

Cultural characteristics of *Rhizoctonia solani* causing sheath blight of rice

Abstract

Sheath blight of rice caused by *Rhizoctonia solani* Kuhn is emerging as a very destructive disease causing heavy yield losses. The present study was conducted at Department of Plant pathology, JNKVV, College of Agriculture, Rewa, (M.P.) with eight culture media viz., Potato Dextrose Agar (PDA), Czapek's Dox Agar (CDA), Richards medium (RMA), Corn Meal Agar (CMA), Oat meal agar (OMA), Rice leaf extract agar (30%), Rice seed extract agar (10%) and Rice seed extract (10%)+ (2%) sucrose were evaluated against *R. solani*. It can be concluded that Potato dextrose agar (88.0 mm) and Czapek's dox agar (87.7 mm) medium supported maximum mycelial growth of *R. solani* isolated from rice. Whereas, least mycelial growth (75.8 mm) was observed in Rice seed extract agar and Richards agar medium. Mycelial growth was excellent, fast, abundant and off white on Potato dextrose agar. Whereas it was fast, abundant and on Czapek's dox agar. Sclerotia of the fungus initiated within 3 - 4 days in different culture media. Maximum number of sclerotia was produced in Oat meal agar (54.0) closely followed by Potato dextrose agar (51.2) and Czapek's dox agar (44.6). Maximum weight of 10 sclerotia was recorded in potato dextrose agar (1030.6 mg) followed by Czapek's dox agar (920.3 mg) and Oat meal agar (884.7 mg). Maximum size of sclerotia were formed in Potato dextrose agar followed by Rice seed extract agar and Corn meal agar.

Key words: Rice, *Rhizoctonia solani*, PDA and culture

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crop grown *kharif* in India and contributes 40% of total food grain production. Productivity of rice can be increased by adopting the hybrid varieties, nutrient management and biotic stresses. It is being grown in India in 42 mha with production 104.32Mt and productivity 2404 kg/ha (Anon, 2016). Among the major diseases of rice, sheath blight caused by *Rhizoctonia solani* Kuhn is emerging as a very destructive disease causing heavy yield losses (Suthinet *al.*, 2018). The centre of lesion becomes grayish white with brown margin, later several spots coalesce and show blight symptoms (Ou, 1985). When humidity exceeds 95 % and temperature ranges from 29 to 32°C, infection spreads rapidly which appears on plant parts, including leaf blades, causing irregularly shaped lesions with brown borders as bands. This symptom is generally referred to as "banded blight". *R. solani* possesses pale to dark brown rapidly growing mycelium with septum in the branch near the point of origin. It produces large numbers of globose sclerotia which initially turn white, later turn brown to purplish brown. Sclerotia formed varying in size but uniform in texture. The outer cells of the sclerotia were darker and thick walled. Sclerotia serve as a major source of primary inoculum. Keeping this background in view, present study was undertaken on cultural characteristics of *R. solani* causing sheath blight of rice.

Materials and Methods

The leaf sheaths of rice infected with *R. solani* showing characteristic sheath blight symptoms were collected and standard technique used. Eight culture media viz., Potato Dextrose Agar (PDA), Czapek's Dox Agar (CDA), Richards medium (RMA), Corn Meal Agar (CMA), Oat meal agar (OMA), Rice leaf extract agar (30%), Rice seed extract agar (10%) and Rice seed extract (10%) + (2%) sucrose. Five days old culture of *Rhizoctonia solani* was inoculated in triplicate at the centre of 90 mm media plate containing test media. Inoculation was done using 5 mm mycelial discs taken from margin of colonies grown on 3 plates of each culture media. The plates were incubated at ambient temperature (25°C) and the mycelial growth was measured (in mm) 48, 72, 96 and 120 hrs after incubation. Colony characteristics (Growth type, growth pattern, colony colour etc.) and sclerotial characteristics (size, colour, weight, number etc.) were observed by visual observation of the growth pattern of *R. solani* after 120 hrs of inoculation.

Results

Significant differences in mycelial growth diameter (mm) at different culture media and time intervals recorded (Table 1). Potato Dextrose Agar (PDA) supported maximum mycelia growth (88.0 mm) of the fungus followed by Czapek's dox agar (87.7 mm), Corn meal agar (84.8 mm), Oat meal agar (84.2), Rice seed extract agar (10%) + 2% sucrose (83.3 mm), Rice leaf extract agar (30%) (80.0 mm), Richard's agar (76.0 mm) and Rice seed extract agar (10%) (75.8 mm) after 120 hrs of incubation. Lowest mycelia growth of (75.8 mm) in Rice seed extract agar which was at par with Richard's agar medium (76.0 mm).

