Int.J.Curr.Microbiol.App.Sci (2017) *6*(11): 3457-3469

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 11 (2017) pp. 3457-3469**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.611.407

Cultural and Morphological Characterization of *Rhizoctonia solani* f. sp. *sasakii* Isolates Collected from Different Districts of Andhra Pradesh

Bindu Madhavi Gopireddy[1*], G. Uma Devi[2], K.Vijayakrishna Kumar[1], T. Ramesh Babu[1] and T.C.M.Naidu[1]

[1]Acharya N G Ranga Agricultural University, Guntur, Andhra Pradesh, India
[2]Professor Jayasankar Telangana State Agricultural University, Hyderabad, Telangana, India
**Corresponding author*

Keywords

Maize, BLSB, *Rhizoctonia solani* f. sp. *sasakii*, Morphological, Cultural characterization.

Article Info

Accepted:
26 September 2017
Available Online:
10 November 2017

A B S T R A C T

Sixty BLSB affected maize samples were collected from three districts and pathogen was isolated and identified based on morphological, cultural and sclerotial characters using standard descriptions of IMI. Light microscopic studies revealed that all the isolates of *R. solani* f. sp. *sasakii*are characteristically branched out at right angle in the distal end of the cell and showed a characteristic constriction at the point of branching. Formation of septum near the point of origin of the branch / adjacent to branch was present in most of the isolates except for isolates RS 44, RS 48, RS 58 and RS 59, where in the septum was slightly away from the origin of branching. The hyphal width of all the 60 isolates varied from 5.05 µm (RS 52) to 7.98 µm (RS 15). Out of 60 maize isolates, eight isolates *i.e.,* RS 7, RS 8, RS 9, RS 10, RS 11, RS 12 RS 16 and RS 26 produced barrel shaped monilliod cells and the remaining isolates produced irregular shaped monilliod cells. Clamp connections were absent in all the isolates, multinucleate and the number of nuclei per cell varied from five to seven in the isolates.

Introduction

Maize (*Zea mays* L.) is one of the most versatile emerging crops having wider adaptability under varied agro-climatic conditions. Globally, maize is known as queen of cereals due to its high genetic yield potential. Among the potential factors that limit maize production, fungal diseases are reported to cause extensive crop yield reduction in many countries and are considered as a priority in disease management practice (Agrios, 2005). Of different fungal diseases affecting maize cultivation, banded leaf and sheath blight (BLSB) incited by *Rhizoctonia solani* f. sp. *sasakii* Exner (*Thanatephorus sasakii* (Shirai) Tu and Kimbrough) (Tu and Kimbrrough, 1978) is an economically significant disease causing huge losses in all crop growing areas of the world. Increased incidence of BLSB has been observed in rice fallow maize crop (zero tillage) in different districts of Andhra Pradesh. Effective management of BLSB in maize is possible only when the pathogen is eliminated completely or the propagules are brought down below economic threshold limits at field level. Control measures used were partly effective because *R. soiani* is able to produce sclerotia that can persist in the soil for at least two years (Ou, 1985). The pace of

development and durability of resistant varieties had been slow and unreliable despite tremendous advancements in the field of plant genetic engineering. Variability of the pathogen plays major role in resistance breeding hence the cultural and morphological characterization of the isolates was studied.

Materials and Methods

To study the morphology of hyphae of each BLSB pathogenic isolate, four day old-fungal hyphae grown on PDA medium was taken and stained with 0.1 per cent lactophenol cotton blue on microscopic slides for recording, angle of branching, septation, presence of monilioid cells, presence of clamp connections, type of septum and hyphal width and number of nuclei using Olympus CX31 microscope with ProgRes. CT3 image analyser.

Cultural characteristics of *Rhizoctonia solani* f. sp. *sasakii* isolates (Fig. 1)

Growth of fungal pathogen

The isolates were grown on PDA medium in Petri dishes at 27 +2ºC until the hyphae reached the periphery of the petridishes for determination of color, abundance of mycelium, zonation and sclerotial characters.

Colony characters

Abundance of mycelium was compared with the key given by Burpee *et al.,* (1980).

The abundance was characterized into the following four categories.

Slight: Aerial mycelium does not obscure surface mycelium

Moderate: Aerial mycelium obscure surface mycelium and does not touch the cover of Petri dishes

Abundant: Aerial mycelium obscure surface mycelium and touches the cover of Petri dishes

No aerial mycelium

Colony color was determined with the help Munsell's soil colour chart (Munsell color Company, Inc., 1954).

