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MORPHOLOGICAL AND CULTURAL DIVERSITY AMONG *RHIZOCTONIA SOLANI* ISOLATES FROM DIFFERENT GEOGRAPHICAL REGIONS OF INDIA

P Krishna Kumari[1]
[1]Department of Botany & Microbiology,
Acharya Nagarjuna University,
Nambur,A.P,
India

Prof. M Vijayalakshmi[2]
[2]Department of Botany & Microbiology,
Acharya Nagarjuna University,
Nambur,A.P,India

ABSTRACT

Sheath blight disease of rice caused by Rhizoctonia solani is a major production costraint in all rice producing areas of the world. The annual losses due to sheath blight are estimated to be 25% under optimum conditions of disease development. R. solani infects rice plants at any stage of growth. Lesion started at the base of the culms near the water level on artificial inoculation and ascended to the upper parts of the plants. The lesions on sheath were first greenish-grey, ellipsoidal or oval, 2-3 cm long and gradually became greenish white with black brown margin. The sheath blight of rice is a typical disease starting from seedling stage to harvest, indicating all the stages of a crop are susceptible to infection by R. solani.Mycelial and sclerotial morphology of the fungus was studied from one week old culture. A total of 28 strains isolated from different geographic regions and are designated as RS- 1 to RS- 28. Diversity in morphological characters of 28 isolates of R. solani was studied. Morphological characterization of different isolates indicated that, there existed much variation among the isolates, in mycelium colour, appearance, type of margin, mycelial width diameter and sclerotial number, colour, shape, growth pattern number diameter and texture. The branching of mycelium, constriction and septum remained same in all the isolates tested. The colour of the fungal colony varied from light brown to brown and it was found to be dark brown in four isolates. The right angled branching of mycelia was found with all twenty eight isolates. Surface of colony was flat in sixteen isolates and the remaining were fluffy. The margins of the colonies varied from smooth to irregular. The smooth margin was observed in the majority of isolates and only few were with irregular margin .The constriction at the point of origin and the formation of septum in the branch near the point of origin were found in all the isolates. Among the 28 isolates, maximum growth was noticed in 15 isolates with 89 mm colony diameter after 13 days of incubation on PDA, while, the least growth was recorded in RS- 3 and RS- 24 (79 mm). Further, among the isolates mycelial width was larger in the isolate RS- 13 (2.76 µm) where as, it was least in RS- 2 and RS- 7 (1.81 µm).The colour of sclerotium varied from brown to blackish brown in 24 isolates while it was greyish in two isolates (RS-11 and RS-13). In many of the isolates (16) distribution of sclerotia over the colony was found to be central, in some (ten isolates) they were scattered. The number of sclerotia produced also varied in different isolates. Eleven isolates produced higher number of sclerotia followed by moderate number of sclerotia in nine isolates. Further, low level of sclerotial formation was observed in six isolates. On the contrary, isolates RS-1 and RS-6 failed to produce sclerotia on the PDA medium. Shape of sclerotia ranged from globose to irregular.The texture of sclerotia ranged from fine to coarse. Majority of the isolates (seventeen) showed coarse texture and only nine isolates showed fine texture. The maximum sclerotial size was observed in isolate RS- 23 (180 µm). On the other hand, least sclerotial size was exhibited by isolate RS- 7 (47 µm).