The culture characteristics i.e. colour, growth pattern and growth rate of *R. solani* were studied in eight culture media and observations are presented in (Table 2). Differentiation in colour of culture viz., off White, Pale brown, Yellowish brown was recorded. Off White colony was observed in Potato dextrose agar, Czapek's dox agar, Richard's agar and Corn meal agar media. Whereas, Pale brown colony was recorded in Oat meal agar, Rice seed extract agar and Rice Seed extract agar + 2% sucrose. Yellowish brown colony was found in Rice leaf extract agar medium. Abundant growth pattern was recorded in Potato dextrose agar, Oat meal agar, Richard's agar, Czapek dox agar media and moderate was in Rice leaf extract agar media. Slight growth pattern was observed in Corn meal agar, Rice seed extract agar and Rice seed extract agar + 2% sucrose. Fast growth rate was recorded in Potato dextrose agar Czapek dox agar media and Corn meal agar. and Moderate growth rate was in Oat meal agar, Rice leaf extract agar, Rice seed extract agar and Rice seed extract agar + 2% sucrose. Whereas, slow growth rate was recorded in Richard's agar media.

Data on sclerotial formation viz., Time taken for initiation of sclerotia, number of sclerotia, weight of sclerotia and size of sclerotia on eight culture media are presented in (Table 3). Sclerotia formation was initiated within 3 to 4 days in different tested media. Sclerotia formation was started in three days on Potato dextrose agar, Oat meal agar, Czapek's dox agar, Richard's agar and Rice seed extract agar. Whereas, sclerotial formation initiated in four days on Corn meal agar, Rice leaf

extract agar and Rice seed extract agar +2% sucrose. Significant differences in number of sclerotia formed on different media ranging from 12.3 to 54.0 were recorded. Highest number of sclerotia were formed in Oat meal agar (54.0) followed by Potato dextrose agar (51.2) and Czapek's dox agar (44.6) and these results are at par with each other. Number of sclerotia formed in Corn meal agar and Richard's agar were 34.6 and 28.2, respectively. Least number of sclerotia were formed in Rice seed extract agar (12.3) followed by Rice leaf extract agar (15.3) and Rice seed extract agar +2% sucrose. (16.5). Weight of 10 sclerotia ranged from 135.0 to 1030.6 mg produced on different media. Highest weight of sclerotia was recorded produced on Potato dextrose agar (1030.6 mg) followed by Czapek's dox agar (920.3 mg), Oat meal agar (884.7 mg). Whereas lowest weight of sclerotia was recorded on Corn meal agar (135.0 mg) followed by Richard's agar (215.3 mg) and Rice seed extract agar +2% sucrose. (345.0 mg). Significant variation in size of sclerotia ranging from 0.4 to 1.3 mm was recorded. Maximum sclerotial size was recorded in Potato dextrose agar (1.3 mm) followed by Rice seed extract agar (1.2 mm). Average size of sclerotia was recorded on Corn meal agar (0.8 mm), Oat meal agar (0.8 mm), Rice leaf extract agar (0.4 mm) and Rice Seed extract agar +2% sucrose (0.7 mm). Minimum size of sclerotia was recorded on Czapek's dox agar (0.5 mm), Richard's agar (0.4 mm).

Observations on Sclerotial characteristics was recorded on eight culture media and data of sclerotial topography, colour, arrangement and clump formation are presented in (Table 4). Sclerotia pattern was superficial in Potato dextrose agar, Richard's agar, Corn meal agar, Rice seed extract agar and Rice Seed extract agar +2% sucrose. Whereas, immersed sclerotia were formed in Oat meal agar, Czapek's dox agar and Rice leaf extract agar media. Variations in sclerotial colour from light brown, brown to Deep dark brown were recorded. Light brown sclerotia were formed in Potato dextrose agar, Oat meal agar, Richard's agar, Rice leaf extract agar and Rice Seed extract agar +2% sucrose. Whereas, brown sclerotia was formed on Rice seed extract agar and Deep dark brown on corn meal agar and Czapek dox agar. Peripheral, central and scattered arrangement of sclerotia were observed in tested media. All three patterns were recorded in Richard's agar and Czapek dox agar media. Central and scattered pattern was observed in oat meal agar, peripheral and scattered pattern was observed in corn meal agar. Only scattered pattern was observed in potato dextrose agar, central in Rice leaf extract agar and peripheral pattern in Rice seed extract agar and Rice Seed extract agar +2% sucrose were recorded.