The culture and key color cards were placed side by side against white background under sunlight for comparison (Burpee *et al.,* 1980).

Observations for colony color were recorded 10 days after incubation.

Based on the colony pigmentation, the cultures were assigned to different groups based on dominant spectral color.

Sclerotial characteristics of *Rhizoctonia solani* f. sp. *sasakii* isolates

Sclerotial characteristics were also compared with the key given by Burpee *et al.,* (1980).

Location of sclerotia

Based on location of sclerotial production in the culture the isolates of *R. solani* f. sp. *sasakii* were categorized into following groups

Aerial: Sclerotia formed with in aerial mycelium

Embedded: Sclerotia formed with in substrate

Size of sclerotia

The size of sclerotia of the sixty isolates that were 10-day-old were observed by keeping

the Petri dishes under the stereo binocular microscope and were classified as (a) Large (b) Small

Colour of the sclerotia

It was categorized in to 4 groups a) Light brown (b) Brown (c) Dark brown (d) Deep dark brown

Location and Pattern of sclerotial formation

Location of sclerotia produced by different isolates in the Petri dishes containing PDA were observed and recorded as sclerotia produced on the surface of agar (aerial) or submerged in the medium. The pattern of the sclerotial production was also studied and the isolates were divided into different categories based on their distribution on the culture. The production of sclerotia by different isolates was recorded as more or less circular manner concentrated towards periphery; irregularly scattered but more towards the centre of the colony; irregular very sparsely scattered and scattered irregularly all over the colony surface. Sclerotial types were mainly divided into two categories as follows.

Sclerotia with rough border
Sclerotia with smooth border

Results and Discussion

Morphological variability among the isolates of *Rhizoctonia solani* f. sp. *sasakii* of maize

Morphological characters are the important basic factors for identification of a fungus and its variability. Studies on morphological characteristics of *R. solani* f. sp. *sasakii* maize isolates (60 numbers) and RS 61 were studied and the results are presented in Table 1.

Light microscopy studies revealed that all the isolates of *R. solani* f. sp. *sasakii* characteristically branched out at right angle in the distal end of the cell (Plate 1).

Constriction at the point of branching

All isolates showed a characteristic constriction at the point of branching. Formation of septum near the point of origin of the branch / adjacent to branch was present in most of the isolates while in isolates RS 44, RS 48, RS 58, RS 59 the septum was slightly away from the origin of branching.

Hyphal width

The hyphal width of all the 60 isolates varied from 5.05 µm (RS 52 from Krishna district) to 7.98 µm (RS 15 from Prakasam district). The hyphal widths of most of the isolates were at par with each other. However, the differences in hyphal width observed among the other isolates were non- significant.

Monilioid cells

In addition to ordinary vegetative hyphae, *R. solani*produces simple or branched chains of short broad cells, which may be hyaline or brown, barrel shaped, pyriform, irregular, or lobate known as monilioid cell (Plate1). Out of 60 maize isolates, eight isolates *i.e.*, RS 7, RS 8, RS 9, RS 10, RS 11, RS 12 RS 16 and RS 26 produced barrel shaped monilioid cells. The remaining isolates produced irregular shaped monilioidcells .

Clamp connections: were absent in all the isolates

Observation for the mycelium branching out at right angles, presence of characteristic constriction at the point of branching and formation of septum near the point of origin of the branch, hyphal width >5.00µm, presence of monilioid cells, absence of clamp connections were the characters of immense

taxonomical importance which were described by the previous workers Duggar (1915), Matsumato (1921), Thomas (1925).

Number of nuclei

All the isolates under present investigation were found to be multinucleate and the number of nuclei per cell varied from five to seven. Isolates RS 21, RS 23, RS 25, RS 26, RS 27 had statistically maximum number (7) of nuclei per cell (Table 1).

The classification of *Rhizoctonia solani*has been done on the basis of hyphal and cultural, morphology, nuclear condition, hyphal anastomosis and morphology of teleomorphs. The present findings on morphological variability among *R. solani* isolates are in accordance with Sneh *et al.,* (1991), Amita Singh *et al.,* (1999), Meena *et al.,* (2001) and Srinivas (2002) in maize, Singh *et al.,* (2002) and Basu *et al.,* (2004) in rice.

