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CULTURAL AND MORPHOLOGICAL VARIABILITY IN *RHIZOCTONIA SOLANI* ISOLATES OF RICE AND MAIZE

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ABSTRACT
Sheath Blight of rice and Banded leaf and sheath blight of maize caused by *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*), is a complex pathogen and worldwide in distribution, a very destructive disease under favorable weather conditions causes substantial yield losses. Cultural and Morphological variability was studied among isolates of rice and maize collected from N.E. Borlaug Crop Research Centre, Pantnagar (29º N latitude, 79.3º E longitude), Uttarakhand, India.Colony size, colony growth, colour and sclerotia formation (central, peripheral or scattered), location (aerial or surface) and texture (smooth or rough) varied in isolates of rice and maize. Among different media tested PDA was found best for the sclerotial development in both the isolates followed by Czapek's dox media. Sclerotia of *Rhizoctonia solani of* rice isolate were found brown in color which was formed at the center and periphery of the plates, whereas *R. solani* of maize isolate was fast growing, white in color and sclerotia formed scattered in the plates with larger size and more in numbers.The *R.solani* pathogen grew best at temperature between 25-30ºC and maximum at 30ºC with 7 pH and have great diversity in cultural or morphological characters when compared with each other.

INTRODUCTION

Rice (*Oryza sativa L.)* and Maize *(Zea mays)* is a graminaceous crop. They are one of the important staple foods for Asian and Europian countries. Sheath blight disease in rice and Banded leaf and sheath blight in maize are important fungal diseases which is caused by *Rhizoctonia solani*. The disease is alarming due to its intensive cultivation of modern high yielding varieties with high doses of nitrogenous fertilizers. Yield losses due to this disease are estimated to range from 1.2 to 69 per cent (Rajan 1987; Naidu 1983) in rice and 23.9 to 31.9 per cent (Lal *et al.,* 1980) in maize, depending on cultivars, environmental conditions, crop stages at which the disease appears and cultivation practices. Grain yield can be increased if there is an increase in traits showing positive and significant association with grain yield hence genetic characters can be given priority while selection for higher yield as these was mutually and directly associated with yield (Mamta *et al.,* 2015). Currently, this pathogen is distributed in almost all the rice and maize growing areas. *R. solani* has potential to show variation in their characters. Cultural and morphological analysis of West Bengal isolates of rice has indicated that the diversity among the isolates does not correlated with their origin. On the basis of morphological characteristics, *R. solani* isolates could be easily separated from R. oryzae-sativae isolates (Kuiry *et al*., 2014). After analyzing the morphological and cultural characters of the isolates, it was found that there was no relations between the isolates with respect to their origin from where they were collected. There is a significance importance of mycelial and sclerotial characteristics in studying the variations among different *R. solani* isolates (Sriram *et al*., 1997), (Meena *et al*., 2001), (Neeraja *et al*., 2002) and Singh *et al*. (2003). Different isolates may produces different types of symptoms on different hosts. Isolate of Web blight fungus of mung bean (*R. solani*) are also virulent on rice but causes different type of symptom on it than do the isolates of *Rhizoctonia solani,* the casual agent of Rice sheath blight(Singh *et al.,* 2014). Efforts were made to study the variation among rice and maize isolates of *Rhizoctonia solani* and to develop suitable management strategy. Keeping this information in mind, the present investigation was carried out to find out cultural and morphological variability in *R. solani* isolates of rice and maize.

MATERIALS AND METHODS

Infected leaves of rice and maize exhibiting typical symptoms of sheath blight of rice and banded leaf and sheath blight of maize were collected in a paper bag from pathology block of N.E. Borlaug Crop Research Centre, GBPUA & T, Pantnagar, Uttarakhand, India. The leaves sample were brought to the laboratory for its microscopic examination and isolation. The pathogen was isolated on potato dextrose agar (PDA) and purified through hyphal tip/single sclerotial method (Rangaswami and Mahadevan, 2005). Pure culture maintained and stored in refrigerator at 5ºC for further studies. Fungi derives energy from the substrate on which they grow. They need nearly the same essential elements for their proper growth and development but their ability to utilize the compound present in the medium is different. No universal medium is