KEYWORDS: *Rice, Sheath blight, Rhizoctonia solani, sclerotium.*

INTRODUCTION

Sheath blight of rice caused by *Rhizoctonia solani* is one of the most important diseases which cause significant loss in almost all the rice growing tracts of India. In India, majorly rice is subjected to one or more diseases caused by bacteria, fungi and viruses (Sridhar *et al.* 1975; Kalita *et al.* 1996; Gnanamanickam *et al.* 1999; Chakrabarti, 2001). Sheath blight incited by basidiomycetous fungus *Rhizoctonia solani* Kuhn anamorph once considered a minor disease, is now a major threat to rice crop inflicting substantial yield losses in most of the Asian countries. Various estimates of crop losses due to sheath blight have been made and estimates from Southern India suggested that yield losses were 5.2 to 50% in 1978 and were higher than 70% in severe conditions during 1992 (Baby, 1998). In other countries, average yield losses have been reported to be in the range of 20 to 40%. (Cu *et al.* 1996). Hence an urgent need has been created for the management of the disease. Rice sheath blight, one of the most serious fungal diseases of rice, is caused by multinucleate *R. solani* Kuhn, a ubiquitous pathogen. Diversity within rice sheath blight isolates has been studied by morphological characterization (Sherwood, 1969; Vijayan and Nair, 1985) and pathogenicity testing (Jones and Belmar, 1989; Banniza *et al.*1996). Morphological and pathological variations of sheath blight inciting South Indian Rhizoctonia solani isolates (Jayaprakashvel *et al.* 2012). Morphological variability in *Rhizoctonia solani*isolates from different agro-ecological zones of West Bengal was studied (S.P. Kuiry *et* al.2014),

MATERIALS AND METHODS

Collection of pathogenic strains of *Rhizoctonia solani* causing rice sheath blight from various geographic regions

Samples from four rice-growing states viz. Andhra Pradesh, Tamilnadu, Karnataka, and Punjab in India were collected using transect sampling by walking through the field diagonally. Ten samples were collected per field all along the path of the diagonal. A sample usually consisted of a single rice tiller, which either had sheath blight lesions on the sheath, on the leaf blades, or on both.

Isolation of the fungus:

Rice sheaths and leaf blades with sheath blight symptoms were surface-disinfected with 0.5% sodium hypochlorite for 45 sec and rinsed three times with sterile distilled water. Pieces of sheath or leaf blade dried on sterilized filter paper were placed on a Petri dish containing acidified water agar (PH 4.5) with 10% lactic acid (AWA), and incubated at 28°C in the dark. After 2 to 3 days, cultures developed from infected materials were examined microscopically for hyphal characteristics typical of *Rhizoctonia* spp. (Shew, 1985; Sneh *et al.* 1991). All plated samples yielded *Rhizoctonia* spp. and a hyphal tip of each isolate was subcultured onto AWA for further purification. Isolates were transferred to potato dextrose agar (PDA) slants and maintained at 28°C. Following sufficient growth and production of sclerotia, culture tubes were kept at 4°C for short-term storage. Fungal isolates consisting either mycelium or sclerotia were lyophilized and stored at 4°C for long-term storage.

Morphological and cultural diversity of *Rhizoctonia solani*:

Isolates were subcultured onto PDA Petri dishes in triplicate, incubated at 28°C for 3 weeks, and studied for the cultural characteristics *viz.,* colony characters (colour, appearance, margin, diameter) and sclerotial characters (shape, growth pattern, number, diameter and texture).

RESULTS AND DISCUSSION

The present investigation has focused on the survey on incidence of sheath blight of rice in four states of India. Collection of sheath blight causing *R. solani* isolates from different geographic regions to study the cultural, morphological variability and isolation, identification.

Symptomatology, isolation and identification of the plant pathogen

R. solani infects rice plants at any stage of growth. Lesion started at the base of the culms near the water level on artificial inoculation and ascended to the upper parts of the plants. The lesions on sheath were initially greenish-grey, ellipsoidal or oval, 2-3 cm long and gradually became greenish white with black brown margin (Plate 1). The sheath blight of rice is a typical disease starting from seedling stage to harvest, indicating the susceptibility of crop at all the stages to infection by *R. solani* which is a soil inhabitant. The similar symptomatic observations were made by several workers (Singh *et al.,* 1990; Roy, 1993; Padhi and Gangopadhyaya, 1998).

The field survey of disease in North-Eastern Dry Zone of Karnataka showed a varied incidence from moderate to severe form of sheath blight. Quite often, the variations in severity could also be due to the presence of pathogenic variability apart from environmental conditions. Therefore, samples of rice plants showing typical sheath blight symptoms were collected from different locations of Karnataka *viz.,* Raichur and Koppal districts during the survey. In addition, isolates of *R. solani* were also obtained from Ludhiana of Punjab, Coimbatore of Tamil Nadu and different rice growing areas of Andhra Pradesh for variability study. The isolates collected from different regions were maintained in pure form on potato dextrose agar (Plate 2).