Cultural variability of *R. solani* isolates

Colony colour

The colour of the colony varied from white to dark brown. Based on pigmentation dominant spectral colour from Munsell's soil colour chart (1954), the cultures were assigned to five colour groups with respective shade numbers *i.e,* Group I- white, Group II-yellowish white, Group III-pale brown and Group IV brown and Group V dark brown (Table 1).

Among the 60 isolates studied, 11 isolates belonged to group I, 9 isolates with yellowish white were assigned in group II, 22 isolates in Group III, ten isolates in group IV and eight in Group V. The variation in the colour of the colony might be attributed to the production of pigments by the pathogen. The differences in the intensity of the colour might also correspond to the amount of pigments released by respective isolate in the media. The colour production may also be due to release of other secondary metabolites like toxins. Amita Singh *et al.,* (1999) assigned Munsell'ssoil colour chart shade number to the colony colour of *R. solani* isolates from rice, maize, soybean, mung beans and cotton. Further, Akhtar *et al.,* (2009) stated that the colony colour of *R. solani* maize isolates *Hc* and *It* were brown whereas the isolates *Bc, Jr* and *Rf* had white colour. Studies on cultural characteristics revealed that the colony colour of different *R. solani* isolates varied from white to brown on PDA (Khodayari *et al.,* 2009). The results are also in agreement with the observations of other researchers (Sneh *et al.,* 1991; Sweetingham and Mac Nish, 1994; Amita Singh *et al.,* 1999). Srinivas (2002) categorised maize isolates of *R. solani*causing BLSB disease based on colony pigmentation.

Abundance of mycelium

Among 60 isolates, 27 isolates produced abundant mycelium, while 18 isolates have moderate mycelium and the remaining 15 isolates recorded slight/ sparse mycelium.

Colony diameter and growth rate

The data presented in Table 1 on colony diameter and growth rate revealed that there were significant differences among the isolates after 72 hours of incubation on PDA medium. Among the 60 isolates, 29 isolates recorded as fast growers (more than 40 mm growth) and 21 as moderate (35-40mm growth) and ten recorded slow growth (30-35 mm) after 72 h of incubation.

The cultural characteristics studied among the *R. solani* isolates with respect to the colony colour, abundance of mycelium, colony diameter and growth rate revealed the existence of significant variation.

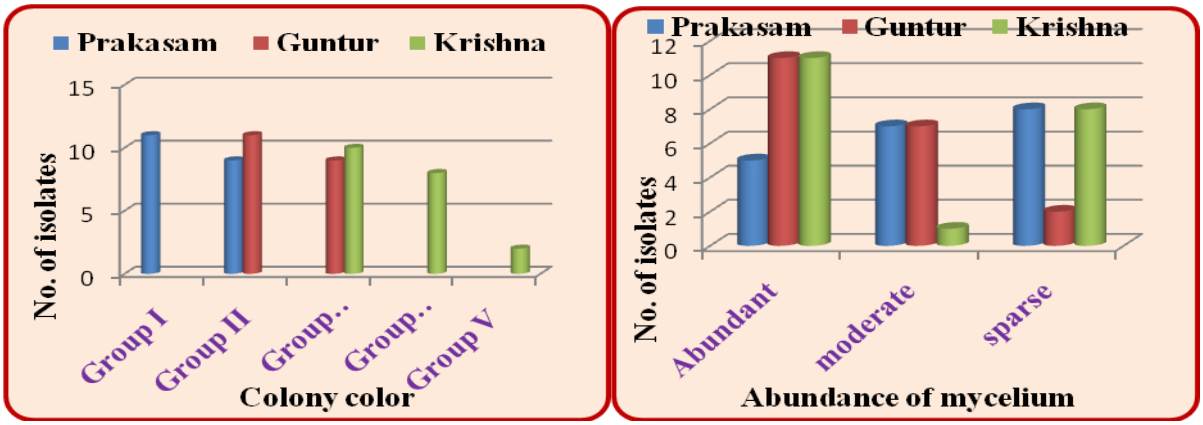
Distinct differences were observed in the colony appearance and the isolates were categorised into different groups based on texture and abundance of mycelium. The difference in the colony growth was distinct in 27 isolates. These isolates produced abundant aerial cottony mycelial growth which may be due to the inherent nature of these isolates to go for quick and profuse mycelial growth in early stages of growth before setting the sclerotia.

