Original Research Article

Cultural and Morphological Variability in isolates of *Rhizoctonia solani* Kuhn causing Sheath Blight of Rice

.

ABSTRACT

Sheath blight of rice caused by *Rhizoctonia solani* (telomorph: *Thanatephorus cucumeris*) has become a major constraint to rice production during the last two decades. Eleven *R. solani* isolates were collected from various rice growing states of India. Studies on cultural variability revealed that colony colour varied from whitish brown to pale brown with slow, moderate and abundant growth patterns. Among the eleven isolates, majority were fast growing followed by medium growth. Size of sclerotia ranged from minimum of 1.00mm (Rs-9) to maximum of 1.97mm (Rs-8). Maximum isolates had excellent number of sclerotia (>60) produced per petridish. All isolates took 3-4 days for initiation of sclerotia formation. Based on pattern of sclerotia formation, isolates produced sclerotia in central ring, scattered, central & scattered and central & peripheral manner. Sclerotia is formed either in aerial or surface mycelium or on both aerial and surface mycelium. Colour of sclerotia ranged from light brown to dark brown with rough texture.

Comment [h1]: Mention place where is experiment was conducted in Abstract

Keywords: Rhizoctonia solani, Sclerotia, Isolates, colony

1. INTRODUCTION

Rice (*Oryza sativa* L.) is second most important cereal and staple food for more than half of the world's population. Extensive rice cultivation systems inorder to meet the increasing demand, have brought a shift in pest and disease problems in rice. These biotic factors especially fungal pathogens are limiting the rice productivity to a greater extent. Sheath blight caused by *Rhizoctonia solani* Kuhn (*Thanatephorus cucumeris* (Frank) Donk) is one such disease which is having a major contribution for crop loss both in India and world (Lee and Rush, 1983; Webster and Gunnell, 1992). Isolates of *Rhizoctonia solani* show tremendous variation in morphological and pathogenic characteristics (Ogoshi, 1996). Meena *et. al.* (2001) reported great variation among the isolates with respect to mycelia and sclerotial characters. Variation of sclerotial characters like colour, size, texture were also studied by Kumar *et. al.* (2008). Seeing this wide range of variability, study was conducted to assess variability with respect to morphological and sclerotial characters among isolates.

2. MATERIAL AND METHODS

Sheath blight infected paddy samples were collected from various rice growing regions of India. The pathogen was isolated and purified through hyphal tip/ single scelrotial method (Rangaswami and Mahadevan, 2004). Pure cultures were maintained in PDA slants and stored in refrigerator at 5^0C for further studies. Cultural characters like colony colour, growth pattern and colony diameter were studied for all isolates. The colony colour was determined with the help of Munsell's soil color chart (Munsell, 1954). The colour of the colony was observed from bottom side of the culture plate. Growth pattern was recorded by observing visually according to growth of hyphae – as abundant, aerial mycelium obscured surface mycelium and touched the cover of the Petridish; moderate, aerial mycelium obscured surface mycelium without touching the cover, and slight- aerial mycelium did not obscure

surface mycelium. Mycelial discs of 6mm diameter from 3 day old cultures of each isolates were transferred into the centre of sterilized PDA plates and incubated for 10 days at 27±2^{0}C.

Sclerotial characters *viz.,* colour, texture, number, size, time taken for initiation of sclerotial formation, pattern of sclerotial production and location of sclerotia were studied. Texture of sclerotia was grouped as smooth and rough category. Number of sclerotia was categorized as group-1 (poor), group-2 (1-10, fair), group-3 (11-20, moderate), group-4 (21-40, good), group-5 (41-60, very good) and group-6 (>60, excellent). The diameter of the sclerotia was measured with the help of Digital Vernier Calipers by harvesting 20 sclerotia randomly from each petriplate. Time taken for initiation of sclerotial formation was recorded in days. Pattern of sclerotial formation classified into 3 groups *viz.,* central, peripheral and scattered. Location of sclerotia was observed on basis where actually the sclerotia is formed in the fungal colony *i.e.* Sclerotium formed within aerial mycelium, sclerotia formed at surface of the mycelium and other is sclerotia embedded in fungal mycelium itself.