PLATE 1

PLATE 2

Twenty-eight isolates obtained from different geographical regions were designated as RS-1 to RS-28(Table 1). Further, isolates were subjected to Koch's postulates and identified as *R.solani* based on morphological characters described by Ou *et al.* (1972). Sclerotia were superficial, more or less globose but flattened below, white when young, became brown or dark brown later. Individual sclerotia measured up to 5 mm and sometimes united to form a larger mass (Ou, 1972).

Table 1. List of *Rhizoctonia solani* isolates collected from four states of India

S.No.	Isolates	Place of Collection	State
1	RS-1	Hyderabad	Andhra Pradesh
2	RS-2	Maruteru	Andhra Pradesh
3	RS-3	Ponnuru	Andhra Pradesh
4	RS-4	Tenali	Andhra Pradesh
5	RS-5	Avanigadda	Andhra Pradesh
6	RS-6	Gannavaram	Andhra Pradesh
7	RS-7	Tenali	Andhra Pradesh
8	RS-8	Tanuku	Andhra Pradesh
9	RS-9	Eluru	Andhra Pradesh
10	RS-10	Bhimadolu	Andhra Pradesh
11	RS-11	Raichur	Karnataka
12	RS-12	Koppal	Karnataka
13	RS-13	Ghantasala	Andhra Pradesh
14	RS-14	Manavi	Karnataka
15	RS-15	Manavi	Karnataka
16	RS-16	Sindhanur	Karnataka
17	RS-17	Tiruchnapalli	Tamilnadu
18	RS-18	Gangavathi	Karnataka
19	RS-19	Kakinada	Andhra Pradesh
20	RS-20	Ludhiana	Punjab
21	RS-21	Aduthurai	Tamil Nadu
22	RS-22	Coimbattore	Tamil Nadu
23	RS-23	Ramachandrapuram	Andhra Pradesh
24	RS-24	Thiruchi	Tamil Nadu
25	RS-25	Tanguturu	Andhra Pradesh
26	RS-26	Narsapuram	Andhra Pradesh
27	RS-27	Nellore	Andhra Pradesh
28	RS-28	Bapatla	Andhra Pradesh

Morphological cultural and pathogenic diversity of *R. solani*

Diversity in morphological characters of 28 isolates of *R. solani* was studied. Morphological characterization of different isolates indicated much variation among the isolates in mycelium colour, appearance, mycelial width, colony margin and diameter and sclerotial number, colour, shape, distribution and texture. The branching of mycelium, constriction and septum remained same in all the isolates tested. Among the 28 isolates, maximum growth was noticed in 15 isolates with 89 mm colony diameter after 13 days of incubation on PDA, while, the least growth was recorded in A.P isolate RS-3 (79 mm) and RS-24 (79 mm) of Tamil Nadu isolate. Further, among the isolates mycelial width was larger in the isolate RS-13 (2.76 µm) whereas least mycelial width was observed in RS-2 and RS-7 (1.81 µm).

The colour of the fungal colony varied from light brown to brown and it was found to be dark brown in four isolates. The right angled branching of mycelia was found with all twenty eight isolates. Surface of colony was flat in sixteen isolates and the remaining were fluffy. The margins of the colonies varied from smooth to irregular. The smooth margin was observed in the majority of isolates and only few were with irregular margin (RS-5, RS-8, RS-13, Rs-17, RS-23 and Rs-26).

The number and size of sclerotia also exhibited variation among the isolates. Most of the Andhra Pradesh isolates and two Tamilnadu isolates i.e., RS-3, RS-4, RS-7, RS-9, RS-10, RS-15, RS-19, RS-21,RS-23, RS-25 and RS-28 produced higher number of sclerotia whereas less sclerotial number was found in isolates RS-2, RS-12, RS-14, RS-17, RS-20 and RS-27. The maximum sclerotial size was observed in isolate RS-23 (180 µm). On other hand, least Sclerotial size was exhibited by isolate RS-7 (47µm). Similarly, isolates RS-1 and RS-6 failed to produce any sclerotia on the medium. The texture of sclerotia also varied from fine to coarse and their distribution varied from clustered to scattered type (Table 2 & Table 3).