Similar observations have been made by Toda *et al.*, (1999) who divided *Rhizoctonia* AG-D isolates into two subgroups AG-D (I) and AG-D (II), based on the results of cultural characteristics; Srinivas (2002) categorised the *R. solani* f. sp. *sasakii* isolates from maize based on texture and abundance of their mycelia growth and colony appearance. Similarly Guleria *et al.*, (2007) used cultural characters for differentiating the *R. solani* isolates from rice.

Significant variations with respect to colony growth and growth rate were also recorded among the isolates under the study. The isolates RS 34, RS 26, RS 25 having fast growth rate were found more virulent as they induced susceptible reaction on maize. Meena *et al.*, (2003) observed that the fast growing isolate of *R. solani* from maize was found to be more virulent on a susceptible maize cultivar.

Similarly, Guleria *et al.*, (2007), Thind and Aggarwal (2008) and Khodaryari *et al.*, (2009) stated that the *R. solani* isolates from rice were fast growing with >20 mm mycelia growth rate per day indicating their fast growing nature. Rapid growth rate among *R. solani* isolates have also been reported by Peltier (1916), Matz (1921), Matsumoto (1934) and Parmeter and Whitney (1970).

Fig.1 Cultural and sclerotial characteristics of different isolates of *Rhizoctonia solani* f. sp. *sasakii*



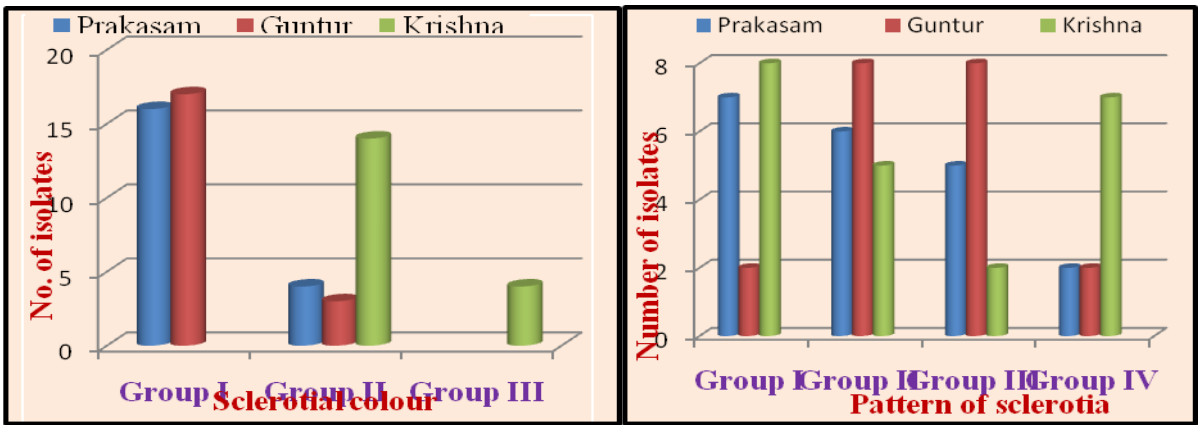


Table.1 Cultural characteristics of different isolates of *Rhizoctonia solani* f.sp. *sasakii* collected from Prakasam, Guntur and Krishna districts of Andhra Pradesh

Isolate	Hyphal width (µm)	Number of nuclei	Colour of the colony	Colony diameter after 72h (mm)	Growth rate*	Growth pattern	Time taken to initiate Sclerotia (days)
RS 1	5.35	6	Pale brown	66	Fast	Moderate	4
RS 2	5.40	5	Pale brown	57	Fast	Moderate	4
RS 3	6.05	5	White	45	moderate	Abundant	4
RS 4	5.83	5	White	69	Fast	Moderate	5
RS 5	7.05	6	White	44	moderate	Slight	5
RS 6	7.27	6	White	29	slow	Slight	6
RS 7	6.45	6	Pale brown	75	Fast	Moderate	4
RS 8	5.95	5	Pale brown	78	Fast	Abundant	4
RS 9	6.10	5	White	42	Moderate	Slight	5
RS 10	5.12	6	White	30	Slow	Slight	6
RS 11	5.37	6	White	45	Moderate	Slight	5
RS 12	5.55	5	White	44	Moderate	Slight	5
RS 13	6.65	6	Pale	45	Moderate	Abundan	5
RS 14	7.23	6	Pale brown	22	slow	Moderate	7
RS 15	7.98	6	Pale brown	29	slow	Slight	7
RS 16	6.45	6	White	64	Fast	Abundant	4
RS 17	5.08	5	Pale brown	40	Moderate	Abundant	5
RS 18	5.11	5	White	39	Moderate	Moderate	5
RS 19	5.86	5	White	28	slow	Slight	6
RS 20	6.65	7	Pale brown	39	Moderate	Moderate	5
RS 21	5.84	7	Yellowish white	65	Fast	Abundant	4
RS 22	5.48	6	Pale brown	69	Fast	Abundant	4
RS 23	6.44	7	Pale brown	56	Fast	Abundant	4