Discussion

In the present study, colony characteristics of *R. solani* were studied in eight culture media. Average colony diameter ranged from 75.8 to 88.0 mm after 120 hrs of incubation, respectively. The maximum mycelial growth of *R. solani* was observed on Potato dextrose agar (88.0 mm) closely followed in Czapek's dox agar (87.7 mm), Corn meal agar (84.8 mm) and Oat meal agar (84.2 mm). Least growth was recorded in Rice seed extract agar (75.8 mm) and Richard's agar (76.0 mm). This may be due to presence of antifungal substances in seeds of Rice. Maximum mycelia growth of *R. solani* on Potato dextrose agar and Czapek's dox agar was also reported by

Sharma (2013) in soybean, Chouhan (2014) and Jain *et al.* (2017) in little millet, in rice and maize. Kumar *et al.* (2014) found maximum growth of *R. solani* isolated from urd bean in Potato dextrose agar as well as in Czapek's dox agar and least growth in Richard's medium. Hase and Nasreen (2017) reported maximum growth of *R. solani* in Corn meal agar and Czapek's dox agar. All these results are in agreement with the present findings.

Variation in colour, growth pattern and growth rate of *R. solani* in different culture media was observed. Colour of the culture varied from white, pale brown to yellowish brown with abundant, moderate to slight growth pattern and fast, moderate to slow growth rate of the fungus in tested media. The excellent fast, abundant and off white mycelial growth was recorded on Potato dextrose agar. Whereas fast, abundant and white growth was observed on Czapek's dox agar. In Oat meal agar, fungal growth rate was moderate, abundant and pale brown, while moderate growth rate, slight growth pattern and off white colony was observed in Corn meal agar. Moderate growth rate, slight growth pattern and pale brown colony was observed in Rice seed extract agar and Rice seed extract agar +2% sucrose. In Richards agar medium growth rate was slow, growth pattern was abundant and off white colony was recorded. Lal and Kandhari (2009) reported great diversity in colony colour, growth pattern and colony diameter of *R. solani*.

Sclerotial characteristics and pattern was studied in eight culture media and significant variation was recorded. Time taken for initiation of sclerotia varied 3 to 4 days in different culture media. Variation in number of sclerotia, weight of 10 sclerotia and size of sclerotia was 12.3 to 54.0, 135.0 to 1030.6 mg and 0.4 to 1.3mm, respectively. Maximum number of sclerotia was recorded in Oat meal agar (54.0) closely followed by Potato dextrose agar (51.2) and Czapek's dox agar (44.6). Whereas least sclerotia were formed in Rice seed extract agar (12.3) followed by Rice leaf extract agar (15.3) and Rice seed extract agar + 2% sucrose (16.5). Maximum weight of 10 sclerotia was recorded in Potato dextrose agar (1030.6 mg) followed by Czapek's dox agar (920.3 mg) and Oat meal agar (884.7 mg). Minimum weight of sclerotia was observed in Corn meal agar (135.0 mg) followed by Richard's agar (215.3 mg) and Rice seed extract agar + 2% sucrose (345.0 mg). Significant variation in size of sclerotia was observed, which ranged 0.4 mm to 1.3 mm with maximum size in Potato dextrose agar followed by Rice seed extract agar (1.2 mm) and Corn meal agar (0.8 mm). Smallest sclerotia were formed in Richard's agar (0.4mm) and Rice leaf extract agar (0.4) followed by Czapek's dox agar (0.5 mm). Sharma *et al.* (2013), Kumar *et al.* (2014) and Husain *et al.* (2016) also reported maximum sclerotial formation of *R. solani* in Oat meal agar, Potato dextrose agar and Czapek's dox agar. These results confirm the present findings.