Good variation was observed in sclerotial colour ranging from brown to blackish brown in 24 isolates while it was greyish in two isolates (RS-11 & Rs-13) and the shape ranged from globose to irregular shape. Diversity in morphological characters such as colony colour, mycelial growth, its margin and topography were appreciable among 28 isolates of *R. solani* under study. The differences were striking particularly in mycelial colour .

A glance towards previous investigations supports our present findings. Basu and Gupta (1992) reported that positive correlation was found between the size of sclerotia and pathogenicity. Isolates with larger sclerotia were significantly more virulent than those with smaller sclerotia and without sclerotia. Similarly in the present study variation with respect to sclerotia was quite convincing, with maximum sclerotial size in RS-23 and the least in RS-7 and interestingly RS-1and RS-6 did not produce any sclerotia, which might inturn reflect their pathogenicity as observed by Gupta and Kolte (1992). The isolates of *R. solani* are known to differ in their host.

Table 2. Colony Charecters Of Isolates

ISOLATES	COLONY CHARACTERS				
	COLOUR	APPEARANCE	MARGIN	MYCELIAL WIDTH(µm)	DIAMETER(mm)
RS-1	LIGHT BROWN	FLUFFY	SMOOTH	1.84	89
RS-2	LIGHT BROWN	FLUFFY	SMOOTH	1.81	89
RS-3	BROWN	FLAT	SMOOTH	1.85	79
RS-4	BROWN	FLAT	SMOOTH	2.24	80
RS-5	LIGHT BROWN	FLUFFY	IRREGULAR	2.2	89
RS-6	LIGHT BROWN	FLUFFY	SMOOTH	2.16	89
RS-7	BROWN	FLAT	SMOOTH	1.81	82
RS-8	LIGHT BROWN	FLUFFY	IRREGULAR	2.15	84
RS-9	BROWN	FLAT	SMOOTH	2.1	84
RS-10	BROWN	FLAT	SMOOTH	2.13	80
RS-11	LIGHT BROWN	FLAT	SMOOTH	2.12	89

RS-12	LIGHT BROWN	FLUFFY	SMOOTH	2.4	89
RS-13	LIGHT BROWN	FLAT	IRREGULAR	2.76	86
RS-14	DARK BROWN	FLAT	SMOOTH	2	89
RS-15	BROWN	FLAT	SMOOTH	1.96	84
RS-16	LIGHT BROWN	FLUFFY	SMOOTH	2.39	89
RS-17	DARK BROWN	FLAT	IRREGULAR	2.49	89
RS-18	LIGHT BROWN	FLUFFY	SMOOTH	2.26	89
RS-19	BROWN	FLAT	SMOOTH	2.44	82
RS-20	DARK BROWN	FLAT	SMOOTH	2.33	89
RS-21	BROWN	FLAT	SMOOTH	1.92	80
RS-22	LIGHT BROWN	FLUFFY	SMOOTH	1.89	89
RS-23	DARK BROWN	FLAT	IRREGULAR	2.24	89
RS-24	BROWN	FLAT	SMOOTH	1.889	79
RS-25	BROWN	FLAT	SMOOTH	1.85	89
RS-26	LIGHT BROWN	FLUFFY	IRREGULAR	1.84	83
RS-27	LIGHT BROWN	FLUFFY	SMOOTH	2.15	89
RS-28	LIGHT BROWN	FLUFFY	SMOOTH	2.1	84

