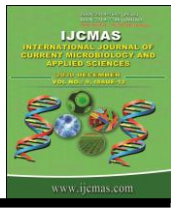
Int.J.Curr.Microbiol.App.Sci (2020) *9*(12): 868-876

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 12 (2020)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2020.912.104

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

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Keywords

Rice, Sheath blight, *Rhizoctonia solani*, survey, Cultural and Morphological characterizations

Article Info

Accepted:
10 November 2020
Available Online:
10 December 2020

A B S T R A C T

The fungal disease sheath blight of rice caused *by Rhizoctonia solani* is an economically important disease of rice in India. A survey was carried out to find the disease severity of sheath blight of rice and collected the specimens from the different locations of Prayagraj district. Thirteen isolates of *R. solani* collected from different locations of Prayagraj district were studied for their morphological and cultural characterization. All isolates showed wide morphological and cultural variations in terms of colours (white, whitish yellow, light cream and pale-yellow) colony behavior (upper touch lid and no-upper touch lid), radial growth (slow, moderate and fast growing), sclerotia colour (brown, light brown, dark brown, and deep dark brown), sclerotia distribution pattern (scattered, peripheral and scattered-peripheral) and sclerotia weight (ranged between 0.123-0.233g).

Introduction

Rice suffers from many diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non-parasitic disorders. Sheath blight is the fungal disease of rice caused by *Rhizoctonia solani* Kuhn considered to be one the most destructive fungal disease. Sheath blight disease of rice is an economically important in many rice growing regions. Its causes up to 50% yield loss under favorable condition around the world (Groth, 2008 and Bernardes, 2009). In Eastern Asia, it is reported that sheath blight disease of rice causes yearly yield loss up to 6 million tons of rice grain by affecting nearly 15-20 million ha of rice under irrigated condition (Bernardes, 2009).

The pathogen *Rhizoctonia solani* is most widely distributed causing diseases in many crops. *Rhizoctonia solani* causes stem rot, fruit rot, fruit and seed decay, foliar blight, damping off, crown rot and stem canker in

various crops (Baker, 1970; Anderson, 1982). Many attempts have been made to organize R. solani isolates into groups on the basis of various morphological, cultural and pathological characteristics (Sherwood, 1969).

The earlier studies suggest that sheath blight pathogen *R. solani* was found homogenous in nature (Kuninaga *et al.,* 1982) but recent investigations revealed that the pathogen is more diverse than previously assumed (Neeraja *et al.,* 2002, Singh *et al.,* 2002, Susheela *et al.,* 2004, Yu *et al.,* 2003) Keeping in view, the present investigation was carried out to determine the morphological and cultural variability in 13 isolates of *R. solani* from rice crop.

Materials and Methods

Survey and collection of *Rhizoctonia solani* isolates from different regions of Prayagraj district

Collection of samples

A survey was carried out to collect different samples of rice for isolation of different *Rhizoctonia solani* isolates from selected areas of Prayagraj district (Table 1). The details of collected isolates given in Table 1. The in which the different isolates were collected and denoted as PRS_1 to PRS_20. Due to unfavourable conditions some isolates (*viz.*, PRS_4, PRS-5, PRS_6, PRS_11, PRS_15, PRS_17 and PRS_20) were contaminated and unable togrow as such they were not used for further study.

Isolation of pathogen

The diseased plant parts were washed in running water followed by sterile distilled water to remove surface contaminants. Then the tissues were dissected in to 0.5-1 cm size with a sterile blade. The cut pieces were surface sterilized with 2% sodium hypochlorite solution for 1 min and rinsed three times with sterile distilled water. After surface drying, disease tissues were placed on water agar and incubated at 28 ^{0}C for 24h.

Later, pure cultures for all isolates were prepared by transferring mycelial tips to potato dextrose agar (PDA) medium. After 2 to 3 days, cultures were examined microscopically for hyphal characteristics typical to *R. solani*.

Morphological study

To study about morphological character of isolates, a 5 mm disc from 5 days old active colony of each isolate was inoculated in the center of Petri plate (90 mm diameter) containing Potato Dextrose Agar (PDA). All isolates were replicated thrice.

The observations on colony colour, radial growth and behaviour were recorded at 7 day after incubation (DAI) and sclerotia distribution pattern, its colour, and weight (g) of each isolate were recorded after 15 DAI.

Disease assessment

The disease severity were recorded by following Standard Evaluation System (SES) for rice (IRRI, 1996) and the per cent disease reduction over the control will be calculate. The lesion height and plant height were measured. Relative Lesion Height (RLH) was calculate using the following formula.

$$RLH = \frac{Lesion\ height}{Plant\ height} \times 100$$

For evaluating the efficacy of the antagonistic fungi against sheath blight disease, the following 0-9 scale given by IRRI (1996)was used (Table 2 and Figure 1).