available on which all the fungus can be grown properly, therefore it is important to study the suitable media on which the proper growth and sporulation of the fungi can take place. The experiment was conducted by growing the *R. solani* isolates of rice and maize on four different culture media viz; Potato Dextrose Agar medium, Richard's Agar medium, Czapek's Dox Agar medium and Corn Meal Agar medium to find out the best suited medium for mycelial growth in Petri dishes, the basic cultural characteristics such as colony diameter, colour,growth pattern, time taken for initiation of sclerotial formation, pattern of production (central, peripheral and scattered) sclerotialcolour and location formed were also recorded (Kuiry *et al.,* 2014). All the observations were recorded after 5 days visually, according to growth of hyphae- as abundant, aerial, sparse, dense etc. Growth of each isolates were measured in five replications. For isolation and identification, standard procedures were followed as described by Bhuiyan (1994).

For effect of different temperature

The effect of temperature on the growth of fungus was studied on PDA on different temperature values viz; 15, 20, 25, 30 and 35& 45ºC. Petri plates were poured with about 20 ml sterilized melted medium aseptically and each treatment was replicated thrice. After solidification of the medium, plates were centrally inoculated with 5 mm mycelial disc cut from the margin of 3 days old culture with the help of a sterilized cork borer. Inoculated Petri plates were incubated for 72 hrs for mycelial growth measurement.

For effect of different pH

Five pH level viz., 5.0, 6.0, 7.0, 8.0 and 9.0 were adjusted in PDA with the help of pH meter by using 0.1N acetic acid or 0.1N sodium hydroxide before autoclaving for keeping the pH constant. Each treatment was replicated thrice. After solidification of the medium each plate was centrally inoculated with 5 mm mycelial disc cut from the margin of 3 days old culture of both the isolates with the help of a sterilized cork borer. Inoculated Petri plates were incubated at 27 + 0.2ºC for 72 hrs to record mycelial growth measurement.

RESULTS AND DISCUSSION

Effect of different cultural media on mycelial growth and sclerotia formation of *Rhizoctonia solani*

Rhizoctonia solani is a fast growing and a complex fungi which produces, whitish fluffy growth and later turns brown on PDA medium. It was found that in rice isolates, the growth pattern of colony in each media was sparse with off white in colour, whereas sclerotia are formed only in PDA and Czapek's dox media at the centre of the plates which were brown in colour.

In case of maize isolates of *Rhizoctonia solani*, colony colour was creamy white colour in PDA and Czapek's dox media with cottony fluffy growth, in Richard's and Corn meal agar media colony colour was off white with sparse growth. Sclerotial formation took place in PDA and in Czapek's dox media which was scattered and white in colour at its initial stage and more in numbers as comparison to rice isolates. (Table 1).

Effect of different media on the growth of *R. solani* of rice and maize

Table 2; Fig. 1 reveals that after 72 hrs of incubation at 28 ± 1ºC, maximum growth (87.50 mm) of *R. solani* of rice was recorded in Potato Dextrose Agar medium followed by Czapek' Dox Agar medium (86.83mm) and Corn Meal Agar medium (83.16 mm) while minimum growth (57.16 mm) was recorded in Richard's medium, PDA and Czapek's agar was found best in supporting mycelium growth.In case of maize isolates maximum growth (88.33 mm) was observed in Potato Dextrose Agar followed by Czapek's Dox (56.00 mm). Minimum radial growth (46.50 mm) of the test fungus was found in Richard's

Table1: Effect of different cultural media on mycelial growth and sclerotia formation of *Rhizoctonia solani*

Media	Rice *Rhizoctonia solani*				Maize			
	Colony colour	Presence of sclerotia	*Pattern of Sclerotia formation	Growth patternof colony	Colony colour	Presence of sclerotia	*Pattern of Sclerotia formation	Growth pattern of colony
PDA	Off white	+ +	Central/brown	Sparse	Creamy white	+ + +	Scattered/White	Dense/ Cottony fluffy
Czapek's dox media	Off white	+	Central/brown	Sparse	Creamy white	+	Scattered	Cottony fluffy
Richard's media	Off white	-	-	Sparse	Off white	-	-	Sparse
Corn Meal Agar media	Off white	-	-	Sparse	Off white	-	-	Sparse

* The data was recorded after 5 days of incubation at 28 ± 1ºC.