RS 24	5.23	6	Pale brown	41	Moderate	Abundant	4
RS 25	6.15	7	Pale brown	66	Fast	Moderate	5
RS 26	6.87	7	Yellowish white	69	Fast	Abundant	4
RS 27	5.21	7	Yellowish white	70	Fast	Abundant	4
RS 28	5.65	6	Yellowish white	66	Fast	Abundant	4
RS 29	6.51	6	Pale brown	62	Fast	Abundant	4
RS 30	7.57	6	Pale brown	60	Fast	Moderate	4
RS 31	7.14	5	Pale brown	44	Moderate	Moderate	4
RS 32	6.16	5	Yellowish white	51	Fast	Moderate	4
RS 33	5.20	5	Pale brown	55	Fast	Moderate	4
RS 34	6.80	6	Yellowish white	26	slow	Abundant	5
RS 35	7.12	6	Yellowish white	61	Fast	Abundant	4
RS 36	7.05	6	Pale brown	72	Fast	Slight	6
RS 37	6.55	7	Pale brown	29	Slow	Slight	6
RS 38	5.33	5	Yellowish white	46	Fast	Moderate	4
RS 39	5.84	6	Pale brown	55	Fast	Abundant	4
RS 40	6.81	6	Yellowish white	41	Moderate	Moderate	5
RS 41	6.25	6	Brown	32	Moderate	Slight	5
RS 42	6.13	6	Dark brown	34	Moderate	Slight	5
RS 43	5.68	6	Brown	30	Moderate	Slight	5
RS 44	5.26	6	Pale brown	31	Moderate	Slight	6
RS 45	5.11	5	Dark brown	40	Fast	Abundant	5
RS 46	6.60	5	Dark brown	42	Fast	Abundant	5
RS 47	6.47	6	Dark brown	44	Fast	Abundant	6
RS 48	6.80	6	Pale brown	44	Fast	Abundant	6
RS 49	7.55	6	Brown	45	Fast	Abundant	6
RS 50	5.45	6	Dark brown	50	Fast	Abundant	6
RS 51	5.83	6	Dark brown	54	Fast	Abundant	6
RS 52	5.05	6	Brown	52	Fast	Abundant	6
RS 53	6.25	6	Brown	36	Moderate	Abundant	8
RS 54	7.30	6	Dark brown	32	Slow	Moderate	10
RS 55	7.87	6	Brown	38	Moderate	Abundant	8
RS 56	6.48	5.5	Brown	39	Moderate	Abundant	8
RS 57	6.05	6	Brown	32	Moderate	Slight	8

RS 58	7.20	6	Brown	22	Slow	Slight	No sclerotia
RS 59	7.83	5.5	Dark brown	29	Slow	Slight	No sclerotia
RS 60	5.65	6.5	Brown	35	Moderate	Slight	8
RS61	7.44	6	White	49	Fast	Slight	6
S. Em ±	0.056	0.266					
CD (0.05%)	0.157	0.737					

*Scale
Isolates showing more than 40 mm growth after 72 h (fast growers)
Isolates showing more than 35- 40 mm growth after 72 h (moderate growers)
Isolates showing more than 30- 35 mm growth after 72 h (slow growers)

Table.2 Sclerotial characteristics of different isolates of *Rhizoctonia solani* f. sp. *sasakii*

