is due to the genetic differences only as they were grown in controlled conditions on PDA (Okereke and Wokocha, 2007).

Morphological variability in sclerotial parameters

The sclerotial characteristics like time for their formation, colour, site of production, number and weight of 100 sclerotia varied in the studied isolates of *S. rolfsii* and the results are presented in the Table 3 and Plate 2.

The isolates took five to ten days for sclerotial production. The isolates KCSR 13 and KCSR 18 took 5 days for sclerotial production revealing the early entry into sclerotial formation. The colour of sclerotia showed great variation from dark brown (KSCR 1, KCSR 2, KCSR 5, KCSR 6, KCSR 10, KSCR 13 and KSCR 15), brown (KSCR 7, KSCR 9, KCSR 12 and KSCR 14), light brown (KCSR 3, KSCR 4, KSCR 8, KSCR 11 and KSCR 17) and golden brown in KCSR 18 to black in KSCR 16. The sclerotial production site in the isolates showed huge variation and the isolates, KCSR 1, KCSR 2, KCSR 4 and KCSR 5 produced clustered/ grouped sclerotial bodies; KCSR 3, KCSR 9, KCSR 10, KCSR 13, KCSR 14 and KCSR 17 produced single scattered sclerotial bodies all over the plate; KCSR 6, KCSR 7 and KCSR 8 produced sclerotial bodies to side walls of petriplate; KCSR 12 produced scleotial bodies around periphery; KCSR 11 and KCSR 18 produced sparse sclerotial bodies at periphery; KCSR 15 and KCSR 16 produced scleotial bodies on top of petriplate. Variations in sclerotial parameters have been reported by different scientists on various hosts and media (Sarma *et al.,* 2002; Palaiah and Adiver, 2006 and Srividya *et al.,* 2018).

The number of sclerotia ranged from 25 (KCSR 18) to 124 (KCSR 13) among isolates after 20 days of incubation. The weight of 100 sclerotia varied from 40mg (KCSR 1) to 320mg (KCSR 16). But the number of sclerotia is inversely proportional to the weight of 100 sclerotia as it was clearly seen in the isolates, KCSR 2, KCSR 3, KCSR 16 and KCSR 18 where the number is less but the weight is more while some of the isolates showed high sclerotia number with minimum weight as seen in KCSR 1. Some produced very less number of sclerotia with with minimal weight. The variation in sclerotial forming capacity could be a useful parameter in characterizing the isolates in the present

study as the number of sclerotia showed great variability in the study.

The very fast growing types produced sclerotia in 8-9 days but the number of sclerotia was varied among the isolates. The isolate, KSCR 11, produced sclerotia in 8 days with the number of sclerotia were 75 at 20 DAI. This is in confirmation with the report of Komathi (2002) who reported that highly virulent strains exhibited very rapid growth and produced huge number of sclerotia in the culture. Morphological characterization of isolates based on variability in mycelia and sclerotial parameters in *Sclerotium rolfsii* has been reported by different scientists on different hosts (Punja and Grogan, 1983; Harlton *et al.*, 1995; Punja and Damiani, 1996; Zarani and Christensin, 1997; Butler and Day, 1998; Okabe *et al.*, 1998; Carpenter *et al.*, 1999; Almeida *et al.*, 2001; Sarma *et al.*, 2002; Adandonon *et al.*, 2005; Palaiah and Adiver, 2006; Okereke and Wokocha, 2007; Akram *et al.*,2007; Shukla and Pandey, 2007; Sachin *et al.*, 2009; Rakholiya and Jadeja, 2011; Sharma *et al.*, 2013; Thilaghavathi Rasu *et al.*, 2013; Manjunath *et al.*, 2014; Reddi *et al.*, 2014; Jabbar *et al.*,2014; Manu *et al.*,2018; Poonam *et al.*,2018; Srividya *et al.*, 2018).