Results and Discussion

Sheath blight disease of rice was identified in the farmer's fields based on key symptoms of circular, oblong or ellipsoid, greenish-grey water soaked spots about 1 cm long that occur on leaf sheath near water level. Based on these key symptoms, sheath blight rice Per cent Disease Incidence (PDI) was assessed in farmers' fields of each village nineteen villages and 8 blocks in Prayagraj district.

A survey was carried out for recording the diseases everity and to collect different samples of rice for isolation of different *Rhizoctonia solani* isolates from different areas of Prayagraj district.

Sheath blight of rice was prevalent in all the areas surveyed and the diseases everity varied from 5.10 to 13.5% depending upon the regions. However, highest disease severity was recorded in Leudi village under Jasra block i.e.13.4% followed by village Naini under Chaka block which recorded disease severity of 10.6. The data presented in Table 3 reveals that relative lower disease severity were recorded in Chaka village under Chaka block leaf disease intensity 5.76%.

The variations in the diseases severity among the surveyed regions may be due to different environmental conditions, different practices farmers adopted or can be different varietal or genotypes of rice crop or mean disease incidence which might be attributed to balanced fertilization especially of nitrogenous fertilizers by the farmers of the village. Kapse *et al.,* (2012) surveyed rice growing area in Jabalpur region and recorded the diseases incidence of sheath blight of rice between 2-24%. Thakur *et al.,* (2017) surveyed different rice growing regions of Chhattisgarh to record and analyze disease incidence of sheath blight of rice and maximum disease incidence observed in November 2014 i.e. 82.2%. Pal *et al.,* (2015) surveyed west central table land zone (*viz.,* Sambalpur, Bargarh and Jharsuguda) of Odisha region to record and analyse the incidence of sheath blight of rice. Among the districts surveyed, the highest mean disease incidence recorded in Bargarh district i.e.30.19% and the least mean disease incidence recorded in Jharsuguda district i.e. 21.98%. A positive relation was also observed between dose of nitrogenous fertilizers used by the farmers and disease incidence percentage.

Cultural and morphological variability of different *Rhizoctonia solani* isolates

Cultural and morphological variability includes colony behaviour, radial growth, colony colour, sclerotia colour, sclerotia distribution pattern, and sclerotia weight (g). All the isolates were grown on Petri plates. Then, all the isolates were observed under microscope for identification. The results of this study revealed that growth of *R. solani* was observed in different colours like, white, whitish yellow, light cream and pale yellow. Growth of the pathogen varied from slow growth, moderate growth and fast growth as in some isolates it touched the upper lid of the Petri plates. For ease to study the different *R. solani* isolates characterized as PRS_1, PRS_2 PRS_3 and so on.

Identification and morphological studies of pathogen

The fungus found to be responsible for sheath blight disease of rice and grown Potato Dextrose Agar (PDA) medium. Morphological characters were studied under compound microscope.

The pathogen was isolated in Potato Dextrose Agar (PDA) media and purified. The

pathogen produced white mycelial growth at first but later on it turned into light cream colour to yellowish cream colour. The mycelium was septate and hyaline. Further the mycelia aggregated and formed the sclerotia with respected colour and pattern of distribution.

Cultural variability among *R. solani*Isolates on Potato Dextrose Agar Medium

Colony colour

Colony colour change was recorded on fourteen days after incubation. In the present study, colony colour among the13 isolates varied as white, whitish yellow, light cream and pale-yellow (Table 1 and Fig. 2). Out of 13 isolates, 2 isolates were white (PRS_2 and PRS_9), 5 isolates were whitish yellow (PRS_1, PRS_3, PRS_8, PRS_13 and PRS_18), 4 isolates were light cream (PRS_7, PRS_10, PRS_14 and PRS_16) and 2 isolates were pale yellow (PRS_12 and PRS_19). The colour creation may likewise be because of arrival of other secondary metabolites like toxins (Zhang *et al.*, 1995) examined the cultural and morphological variability of *R. solani* isolates confines and found that the *R. solani* isolates varied pale brown coloured to brownish colony on agar medium.

Colony behaviour

Variations in colony behaviour was recorded among the thirteen isolates. In the present study, colony behaviouramong the 13 isolates varied as upper touch lid and no-upper touch lid (Table 4 and Fig. 2). Out of 13 isolates 3 were upper touch lid (PRS_8, PRS_13 and PRS_14) and 10 were no-upper touch lid (PRS_1, PRS_2, PRS_3, PRS_7, PRS_9, PRS_10, PRS_12, PRS_16, PRS_18 and PRS_19).