Isolate	**Colour of sclerotia**	**Number of sclerotia/ petri dish**	**Manner of sclerotial formation**	**Location of sclerotia**	**Clump formation**	**Size**	**Texture of sclerotia**
RS 1	Light brown	Very good	Centre	Aerial surface	Moderate	Large	Rough
RS 2	Dark brown	Very good	Peripheral	Aerial surface	Moderate	Large	Rough
RS 3	Light brown	Very good	Centre	Aerial surface	Moderate	Large	Rough
RS 4	Light brown	Good	Centre	Aerial surface	Moderate	Large	Rough
RS 5	Light brown	Good	Centre	Aerial surface	Moderate	Micro	Smooth
RS 6	Light brown	Excellent	Peripheral	Embeded	Less	Small	Smooth
RS 7	Light brown	Very good	Peripheral &centre	Aerial surface	Less	Small	Smooth
RS 8	Light brown	Very good	Scatter	Aerial surface	Less	Large	Rough
RS 9	Light Brown	Good	Peripheral &centre	Aerial surface	Moderate	Large	Rough
RS 10	Dark brown	Very good	Peripheral	Embeded	Moderate	Large	Rough
RS 11	Light brown	Very good	Peripheral &centre	Aerial surface	Less	Large	Rough
RS 12	Light brown	Good	Peripheral &centre	Embeded	Moderate	Large	Rough
RS 13	Light brown	Good	Centre	Aerial surface	Moderate	Small	Smooth
RS 14	Light brown	Good	Centre	Aerial surface	Less	Large	Rough
RS 15	Light brown	Good	Peripheral	Aerial surface	Less	Large	Rough
RS 16	Light brown	Very good	Peripheral &centre	Aerial surface	Less	Large	Rough
RS 17	Dark brown	Very good	Scatter	Aerial surface	Less	Large	Rough
RS 18	Light brown	Very good	Centre	Aerial surface	Moderate	Large	Rough
RS 19	Light brown	Very good	Peripheral	Embeded	Less	Large	Rough
RS 20	Dark brown	Good	Peripheral	Aerial surface	Less	Small	Smooth
RS 21	Light brown	Very good	Peripheral	Aerial surface	Moderate	Large	Rough
RS 22	Light brown	Very good	Peripheral	Aerial surface	Moderate	Large	Rough
RS 23	Light brown	Very good	Peripheral	Aerial surface	Moderate	Large	Rough
RS 24	Light brown	Very good	Peripheral	Aerial surface	Moderate	Large	Rough
RS 25	Light brown	Good	Peripheral	Aerial surface	More	Large	Rough
RS 26	Light brown	Very good	Peripheral &centre	Aerial surface	Moderate	Large	Rough
RS 27	Light brown	Excellent	Peripheral	Aerial surface	Moderate	Large	Rough
RS 28	Light brown	Excellent	Peripheral	Aerial surface	Moderate	Large	Rough

			&centre				
RS 29	Light brown	Very good	Peripheral &centre	Aerial surface	Moderate	Large	Rough
RS 30	Light brown	Very good	Peripheral &centre	Aerial surface	More	Large	Rough
RS 31	Light brown	Good	Peripheral &centre	Aerial surface	Less	Large	Rough
RS 32	Light brown	Good	Peripheral	Aerial surface	Less	Large	Rough
RS 33	Light brown	Very good	Scatter	Aerial surface	Less	Large	Rough
RS 34	Light brown	Excellent	Peripheral	Aerial surface	Less	Large	Rough
RS 35	Light brown	Very good	Peripheral	Aerial surface	More	Large	Rough
RS 36	Light brown	Good	Peripheral &centre	Aerial surface	Moderate	Large	Rough
RS 37	Light brown	Good	Peripheral &centre	Aerial surface	More	Large	Rough
RS 38	Dark brown	Good	Peripheral &centre	Aerial surface	Moderate	Large	Rough
RS 39	Dark brown	Good	Peripheral &centre	Aerial surface	Moderate	Large	Rough
RS 40	Dark brown	Good	Scatter	Aerial surface	Moderate	Large	Rough
RS 41	Dark brown	Good	Centre	Aerial surface	More	Large	Rough
RS 42	Dark brown	Good	Peripheral	Embeded	More	Large	Rough
RS 43	Dark brown	Good	Centre	Embeded	More	Large	Rough
RS 44	Dark brown	Good	Centre	Embeded	Moderate	Large	Rough
RS 45	Dark brown	Good	Centre	Aerial surface	Moderate	Large	Rough
RS 46	Dark brown	Good	Scatter	Embeded	More	Large	Rough
RS 47	Dark brown	Very good	Scatter	Aerial surface	More	Large	Rough
RS 48	Deep dark brown	Very good	Scatter	Aerial surface	More	Large	Rough
RS 49	Dark brown	Very good	Peripheral &centre	Aerial surface	More	Large	Rough
RS 50	Deep dark brown	Very good	Centre	Embeded	Moderate	Large	Rough
RS 51	Deep dark brown	Very good	Peripheral &centre	Embeded	Moderate	Large	Rough
RS 52	Dark brown	Very good	Scatter	Embeded	More	Large	Rough
RS 53	Deep dark brown	Good	Scatter	Aerial surface	Moderate	Large	Rough
RS 54	Dark brown	Good	Centre	Embeded	Moderate	Large	Rough
RS 55	Dark brown	Good	Centre	Embeded	Moderate	Large	Rough
RS 56	Dark brown	Good	Centre	Embeded	Moderate	Large	Rough
RS 57	Dark brown	Good	Peripheral	Embeded	More	Large	Rough
RS 58	No sclerotia formation						
RS 59	No sclerotia formation						
RS 60	Dark brown	Good	Scatter	Aerial surface	More	Large	Rough
RS 61	Brown	Good	Peripheral	Aerial surface	More	Large	Rough