Table 3. Sclerotial Charecters of Isolates

ISOLATES	COLOUR	SHAPE	GROWTH PATTERN	NUMBER	SCLEROTIAL DIAMETER(µm)	TEXTURE
RS-1	*	*	*	*	*	*
RS-2	DARK BROWN	IRREGULAR	CLUSTER	+	129	COARSE
RS-3	BLACKISH BROWN	GLOBOSE	SCATTERED	+++	53	FINE
RS-4	BLACKISH BROWN	GLOBOSE	SCATTERED	+++	55	FINE
RS-5	DARK BROWN	IRREGULAR	CLUSTER	++	62	COARSE
RS-6	*	*	*	*	*	*
RS-7	DARK BROWN	GLOBOSE	SCATTERED	+++	47	FINE
RS-8	DARK BROWN	IRREGULAR	CLUSTER	++	160	FINE
RS-9	BLACKISH BROWN	GLOBOSE	SCATTERED	+++	59	FINE
RS-10	BLACKISH BROWN	GLOBOSE	SCATTERED	+++	146	COARSE
RS-11	GREY	IRREGULAR	CLUSTER	++	154	COARSE
RS-12	DARKBROWN	IRREGULAR	CLUSTER	+	159	COARSE
RS-13	GREY	IRREGULAR	CLUSTER	++	164	COARSE
RS-14	BROWN	IRREGULAR	CLUSTER	+	164	FINE

RS-15	BLACKISH BROWN	GLOBOSE	SCATTERED	+++	51	COARSE
RS-16	DARKBROWN	IRREGULAR	CLUSTER	++	151	COARSE
RS-17	BROWN	IRREGULAR	CLUSTER	+	177	COARSE
RS-18	BLACKISH BROWN	IRREGULAR	CLUSTER	++	153	COARSE
RS-19	BLACKISH BROWN	GLOBOSE	SCATTERED	+++	146	COARSE
RS-20	BROWN	IRREGULAR	CLUSTER	+	142	COARSE
RS-21	BLACKISH BROWN	GLOBOSE	SCATTERED	+++	53	COARSE
RS-22	DARKBROWN	IRREGULAR	CLUSTER	++	166	COARSE
RS-23	BROWN	IRREGULAR	CLUSTER	+++	180	COARSE
RS-24	BLACKISH BROWN	GLOBOSE	SCATTERED	++	166	FINE
RS-25	DARKBROWN	GLOBOSE	SCATTERED		53	FINE
RS-26	DARKBROWN	IRREGULAR	CLUSTER	++	146	FINE
RS-27	DARKBROWN	IRREGULAR	CLUSTER	+	160	COARSE
RS-28	DARKBROWN	IRREGULAR	CLUSTER	+++	59	COARSE

* Not formed + Low Level(10-20 Sclerotia per Sq.cm) ++ Moderate(21-30 Sclerotia per Sq.cm)
+++ High Level (More than 30 Sclerotia per Sq.cm)