3. RESULTS AND DISCUSSION

Comment [h2]: Mention photos of various isolates of R. Solani petri plates invitro experiment

3.1 Colony colour: Out of eleven isolates, four isolates (KARS-1, KARS -2, TNRS -2 and TRS-1) of *Rhizoctonia solani* showed whitish brown colour, four isolates (TNRS-1, APRS-1, APRS-2 and KERS-1) were of light brown colour and three isolates (KARS -3, ODRS-1 and ODRS-2) were of pale brown colour (Table 1). Singh *et. al.* (2015) had also reported that colony colour ranged from whitish brown, light brown, yellowish brown, dark brown, pale brown to milky brown. Lal and Kandhari (2009) also reported varied colony colours *i.e.* light brown, yellowish brown, whitish brown, dark brown and very pale brown.

3.2 Growth pattern: Among eleven isolates of *Rhizoctonia solani*, six isolates (KARS -1, KARS -2, KARS -3, TNRS-1, TNRS-2 and APRS-2) were bound to show abundant growth and categorized into the group-1. Two isolates (APRS-1 and TRS-1) were found to show moderate growth pattern and categorized into group-2, whereas remaining three isolates (KERS-1, ODRS-1 and ODRS-2) were categorized into group-3 (Table 1). Lal and Kandhari (2009) and Burpee *et. al.* (1980) had also grouped the growth pattern into same three groups.

3.3 Growth rate: Based on growth rate, eleven isolates were categorized into three groups. Isolates (KARS -1, KARS -2, KARS -3, TNRS-1, TNRS-2 and APRS-2) with mean colony diameter of >65mm were categorized into group-1, medium growing isolate (TRS-1) was categorized into group-2 and remaining slow growing (30-49mm) isolates (APRS-1, KERS-1, ODRS-1 and ODRS-2) were categorized into group-3 (Table-1). Singh *et. al.* (2015) had also reported growth rate of twenty five isolates as fast growing, medium growing and slow growing.

Table 1: Morphological variability studies of isolates of *Rhizoctonia solani* Sheath blight of rice (Mycelial characters)

Isolates	Colony colour	Growth pattern	Colony growth diameter at different intervals (mm)				
			24hr	48hr	72hr	96hr	Mean
KARS -1 (Gangavathi)	Whitish brown	Abundant	52.50	69.20	90.00	90.00	75.43

KARS -2 (Mandya)	Whitish brown	Abundant	49.80	61.10	90.00	90.00	72.73
KARS -3 (Kampli)	Pale brown	Abundant	53.70	67.30	90.00	90.00	75.25
TNRS-1 (Thanjavur)	Light brown	Abundant	48.60	59.10	88.80	90.00	71.63
TNRS-2 (Coimbatore)	Whitish brown	Abundant	50.60	60.30	86.80	90.00	71.93
APRS-1 (Bapatla)	Light brown	Moderate	13.40	26.30	54.80	86.80	45.33
APRS-2 (West Godavari)	Light brown	Abundant	65.00	76.70	89.30	90.00	80.25
TRS-1 (Mokila)	Whitish brown	Moderate	36.30	47.20	78.30	90.00	62.95
KERS-1 (Ambalavayal)	Light brown	Slow	9.00	15.10	36.80	68.90	32.45
ODRS-1 (Jeypore)	Pale brown	Slow	16.25	36.25	59.25	81.63	48.34
ODRS-2 (Cuttack)	Pale brown	Slow	13.75	31.13	49.38	66.13	40.09
Sem±			1.00	1.15	1.04	1.03	
CD (5%)			3.99	4.60	4.15	4.11	

3.4 Size of sclerotia: Based on the diameter of sclerotia, the isolates were categorized into two groups, Group-1 had diameter range from 1.00-1.49mm and group-2 had diameter range from 1.50-1.97mm. Maximum range of sclerotial diameter was observed in Rs-8 (1.97mm) and minimum in KERS-1 (1.00mm). Seven isolates (KARS -1, KARS -2, KARS -3, TNRS-2, KERS-1, ODRS-1 and ODRS-2) were categorized into group-1 with diameter range between 1.00-1.49mm. Remaining four isolates (TNRS-1, APRS-1, APRS-2 and TRS-1) were categorized into group-2 having diameter range between 1.50-1.97mm (Table 2). According to Basu *et. al.* (2004), the size of sclerotia ranged from 0.23 to 1.91mm. Similarly, Dath (1985) and IRRI (1986) also reported that diameter of sclerotia ranged from 1 to 3mm.