Time taken for sclerotia formation

All the isolates under the study showed great variation in the time taken for initiation of sclerotia formation, which ranged from 4- 10 days (Table 1).

Twenty one isolates took 4 days for initiation, 17 isolates took 5 days, 12 isolates took 6 days and 2 isolates took 7days, 5 isolates in 8 days and 1 isolate took 10 days for sclerotia formation. Sclerotial formation was not observed in RS 58 and RS 59 isolates.

Sclerotial variability of *R. solani*isolates

Observations on the variation in the sclerotial characteristics such as the colour of the sclerotia, location of formation, pattern of production, sclerotia number were recorded after 15 days of inoculation.

Sclerotia colour

The mycelia of most of the isolates formed small spherical sclerotia that were initially cream coloured then light yellowish brown and eventually turned to strong brown or dark reddish brown colour. Based on the colour of the sclerotia, they were categorised into three groups,category I- light brown, category II- dark brown and category III- deep dark brown (Table 2). Colour of the sclerotia was observed using Munsell's soil colour chart (1954).

The sclerotia produced by different isolates in the present study were initially white and turned brown with maturity. Among the 60 isolates, 33 isolates produced pale brown sclerotia where as 21 isolates produced dark brown sclerotia and the remaining 6 isolates recorded deep dark brown sclerotia. Prakasam and Guntur district isolates produced pale brown and dark brown sclerotia where as Krishna district isolates produced dark brown and deep dark brown sclerotia. Further it was also observed that two isolates RS 58 and RS 59 did not produce any sclerotia.

Pattern of sclerotial formation

The isolates were divided into 4 categories *i.e.*, in the I category, the sclerotia were produced more towards the centre of the colony, in II category sclerotia were produced more or less in circular manner concentrated towards periphery, in III category, the sclerotia were irregular, sparse and concentrated both in centre and periphery, in IV category, sclerotia were formed in irregular manner scattered all over the colony surface. Among the 60 isolates, 17 isolates produced sclerotia in category I, 15 isolates in category II, 15 in category III and 11 in category IV.

Location of sclerotial formation

On the basis of the location of sclerotial formation in the fungal colony, the isolates were categorized into two groups. The first group included those isolates where sclerotiawere formed in the aerial surface and the second group included the isolates where sclerotia were embedded in the fungal mycelium (Plate 4.5). Out of the 60 isolates, 45 isolates produced sclerotia in group I category whereas the remaining 13 isolates were grouped under group II with sclerotia embedded in the mycelium.

Clump formation

Sclerotia were aggregated in all the isolates and formed clumps. Based on the clump formation the isolates were categorized in to 3 groups. Group I included more clump formation, Group II included moderate clump formation whereas the group III less clump formation. Among the 60 isolates, 16 isolates were assigned in group I (more clump), 30 isolates were included in group II (moderate clump) whereas 14 isolates were included under group III (less clump) (Plate 4.3).