Table 2: Effect of different media on radial growth of *Rhizoctonia solani* of rice and maize isolates after 72 hours of incubation at 28 ± 1°C

S. No.	Media	Rice *Rhizoctonia solani* Colony diameter (mm)	Maize Colony diameter (mm)
1	Potato Dextrose Agar Medium	87.5	88.33
2	Czapek' Dox Medium	86.83	56
3	Richard's Medium	57.16	46.5
4	Corn Meal Agar Medium	83.16	47.16
CD at 5% =		0.48	0.61
SEm ± =		0.16	0.20
CV =		5.06	8.58

Table 3: Effect of different temperature on radial growth of *Rhizoctonia solani* of rice and maize isolateafter 72 hours of incubation at 28 ± 1°C

S. No.	Temperature (ºC)	Rice *Rhizoctoniasolani* Colony diameter (mm)	Maize Colony diameter (mm)
1	15	33.6	36.3
2	20	44.5	47.86
3	25	71.3	75
4	30	80.23	83.56
5	35	65.89	66.63
6	45	00.00	0.00
CD at 5% =		2.95	3.23
CV =		3.36	3.52

Table 4: Effect of pH on radial growth of *Rhizoctonia solani* of rice and maize isolateafter 72 hours of incubation at 28 ± 1°C

S. No.	pH	Rice *Rhizoctonia solani* Colony diameter (mm)	Maize Colony diameter (mm)
1	5	52.9	55.66
2	6	70.3	72.63
3	7	80.83	84.23
4	8	74.3	78.66
5	9	64.86	65.96
CD at 5% =		3.87	4.12
CV =		3.10	3.17

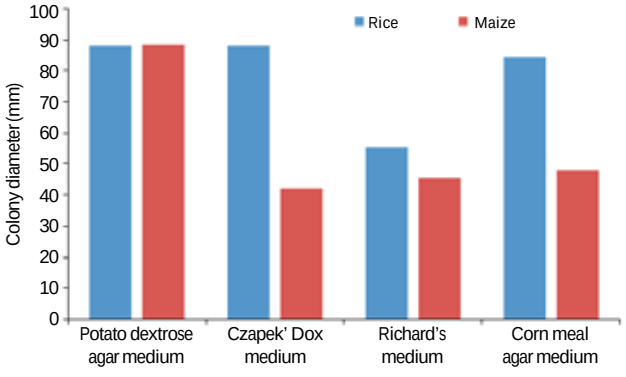

Figure 1: Effect of different media on radial growth of *Rhizoctonia solani* of rice and maize isolates after 72 hours of incubation at 28 ± 1ºC

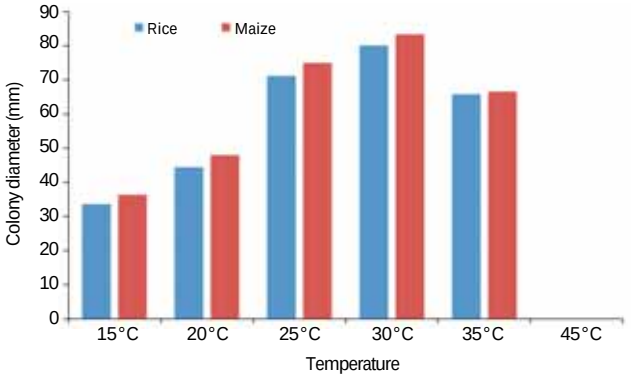

Figure 2: Effect of different temperature on radial growth of *Rhizoctonia solani* of rice and maize isolates after 72 hours of incubation