Table.1 Isolates collected from different regions of Prayagraj district

Isolate codes	Village	Rice varieties	Samples of
PRS_1	SHUATS CRF	SHIATS Dhan-3	**Sheath**
PRS_2	PurwaKhas	Ganga Kaveri	**Sheath**
PRS_3	Indalpur	Balwan	**Sheath**
PRS_7	Naini	1509	**Sheath**
PRS_8	Karchana	Basmati	**Sheath**
PRS_9	Ghatwa	Basmati	**Sheath**
PRS_10	Jhusi	Pant Gold	**Leaf**
PRS_12	Kandhi	Balwan	**Sheath**
PRS_13	Bhaiya	Ganga-Kaveri	**Sheath**
PRS_14	Mamoli	1509	**Sheath**
PRS_16	SHUATS CRF (Trial)	PB-1	**Leaf**
PRS_18	Leudi	1121	**Leaf**
PRS_19	**Rigwan**	**IR66**	**Sheath**

Table.2 The Scale for Disease assessment of rice sheath blight caused by *R. solani*

Score	Disease Description
0	**No infection**
1	**Lesion limited to lower 20 per cent of height of the plant**
3	**Lesion limited to lower 21-30 per cent of the height of the plant.**
5	**Lesion limited to lower 31-45 per cent of the height of the plant.**
7	**Lesion limited to lower 46-65 per cent of the height of the plant.**
9	**Lesion more than 65 per cent of the height of the plant.**

Table.3 Survey for natural occurrence on sheath blight disease incidence of rice in different regions of Prayagraj district

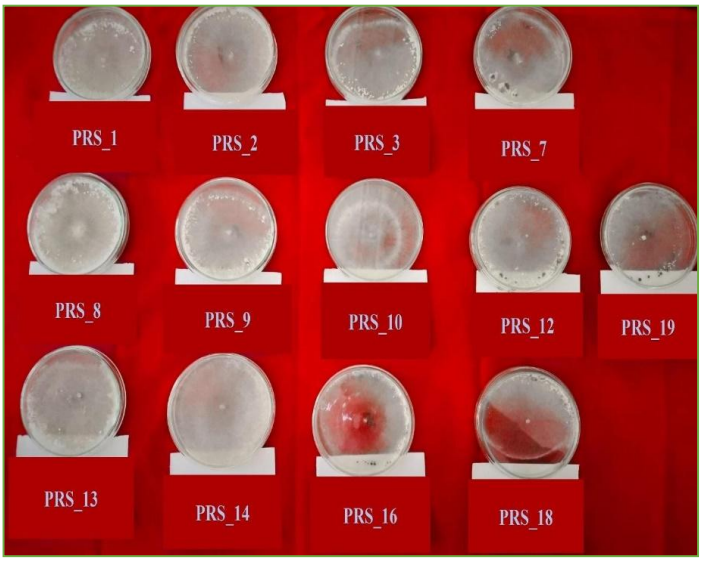
Block	Village	Variety	Crop Stage	Disease Severity (%)	Range DS (%)
Chaka	SHUATS CRF	SHIATS Dhan-3	Max. Tillering	5.93	**5.04-10.60**
	Chaka	Sugandh	Booting	5.04	
	PurwaKhas	Ganga Kaveri	Booting	6.47	
	Baswar	Basmati	Max. Tillering	6.73	
	Mahewa	Basmati	Booting	9.53	
	Indalpur	Balwan	Booting	6.4	
	Naini	1509	Tillering	10.6	
	Dandi	1121	Max. Tillering	6.8	
Karchana	Karchana	NarendraDhan	Max. Tillering	8.8	**6.33-8.87**
	Ghatwa	Basmati	Booting	8.87	
	Jhusi	Pant Gold	Booting	6.33	
	Kandhi	Balwan	Booting	8.13	
Jasra	Leudi	1121	Flowering	13.4	**8.67-13.40**
	Rigwan	IR66	Flowering	8.67	
Meja	Bhaiya	Ganga-Kaveri	Booting	8.87	**8.13-8.87**
	Mamoli	1509	Booting	8.13	
Soraon	Soraon	Pant Gold	Max. Tillering	9.27	**6.48-9.27**
	Jallupur	Basmati	Max. Tillering	6.48	
	Dasapur	**1509**	**Booting**	**8.33**	

Table.4 Cultural and morphological characters of different isolates of *Rhizoctonia solani*