3.5 Number of sclerotia: Based on number of sclerotia, isolates were categorized into various groups. None of the isolates were categorized into group-1 (poor), group-2 (1-10, fair), group-3 (11-20, moderate) or group-4 (21-40, good). One isolate (APRS-1) was categorized into group-5 (41-60, very good) and remaining isolates (KARS -1, KARS -2, KARS -3, TNRS-1, TNRS-2, APRS-2, TRS-1, KERS-1, ODRS-1 and ODRS-2) were

categorized into group-6 (>60, excellent) (Table 2). Lal and Kandhari (2009) and Singh *et. al.* (2015) also categorized number of sclerotia into 6 groups.

3.6 Time taken for sclerotia formation: Seven isolates (KARS -1, KARS -2, KARS -3, TNRS-2, APRS-2, TRS-1 and ODRS-2) took 3 days for initiation of sclerotia formation whereas remaining four isolates (TNRS-1, APRS-1, KERS-1 and ODRS-1) took 4 days for initiation of sclerotia formation (Table 2). According to Meena *et. al.* (2001), time taken for sclerotia formation ranged from 3-11 days. Time taken for sclerotia formation also ranged from 3 to 6 days (Singh *et. al.*, 2015).

Table 2: Morphological variability of isolates of *Rhizoctonia solani* Sheath blight of rice (Sclerotial characters)

Isolates	Size of sclerotia (Avg. Dia in mm)	No. of sclerotia/ petridish	Time taken for sclerotia formation (days)
KARS -1	1.23	112	3
KARS -2	1.38	120	3
KARS -3	1.41	78	3
TNRS-1	1.76	90	4
TNRS-2	1.13	116	3
APRS-1	1.85	50	4
APRS-2	1.85	61	3
TRS-1	1.97	83	3
KERS-1	1.00	91	4
ODRS-1	1.40	98	4
ODRS-2	1.34	86	3
Sem	0.03	0.80	
CD (1%)	0.11	3.18	

3.7 Pattern of sclerotia formation: Based on the pattern of sclerotia formation in the petri dishes among various isolates, they were classified into three groups. Sclerotium formed in the central ring was observed in three isolates (KARS -3, APRS-2 and ODRS-2). Sclerotium were formed in the scattered manner in four isolates (KARS -1, KARS -2, TNRS-2 and KERS-1) whereas three isolates (TNRS-1, APRS-1 and ODRS-1) showed sclerotial formation in both central and scattered manner. None of the isolates showed peripheral manner of sclerotia formation whereas one isolate (TRS-1) showed both central and

peripheral manner of sclerotia formation (Table-3). Singh *et. al.* (2015) also reported sclerotial formation in the same manner *i.e.* central, peripheral and scattered.

3.8 Location of sclerotia formation: Isolates were categorized into three groups, based on the location of sclerotia formation. First group included those isolates where sclerotium formed within the aerial mycelium. None of the isolates showed this pattern. Second group included those isolates where sclerotia formed at the surface of the mycelium (KARS -2, KARS -3, TNRS-1, APRS-2 and ODRS-2). None of the isolates had sclerotia embedded in fungal mycelium itself (Third group) whereas, six isolates (KARS -1, TNRS-2, APRS-1, TRS-1, KERS-1 and ODRS-1) recorded sclerotia formation in both aerial and surface mycelium (Table 2). Location of sclerotia as aerial, surface and embedded was also reported by Lal and Kandhari (2009) and Singh *et. al.* (2002).

3.9 Colour of sclerotia: Based on the pigmentation of the sclerotium, isolates were assigned into two groups. Ten isolates (KARS -1, KARS -2, KARS -3, TNRS-1, TNRS-2, APRS-1, APRS-2, TRS-1, ODRS-1 and ODRS-2) showed dark brown sclerotia whereas one isolate (KERS-1) showed light brown sclerotia (Table 3). Hoa (1994) also reported that sclerotial colour ranged from brown, light/dark brown, black brown, chocolate brown, salmon and dark salmon.

3.10 Texture of sclerotia: Based on the texture of sclerotia, the isolates were classified into two groups *i.e.* smooth and rough. All eleven isolates belonged to rough category of sclerotial texture (Table 3). Sclerotial texture was also classified into two groups – smooth and rough by Hoa (1994) and Singh *et. al.* (2015).