CONCLUSION:

It can be concluded that Potato dextrose agar (88.0 mm) and Czapek's dox agar (87.7 mm) medium supported maximum mycelial growth of *R. solani* isolated from rice. Whereas, least mycelial growth (75.8 mm) was observed in Rice seed extract agar and Richards agar medium. Mycelial growth was excellent fast, abundant and off white on Potato dextrose agar. Whereas it was fast, abundant

andon Czapek's dox agar. Sclerotia of the fungus initiated within 3 - 4days in different culture media. Maximum number of sclerotia was produced in Oat meal agar (54.0) closely followed by Potato dextrose agar (51.2) and Czapek's dox agar (44.6). Maximum weight of 10 sclerotia was recorded in potato dextrose agar (1030.6 mg) followed by Czapek's dox agar (920.3 mg) and Oat meal agar (884.7 mg). Maximum size of sclerotia were formed in Potato dextrose agar followed by Rice seed extract agar and Corn meal agar.

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Table 1. Effect of different culture media on mycelial growth of *R. solani* causing Sheath blight of rice

S. No.	Media	Mycelial diameter (mm)			
		48 hrs	72 hrs	96 hrs	120 hrs
1	Potato dextrose agar	35.8	68.3	84.3	88.0
2	Oat meal agar	27.5	57.7	80.0	84.2
3	Richard's agar	26.9	39.8	71.5	76.0
4	Corn meal agar	28.3	64.7	81.3	84.8
5	Czapek's dox agar	35.4	67.3	83.9	87.7
6	Rice leaf extract agar (30%)	31.0	48.3	76.8	80.0
7	Rice seed extract agar(10%)	33.0	46.3	71.2	75.8
8	Rice seed extract agar(10%) +2% sucrose	36.7	60.7	79.0	83.3
	CD (5%)	4.25	5.90	7.12	5.05

Table 2. Mycelial growth pattern, growth rate and colour of *R. solani* on different culture media

S. No.	Media	Culture characteristics		Growth rate
		Colour	Growth pattern	
1	Potato dextrose agar	Off White	Abundant	Fast
2	Oat meal agar	Pale Brown	Abundant	Moderate
3	Richard's agar	Off White	Abundant	Slow
4	Corn meal agar	Off White	Slight	Fast
5	Czapek's dox agar	Off White	Abundant	Fast
6	Rice leaf extract agar (30%)	Yellowish brown	Moderate	Moderate
7	Rice seed extract agar (10%)	Pale Brown	Slight	Moderate
8	Rice seed extract agar (10%) + 2% sucrose	Pale Brown	Slight	Moderate

Table 3. Effect of different culture media on sclerotial formation of *R. solani* causing Sheath blight of rice

S. No.	Media	Time taken for initiation of sclerotia	Number of sclerotia	Weight of 10 sclerotia (mg)	Size of sclerotia (cm)
1	Potato dextrose agar	3	51.2 (7.46)	1030.6	1.3
2	Oat meal agar	3	54.0 (7.61)	884.7	0.8
3	Richard's agar	3	28.2 (5.55)	215.3	0.4
4	Corn meal agar	4	34.6 (6.08)	135.0	0.8
5	Czapek's dox agar	3	44.6 (7.03)	920.3	0.5
6	Rice leaf extract agar (30%)	4	15.3 (3.63)	528.2	0.4
7	Rice seed extract agar (10%)	3	12.3 (3.51)	536.5	1.2
8	Rice seed extract agar (10%) + 2% sucrose	4	16.5 (3.79)	345.0	0.7
	CD (5%)		0.645	38.80	0.38

Figures in parentheses are square root transformed values

Table 4. Sclerotial characteristics of *R. solani* on different culture media

Media	Sclerotial characteristics			
	Topography	Colour	Arrangement	Clump formation
Potato dextrose agar	Superficial	Light brown	Scattered	Less
Oat meal agar	Immersed	Light brown	Centre, Scattered	More
Richard's agar	Superficial	Light	Peripheral,	Medium

		brown	Centre, Scattered	
Corn meal agar	Superficial	Deep dark brown	Peripheral, Scattered	Less
Czapek's dox agar	Immersed	Deep dark brown	Peripheral, Centre, Scattered	More
Rice leaf extract agar (30%)	Immersed	Light brown	Centre,	Medium
Rice seed extract agar(10%)	Superficial	Brown	Peripheral,	Less
Rice seed extract agar(10%)+2% sucrose	Superficial	Light brown	Peripheral,	Less

UNDER PEER REVIEW