Isolate	Colony Colour	Colony Behaviour	Radial Growth	Sclerotia Colour	Sclerotia Distribution Pattern	Sclerotia Weight (g)
PRS_1	Whitish yellow	No upper lid touch	Moderate growing	Brown	Scattered-peripheral	0.187
PRS_2	White	No upper lid touch	Moderate growing	Light brown	Peripheral	0.147
PRS_3	Whitish yellow	No upper lid touch	Moderate growing	Brown	Peripheral	0.157
PRS_7	Light cream	No upper lid touch	Fast growing	Deep dark brown	Scattered-peripheral	0.123
PRS_8	Whitish yellow	Upper lid touch	Fast growing	Brown	Scattered	0.213
PRS_9	White	No upper lid touch	Moderate growing	Brown	Peripheral	0.183
PRS_10	Light cream	No upper lid touch	Slow growing	Brown	Peripheral	0.206
PRS_12	Pale yellow	No upper lid touch	Moderate growing	Brown	Peripheral	0.163
PRS_13	Whitish yellow	Upper lid touch	Fast growing	Light brown	Scattered	0.207
PRS_14	Light cream	Upper lid touch	Slow growing	Brown	Peripheral	0.217
PRS_16	Light cream	No upper lid touch	Moderate growing	Light brown	Peripheral	0.233
PRS_18	Whitish yellow	No upper lid touch	Slow growing	Brown	Peripheral	0.167
PRS_19	Pale yellow	No upper lid touch	Moderate growing	Dark brown	Scattered	0.187

Fig.1 Rice sheath blight grade chart

Fig.2 Mycelial growth of *Rhizoctonia solani* isolates along with sclerotia



Radial growth

Variations in radial growth was recorded among the thirteen isolates. In the present study, colony behavior among the 13 isolates varied as slow, moderate and fast growing (Table 4 and Fig. 2). Out of 13 isolates 3 isolates were slow growing (PRS_10, PRS_14 and PRS_18), 7 isolates were moderate growing (PRS_1, PRS_2, PRS_3, PRS_9, PRS_12, PRS_16 and PRS_19) and 3 isolates were fast growing (PRS_7, PRS_8, and PRS_13).Thind and Aggarwal (2008) stated that the growth *Rhizoctonia solani* isolates from potato and rice categorized as slow, medium and fast growing. Guleria*et al.*, (2007) reported that the *Rhizoctonia solani* isolates from rice crop were fast growth per day indicating their fast growing nature.

Sclerotia colour

Variations in sclerotia colour was recorded among the thirteen isolates. In the present study, sclerotia colour among the 13 isolates varied as brown, light brown, dark brown, and deep dark brown (Table 4 and Fig. 2). Out of 13 isolates 8 isolates were brown (PRS_1, PRS_3, PRS_8, PRS_9, PRS_10, PRS_12, PRS_14 and PRS_18), 3 isolates were light brown (PRS_2, PRS_13 and PRS_16), 1 isolate was dark brown (PRS_19) and 1 isolate was deep dark brown (PRS_7). Susheela and Reddy (2013) characterized the sclerotia colour 35 isolates of *Rhizoctonia solani* as brown, dark brown and deep dark brown.

Sclerotia distribution pattern

Variations in sclerotia distribution patternwas recorded among the thirteen isolates. In the present study, sclerotia distribution pattern among the 13 isolates varied as scattered, peripheral and scattered-peripheral (Table 4 and Fig. 2). Out of 13 isolates 3 were scattered (PRS_8, PRS_13 and PRS_19), 8 were peripheral (PRS_2, PRS_3, PRS_9, PRS_10, PRS_12, PRS_14, PRS_16 and PRS_18) and 2 were scattered-peripheral (PRS_1 and PRS_7). Ganeshamoorti and Dubey (2015) stated that sclerotia distribution of the *Rhizoctonia solani* isolates showed scattered, peripheral, center ring, peripheral ring pattern of sclerotia distribution. These results were also in agreement with findings

of Thind and Aggarwal (2008), Lal and Kandhari (2009) and Yaduman *et al.,* (2019)

Sclerotia weight

Sclerotia weight was recorded among the thirteen isolates in grams (Table 4 and Fig. 2). In the present study, there was variation in the sclerotia weight among the 13 isolates. Srinivas *et al.,* (2007) and Yaduman*et al.,* (2019) found the sclerotia weight variations among the isolates.

In conclusion the findings of the present study confirmed the variability in *R. solani* isolates from rice crop, according to their morphological and cultural characters, which include different colony and sclerotial characters. Based on results in the present study on variability in *R. solani*, it can be concluded that present results will be useful for resistant breeding and effective management of the diseases in rice.

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How to cite this article:

Rajat Kumar, Abhilasha A. Lal, Sobita Simon and Ravi Teja Naidu, B. 2020. Morphological and Cultural Variability in Rice Isolates of *Rhizoctonia solani* Kuhn causing Sheath Blight Disease of Rice. *Int.J.Curr.Microbiol.App.Sci.* 9(12): 868-876.
doi: https://doi.org/10.20546/ijcmas.2020.912.104