Table.1 Total growth and growth rate of 18 isolates of *S. rolfsii* on PDA after 72 hrs of inoculation

S. No.	Isolate	Growth (mm)	Growth rate (mm/day)
1	KCSR 1	72	19.5
2	KCSR 2	60	20.0
3	KCSR 3	**60**	**20.2**
4	KCSR 4	60	20.0
5	KCSR 5	56	18.0
6	KCSR 6	42	16.0
7	KCSR 7	52	16.0
8	KCSR 8	54	22.0
9	KCSR 9	68	23.0
10	KCSR 10	64	22.0
11	KCSR 11	**60**	**25.0**
12	KCSR 12	50	16.0
13	KCSR 13	58	19.0
14	KCSR 14	52	18.5
15	KCSR 15	44	16.0
16	KCSR 16	**40**	**15.2**
17	KCSR 17	62	21.0
18	KCSR 18	58	19.0
Average		**56.2**	**19.2**

Table.2 Colony characteristics of 18 isolates of *S. rolfsii*

S. No.	Isolate	Growth	Colony colour	Mycelial Appearance and dispersion
1	KCSR 1	Fast	White	Slight cottony, sparse at centre ,dense at margins.
2	KCSR 2	Fast	Extra white	Thin strands at centre, dense at margin upright growth.
3	KCSR 3	Fast	White	Aggregate at centre thick strands towards margin.
4	KCSR 4	Fast	White	Sparse, thread like thin strands.
5	KCSR 5	Moderate	White	Aggregate at centre, light cottony thin strands, branches like pattern towards margin.
6	KCSR 6	Moderate	Extra white	Mat like appearance sparse at centre thick strand towards margin.
7	KCSR 7	Moderate	Extra white	Mat like appearance sparse at centre thin strand towards margin.
8	KCSR 8	Fast	White	Slight cottony upward growth at centre, thin strands, suppressed, wavy like pattern towards margin.
9	KCSR 9	Very Fast	White	Thin strands, suppressed, wavy like pattern.
10	KCSR 10	Fast	White	Flower like appearance thin strands.
11	KCSR 11	Very Fast	White	Flower like appearance dense around centre and sparse toward margin.
12	KCSR 12	Moderate	White	Thick strand branches like pattern.
13	KCSR 13	Moderate	White	Thin hair like sparse growth, dense around centre.
14	KCSR 14	Fast	White	Flower like appearance, sparse growth dense towards margin.
15	KCSR 15	Moderate	Cottony white	Fluffy, dense at margins, aggregate at centre& upright growth.
16	KCSR 16	Moderate	Cottony white	Fluffy, dense at margins, aggregate at centre& upright growth.
17	KCSR 17	Fast	White	Thin strands, suppressed, wavy like pattern.
18	KCSR 18	Fast	White	Sparse at centre thin strands dense towards outer margin.

Table.3 Sclerotial characteristics of 18 isolates of *S. rolfsii*

S. No.	Isolate	Days to formation	Colour	Shape	Arrangement	Number at 20 DAI	100 sclerotia weight (g)
1	KCSR 1	10	Dark brown	Round	Clustered at centre and periphery	96	40
2	KCSR 2	9	Dark Brown	Round	Clustered to sides of petriplate	48	180
3	KCSR 3	9	Light Brown	Irregular	Scattered	32	140
4	KCSR 4	8	Light Brown	Round	Clustered at peripheral	52	220
5	KCSR 5	7	Dark Brown	Oval	Peripheral single	94	140
6	KCSR 6	10	Dark Brown	Spherical	Peripheral clustered	40	180
7	KCSR 7	10	Brown	Oval	To sides of petriplate	65	130
8	KCSR 8	8	Light Brown	Spherical	To sides of petriplate	99.5	190
9	KCSR 9	9	Brown	Irregular	Scattered all over plate	97	90
10	KCSR 10	8	Dark Brown	Round	Scattred all over plate	84	150
11	KCSR 11	8	Light Brown	Round	Peripheral and top of petriplate	75	100
12	KCSR 12	8	Brown	Oval	around peripheral	80	120
13	KCSR 13	5	Dark Brown	Round	Scattered all over plate	124	130
14	KCSR 14	7	Brown	Spherical	Scattered on periphery	50	110
15	KCSR 15	9	Dark brown	Round	Scatterd on top of the petriplate	90	170
16	KCSR 16	8	Black	Spherical	Scatterd on top of the petriplate	72	320
17	KCSR 17	9	Light Brown	Round	Scattered	51	70
18	KCSR 18	5	Golden brown	Round	Peripheral	25	100