Number of Sclerotia

The number of sclerotia was recorded 15 days after incubation and the isolates were categoried into three groups as, good, very good and excellent on the basis of the number of sclerotia produced. Sclerotia forming isolates were categorized as poor, 1-40 sclerotia good, 41-60 very good and above 60 excellent. Accordingly majority of the *R. solani* isolates belonged to good (50per cent isolates), and very good (40 per cent isolates) and only 6.6 per cent isolates produced

excellent number of sclerotia and 2 per cent of isolates did not produce any sclerotia.

Size of sclerotia

In the present study size of sclerotia of *R. solani* f.sp. *sasakii* was categorized into 2 groups, group I large and group II small based on diameter of sclerotia, 1-1.5 mm small and above 1.5 mm large. Out of the 60 isolates, 53 isolates produced large sclerotia and 5 isolates produced small sclerotia and 2 isolates produced no sclerotia.Out of the 20 isolates collected from Prakasam district 15 isolates produced large sclerotia and 5 isolates produced small sclerotia. All the isolates of Guntur district produced large sclerotia. Among the 20 isolates from Krishna District 18 isolates produced large sclerotia and two isolates produced no sclerotia.

Texture of sclerotia

On the basis of texture of sclerotia, *R. solani* isolates were classified into two groups *i.e,* smooth and rough. The smooth type of sclerotia was formed only in 8.3 per cent isolates (five) in the culture media and rough type by 88.3 per cent (53) isolates and the remaining 3.3 per cent (2) isolates produced no sclerotia.

The above results are in agreement with the reports of earlier researchers. Anderson (1982) reported that colour was the only character that clearly distinguished the sclerotia. Srinivas (2002) assigned the sclerotia of *R. solani* isolates causing BLSB in maize into three different colour groups based on pigmentation. Similar categorisation of the sclerotia produced by the *R. solani* isolates in the rice was assigned as per Munsell's soil colour chart by Amita Singh *et al.,* (1999), Thind and Aggarwal (2008) in potato and rice isolates. The arrangement and pattern of formation of sclerotia produced by *R. solani*isolates in the culture varied greatly and were divided into four categories. However most of the isolates had sclerotia which conglomerated at the centre of the colony. Such type of categorisation based on the pattern of formation and arrangement among maize isolates of *R. solani*was done by Srinivas (2002), rice isolates by Amita Singh *et al.,* (1999) and sclerotia from potato and rice isolates by Thind and Aggarwal (2008).

In the present investigation isolates RS 58 and Rs 59 did not produce sclerotia. Sclerotia may be absent in some *R.solani* isolates under certain cultural conditions therefore the absence of sclerotia does not exclude a mycelium from *R. solani.* Similar findings reported by Meyer (1965) and Parmeter and Whitney (1970).

Higher sclerotial aggregation was observed in 16 isolates. The behaviour of the pathogen to grow quickly and produce sclerotia may increase the chances of pathogen to survive in the next season. High sclerotial aggregation, may hinder the quick dispersal of sclerotia and increases the chances of germination whenever they are present. In most of the isolates, (71.6 per cent of isolates) sclerotia were produced on the aerial surface of the colony, while 25 per cent of isolate had embedded sclerotia. Akhtar *et al.,* (2009) revealed that the sclerotia of maize *R. solani* isolates *lt* and *Rf* were located on the surface of the colony; isolate *Hc* produced on subsurface while in isolates *Be* and *Jr*, sclerotia were embedded in the medium. Zhang *et al., (*1995) observed variation based on sclerotiacolour, shape or formation patterns AG-1 IA, Ag-2-2 IIIB cool- season turf grasses and AG-4: assigned the isolates of the *R. solani* tentatively to an AG based on sclerotial characteristics

The results with respect to morphological, cultural and sclerotial characters of *R. solani*

observed in the present investigations have also been recorded and described by several workers (Duggar, 1915; Reyes, 1941; Singh and Sharma, 1976; Maiti, 1978, Srinivas, 2002, Sharma *et al.,* 2004 and Akhtar *et al.*, 2009 in maize and other crops).

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How to cite this article:

Bindu Madhavi Gopireddy, G. Uma Devi, K.Vijayakrishna Kumar, T. Ramesh Babu and Naidu, T. C. M. 2017. Cultural and Morphological Characterization of *Rhizoctonia solani* f. sp. *sasakii* Isolates Collected from Different Districts of Andhra Pradesh. *Int.J.Curr.Microbiol.App.Sci.* 6(11): 3457-3469. doi: https://doi.org/10.20546/ijcmas.2017.611.407