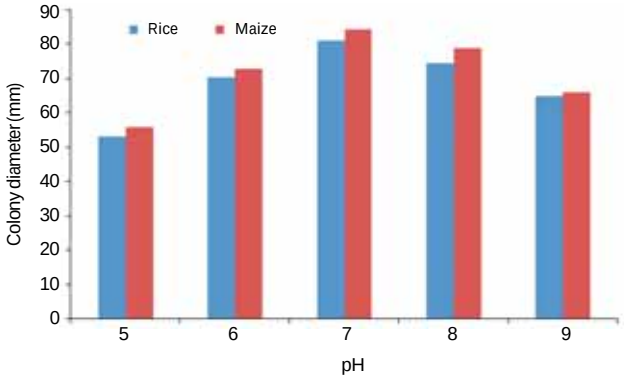

Figure 3: Effect of different pH on radial growth of *Rhizoctonia solani* of rice and maize isolates after 72 hours of incubation at 28 ± 1ºC

medium which was followed by Corn Meal Agar medium (47.16 mm). It was also found that mycelial growth of maize isolates was more in PDA as comparison to *R.solani* isolates of rice but it was less in case of other test mediums.

Effect of temperature on the growth of *R. solani* isolates of rice and maize

The growth pattern of *R. solani* of rice and maize isolates were recorded at different temperature *i.e.* 15ºC, 20ºC, 25ºC, 30ºC 35ºC and 45ºC. After 72 hrs of incubation it was found that maximum growth was recorded at 30ºC (80.23 mm) in case of rice isolates followed by 25ºC (71.30 mm) and 65.89 mm at 35ºC while minimum growth (33.60mm) was recorded at 15ºC followed by 44.50 mm mycelial growth at 20ºC, no growth was observed at 45ºC. In case of maize isolate maximum growth (83.56 mm) was observed at 30ºC while 75.00 mm growth was found at 25ºC. Minimum growth (36.30 mm) was

at 15ºC which was followed by 47.86 mm of radial growth at 20º C.No growth was recorded at 45ºC (Table 3; Fig 2).This result reveals that the *R.solani* pathogen grows best at temperature between 25-30ºC and maximum at 30ºC.

Effect of different pH levels

During evaluation of different pH levels suitable for growth of *R. solani* isolate of rice and maize, it was observed that the maximum growth (80.83 mm) was recorded at pH 7.0 followed by pH 8.0 (74.30 mm) and 70.30 mm radial growth at pH 6.0 while minimum colony diameter was observed at pH 5.0 (52.90 mm),whereas in case of maize isolate the maximum growth (84.23mm) of the fungus was observed at pH 7 followed by pH 8 favoring 78.66 mm growth. Minimum radial growth was observed at pH 5 *i.e.* 55.66 mm followed by pH 9 having 65.96 mm radial growth. (Table 4; Fig. 3).The study showed that the colony diameter increased from pH 5-7, thereafter further increase in the pH there was a decreased trend in colony diameter.

The study also showed that the colony diameter of maize isolate is more as comparison to rice isolates at different temperature and pH.

Gangopadhyay and Chakrabarti (1982) reported that the sclerotia of *R. solani* were produced as compact mass of cells, were first greyish- white, later brown or greyish-black in colour at maturity. According to Huang-ShiChen and Li-XiYing (2004) Potato dextrose agar was the most appropriate growth medium for *R. solani* he also revealed that optimum hyphal growth of *R. solani* was between pH 6-8.Ritchie *et al.* (2009) showed that isolates of *R. solani* produced more sclerotia when initially grown on or transferred to a nutrient rich medium i.e. PDA and the optimum temperature and pH required for mycelial growth and germination was between 20 to 30ºC with broad pH range, *i.e.* pH 4 to 8 with optimum growth between pH 5 to 6. Harikrishnan, R and Yang, X.B. (2004) while describing the effects of temperature on the growth among the different isolates of *R. solani* reported that the optimum growth rate recorded temperature is between 25°C and 30°C. These findings are accordance with our results but it was observed that *R. solani* isolates of rice and maize have great diversity in cultural or morphological characters when compared with each other.

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