Table 3: Morphological variability of isolates of Rhizoctonia solani causing Sheath blight of rice (Sclerotial characters)

Isolates	Pattern of sclerotia	Location of sclerotia	Colour of sclerotia	Texture
KARS-1	Scattered	Aerial & surface	Dark brown	Rough
KARS-2	Scattered	Surface	Dark brown	Rough
KARS-3	Central	Surface	Dark brown	Rough
TNRS-1	Central & scattered	Surface	Dark brown	Rough
TNRS-2	Scattered	Aerial & surface	Dark brown	Rough
APRS-1	Central & scattered	Aerial & surface	Dark brown	Rough
APRS-2	Central	Surface	Dark brown	Rough
TRS-1	Central & peripheral	Aerial & surface	Dark brown	Rough
KERS-1	Scattered	Aerial & surface	Light brown	Rough
ODRS-1	Central & scattered	Aerial & surface	Dark brown	Rough
ODRS-2	Central	Surface	Dark brown	Rough

4. CONCLUSION

Sheath blight disease of rice caused by a soil borne pathogen, *Rhizoctonia solani* is having high economic importance in India. From the experimental results, it is quite evident that all the eleven isolates of *R. solani* were highly different from each other with respect to all the characters examined *viz.,* colony colour, growth pattern, growth rate, size of sclerotia, number of sclerotia, time taken for sclerotia formation, pattern & location of sclerotia formation, colour & texture of sclerotia.

REFERENCES

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1. Basu, A., Podder, M., Prasanta, K and Sengupta. 2004. Variability and anastomosis among the rice isolates of *Rhizoctonia solani*. *Indian Phytopathology*. 57 (1): 70-72.

2. Burpee, L.L., Sanders, H.C., Sanders, Jr and Sherwood, R.T. 1980. Anastomosis groups among isolates *of Ceratobasidium cornigerum* (Bourd) Rogers and related fungi. *Mycologia.* 72 : 689-701.

3. Dath PA. 1985. A better criterion in rating the reaction of rice cultivars against sheath blight. *Indian Phytopath* 38: 678-682.

4. Hoa TTC. 1994. *Characterization and pathogenicity of Rhizoctonia solani Kuhn isolates from different rice zone and management of sheath blight of rice.* Ph. D Thesis, Indian Agricultural Research Institute, New Delhi-12, 122p.

5. IRRI 1986. *Annual Report* (1985). International Rice Research Institute, P.O. Box 933. Manila Philippines. p143-146.

6. Kumar, Singh, V., Prashant and Vikram, K.N. 2008. Morphological and virulence characterization of *Rhizoctonia solani* causing sheath blight of rice. *Environment and Ecology*. 26 (3): 1158-1166.

7. Lal, M. and Kandhari, J. (2009). Cultural and Morphological Variability in *Rhizoctonia solani* Isolates Causing Sheath Blight of Rice. *J Mycol Pl Pathol* 39 (1): 77-81.

8. Lee, F.N and Rush, M.C. 1983. Rice sheath blight: a major rice disease. *Plant Disease.* 67: 829-832.

9. Meena, B., Ramamoorthy, V and Muthusamy, M. 2001. Morphological and pathological variations in isolates of *Rhizoctonia solani* causing sheath blight of rice. *Plant Disease Research.* 16 (2): 166-172.

10. Munsell 1954. Munsell's Soil Colour Chart. 1954.Munsell Colour Co. Inc. Baltimore, Maryaland, U.S.A.

11. Ogoshi, A. 1996. Introduction - the genus *Rhizoctonia solani*. In: Sneh B, Jabaji Hare S, Neate SM, Dijst G (eds), *Rhizoctonia species, Taxonomy, Molecular Biology, Ecology; Pathology and Disease Control*. Kluwer, Dordrecht. 1-9.

12. Rangaswami G and Mahadevan A. (Eds) 2004. *Disease of crop plants in India*. Prentice-Hall of India Private Limited Publisher, New Delhi, India. 507 pp.

13. Singh V, Singh US, Singh KP, Singh M and Kumar A. 2002. Genetic diversity of *R. solani* isolates from rice: Differentiation by morphological characteristics, pathogencity, anastomosis behavior and RAPD finger printing. *J Mycol Pl Pathol* 32: 332-344.

14. Singh, R., Murti, S., Mehilal, Tomer, A. and Prasad, D. (2015). Virulence Diversity in *Rhizoctonia solani* causing Sheath Blight in Rice. *J. Plant Pathol & Microbio.* 6 : 296.

15. Webster, R. Kand Gunnel, P. S. *Compendium of Rice Diseases*. American Phytopathological Society Press. St. Paul, Minnesota. USA. 14-18.