REFFERENCES

1. *BABY, U.I., 1998. Biocontrol potential of fungicide resistant mutants of Trichoderma spp.* ***Indian J. Microbiol****, 38: 165-166.*
2. *BANNIZA, S., RUTHERFORD, M.A., BRIDGE, P.D., HOLDERNESS, M. AND MORDUE, J.E.M., 1996. Biological characterization of Rhizoctonia solani in rice-based cropping systems.* ***In: Proc. Brighton Crop Prot. Conf. Vol. 1. The British Crop Protection Council, Farnham, United Kingdom.*** *pp. 399-404.*
3. *BANSAL, R.K., SOBTI, A.K. AND MEHTA, S.M., 1990. Cultural variability among seven isolates of Rhizoctonia solani.* ***Indian Phytopath****, 43: 448-450.*
4. *BASU, A., AND GUPTA, P.K.S. 1992. Cultural and pathogenic variation in rice isolates of Rhizoctonia solani Kuhn.Beitrage Zur Tropischen Landwirtschoft and veterinarmedizin, 30: 291-297.*
5. *CHAKRABARTI, N.K., 2001. Epidemiology and disease management of brown spot of rice in India.* ***In: Major fungal diseases of Rice: Recent advances****. (eds. S. Sreenivasa prasad, and R. Johnson)* ***Kluwer Academic Publishers****, pp. 293-306.*
6. *CU, R.M., MEW, T.W., CASSMAN, K.G. AND TENG, P.S., 1996. Effect of sheath blight on yield in tropical, intensive rice production system.* ***Plant Dis,*** *80 : 1103-1108.*
7. *DASGUPTA, M.K., 1992. Rice sheath blight: the challenge continues. In Plant diseases of international importance: diseases of cereals and pulses. Edited by U.S., Singh, A.N.,*
8. *Mukhopadhyay, J., Kumar and H.S. Chaube. Prentice Hall, Englewood Cliffs, N.J. pp. 130–150*
9. *GNANAMANICKAM, S. S., PRYIYADARASANI, V., NARAYANAN, N.N., VASUDEVAN, P. AND KAVITHA., 1999. An overview of bacterial blight disease of rice and strategies for management.* ***Curr. Sc****i, 77: 1435-1444.*
10. *JAYAPRAKASHVEL, M., MATHIVANAN,N., 2012, Morphological and pathological variations of rice sheath blight inciting South Indian Rhizoctonia solani isolates. Archives of phytopathology and plant protection, Vol 44, Issue 4.*
11. *JONES, R.K. AND BELMAR, S.B., 1989. Characterization and pathogenicity of Rhizoctonia spp. isolated from rice, soybean and other crops grown in rotation with rice in Texas.* ***Plant Dis****, 73: 1004-1010.*
12. *KALITA, M.K., SAIKIA, A.K. AND BHAGABATI, K.N., 1996, Reaction of some rice cultivars to tungro virus disease in Assam.* ***J. Virology****. 12: 63-64.*
13. *MANOJ KUMAR., VINEETA SINGH., SINGH.K.N., PRASHANT VIKRAM., 2008. Morphological and virulence characterization of Rhizoctonia solani causing sheath blight of rice.* ***Environment and Ecology****, 26:1158-1166.*
14. *OGOSHI., 1995. Ecology and pathogenicity of anastomosis intra specific group of R.solani Kuhn. Indian Journal of Mycology and plant pathology.* ***Ann. Rev.Phytopath,*** *25 : 125-143.*
15. *Ou, SH., 1972. 'Rice diseases.'* ***Commonweath Mycological Institute: Kew, Surrey, England.*** *256*
16. *PADHI, B. and GANGOPADHYAY, G. Ch., 1998. Diseases of rice and their management. Diseases of field crops and their management.* ***Ed. T.S.Thind.,***

National Agricultural Technology Information Centre, Ludhiana.

17. *ROY, A. K. 1993. Sheath blight of rice.* ***Indian Phytopathol****, 46:197-205.*
18. *SHERWOOD, R.T., 1969. Morphology and pathology of four anastomosis groups of Thanatephorus cucumeris.* ***Phytopathology,*** *59: 1924-1929.*
19. *SHEW, H.D., 1985. Rhizoctonia leaf spot of flue-cured tobacco in North Carolina.* ***Plant Dis,*** *69: 901-903.*
20. *SINGH, S.K., SATYANARAYANA, K. AND REDDY, A.P.K., 1990. Studies on morphology, growth habit, hyphal anastomosis and virulence pattern of five isolates of sheath blight pathogen of rice.* ***Indian Phytopath****, 43: 368-371.*
21. *S.P. KUIRY,A. MONDAL,S. BANERJEE &* ***S. DUTTA*** *, 2014 Morphological variability in Rhizoctonia solaniisolates from different agro-ecological zones of West Bengal, India,*
22. *Archives of phytopathology and plant protection, Vol 47, Issue 6.*
23. *SNEH, B., BURPEE, L. AND OGOSHI, A., 1991. Identification of Rhizoctonia Species. The American Phytopathology Society, St. Paul, MN.*
24. *SRIDHAR, R., RANGA REDDY, P. AND ANJANEYULU, A., 1975. Physiology of rice tungro virus disease: changes in chlorophyll, carbohydrates, amino acids and phenol contents.* ***J. Phytopathol****, 86: 136-143.*
25. *VIJAYAN, M. AND NAIR, C.M., 1985. Anastomosis grouping of isolates of Rhizoctonia solani Kuhn (Thanatephorus cucumeris (Frank) Donk) causing sheath blight of rice.* ***Curr. Sci****, 54: 289-291.*