Table.4 Aggressiveness of different isolates of *S. rolfsii* at 20th DAI on Chickpea variety L 550

S. No.	Isolate	Time taken for disease expression (DAI)	Per cent of disease incidence (PDI)
1	KCSR 1	11	75.00
2	KCSR 2	15	44.44
3	KCSR 3	10	84.50
4	KCSR 4	9	90.75
5	KCSR 5	12	77.56
	KCSR 6	**18**	**14.28**
7	KCSR 7	17	25.00
8	KCSR 8	12	81.55
9	KCSR 9	12	79.50
10	KCSR 10	16	37.78
11	KCSR 11	11	71.60
12	KCSR 12	9	90.00
13	KCSR 13	10	87.00
14	KCSR 14	9	91.00
15	KCSR 15	13	69.78
16	KCSR 16	**8**	**93.33**
17	KCSR 17	14	52.22
18	KCSR 18	10	81.00
19	Control	-	0.00

Mean of two replications

Plate.1 Mycelial growth of *S. rolfsii* isolates on PDA after 7 DAI

(A) **(B)** **(C)**

KCSR 1- KCSR 6 **KCSR 7 – KCSR 12** **KCSR 13 –KCSR 18**

Plate.2 Sclerotia production of *S. rolfsii* isolates on PDA after 20 DAI

(A) **(B)** **(C)**

KCSR 1- KCSR 6 **KCSR 7 – KCSR 12** **KCSR 13 –KCSR 18**

Assessment of aggressiveness of isolates by pot culture method

The pot culture experiment was conducted to test the aggressiveness *i.e.*, quantitative variation of pathogenicity on susceptible host, of each isolate on chickpea susceptible variety, L 550 through Percentage of Disease Incidence (PDI). The pathogen was inoculated in jowar grains for mass multiplication and the multiplied isolates were mixed with the soil in pots. The seedling mortality was recorded at 20 days after pathogen inoculation and calculated the per cent of disease incidence (PDI).

The aggressiveness of isolates was studied on chickpea variety L 550 along with a control and the results are presented in the Table 4. The PDI values ranged from 14.28 (KCSR 6) to 95.67 (KCSR 16) among the isolates indicating huge variability among the isolates in disease causing nature. The isolates, KCSR 14, (91%) KCSR 4 (90.75%) and KCSR 12 (90.00%) also showed very high PDI values while, the isolates, KCSR 6 (14.28), KCSR 7 (25%) and KSCR 10 (37.78%) recorded lower values of PDI. The isolate KCSR 16 took eight days for the disease expression whereas the isolate KCSR 6 took 18 days indicating the less aggressiveness of the isolate in causing disease. Thus, the isolate, KCSR 16 is considered as the most aggressive isolate as it produced the symptoms in eight days with high PDI among the isolates followed by the isolates, KCSR 14, KCSR 4, KCSR 12 and KSCR 13. Similar reports of identification of aggressive isolates were reported in the studies of Santha Lakshmi *et al.*, (2010), Muthukumar and Venkatesh, (2013), Sivakumar *et al.*, (2016), Daniel Jebaraj *et al.*, (2016), Dabbas and Shrawan Kumar (2016), Ranga Rani *et al.*, (2017), Durga Prasad *et al.*, (2018) and Anitha Kumari and Abhjeet Ghatak (2018).

The presence of huge variations among the isolates of *S. rolfsii* can be attributed to the differences in physiological and metabolic rates exist in the isolates arising from different geographical and ecological systems and also their biochemical variability to adapt

to the local conditions. The existence of variability among *S. rolfsii* populations was reported by earlier workers (Harlton *et al.,* 1995 and Okabe *et al.,* 1998). The studies on variability within the population of a geographical region are crucial as these studies documented the changes occurring in the population over time periods and facilitate us to design a effective strategy to manage the disease.

Thus, the study reported great variability in the pathogen in a limited area. The variation in cultural, mycelial and sclerotial morphological traits and pathogenicity amongst the isolates revealed that *S. rolfsii* can be characterized by a combination of these traits. The differences in morpho and cultural traits can be taken as potential parameters for differentiating isolates. Further, morphological characterization is a basic key for identification and for devising effective strategies to manage the disease including resistance breeding programmes.

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