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Physiological and nutritional variability of different *Pyricularia grisea* isolates isolated from various hosts inciting blast disease of rice

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Abstract

Different isolates of *Pyricularia grisea* from rice (RPg), *Panicum* grass (PPg) and finger millet (FPg) were isolated for variability study of the pathogen. *Pyricularia grisea* was grown on nine different solid media viz., Potato dextrose agar, Potato sucrose agar, Czapek's dox agar, Sabouraud agar, Richard's medium, Potato carrot agar, *Panicum* leaf extract agar, Rice leaf extract agar and Oat meal agar to study the cultural characteristics of the pathogen. Oat meal agar supported maximum radial growth (83.33 mm) followed by Potato dextrose agar (70.17 mm) and Potato carrot agar (66.17 mm) in case of rice blast. In case of *P. grisea* from *Panicum* and finger millet, PDA supported highest radial growth of 84.00 mm and 82.50 mm respectively followed by Potato sucrose agar (83.33 mm and 80.33 mm) respectively. Highest radial growth of the pathogen (70.13 mm) was found at 30 °C followed by 25 °C (61.88 mm). Sodium nitrate produced highest growth of mycelial dry weight (12079.0 mg) followed by ammonium oxalate (4971.0 mg).

Keywords: *Pyricularia grisea*, rice blast, physiological and nutritional variability

Introduction

Rice (*Oryza sativa* L.) is considered as staple food and mainstay for the human beings for their food security. Genus *Oryza* has 24 different species out of which only two species have been cultivated for staple food, i.e. *Oryza sativa* and *Oryza glaberrima* and rest 22 species are wild types. *Oryza sativa* is grown all over the world while *Oryza glaberrima* has been cultivated in West Africa. Rice is cultivated in 114 countries across the world. The global annual production of rice has been estimated at 697.22 million tons with productivity of 4.4 tons ha⁻¹ cultivated in 158.43 million ha.

The potential yield of rice is affected by many diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non-parasitic disorders. Among the fungal diseases, rice blast caused by *Pyricularia grisea* is considered as a major threat to rice production because of its wide spread distribution and its destructiveness under favourable conditions. The fungus *Pyricularia grisea* attacks at all stages of the crop and symptoms appear on the following parts of the rice plant: leaf (leaf blast), leaf collar (collar blast), panicle neck node (neck rot) and panicle (panicle blast). The symptoms are more severe in case of neck blast that is characterized by the infection at the panicle base followed by rotting.

Materials and Methods

Collection of disease samples from different hosts

Blast infected rice leaves were collected from different fields of OUAT farm during the month of October and November 2018. Finger millet leaves infected with blast were collected from MULLaRP, CPR (Centre for Pulses Research) Station, Berhampur, OUAT. Infected *Panicum* grasses were also collected from farm bonds of OUAT. All the samples collected carefully in polythene bags separately and brought to the laboratory for further study.

Isolation of *Pyricularia* isolates from different hosts

The necrotic patches on the diseased leaves of rice, *Panicum repens* and finger millet were cut into small pieces having both healthy and diseased portion.

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These pieces were surface sterilized by dipping in 0.1% mercuric chloride solution for 40 seconds and then washed three times with sterilized water. The cut pieces were then transferred to a sterilized petri dish containing oat meal agar medium. Four pieces were inoculated at the four corners of the plates. These plates were then incubated at 27 ± 1 °C for the development of fungal growth.

After seven days of incubation mycelial growth of the fungus was seen on the plates. Hyphal tip of the fungus from the mycelial colony was transferred into the petriplates with the help of inoculation needle. This hyphal tip method was subsequently followed two to three times to get pure culture of the pathogen. Pure culture of the fungus was transferred to the oat meal agar slants from the petriplate in aseptic condition. These slants were incubated at room temperature for the growth of the fungus and then stored in refrigerator for future study and use.

Study of cultural characteristics of different *Pyricularia grisea* isolates

Cultural characteristics of *Pyricularia grisea* were studied on nine solid media viz., Potato dextrose agar, Potato sucrose agar, Czapek's dox agar, Sabouraud agar, Richard's medium, Potato carrot agar, *Panicum* leaf extract agar, Rice leaf extract agar and Oat meal agar. About 20ml of each media was poured into sterilized petridish and solidified in laminar airflow chamber. Fungal disc of the pathogen (8mm) was inoculated at the centre of the media from an actively growing culture plate. These plates were replicated thrice and incubated at 25 ± 1 °C. When the maximum growth of the fungus was obtained in any one of the media, the radial growth of the colony was measured. The colony diameter was measured and recorded in the table.

Effect of temperature

The effect of different temperatures on the growth of *Pyricularia grisea* isolate from rice was studied at six temperature levels viz., 10, 15, 20, 25, 30 and 35 °C. 20 ml of potato dextrose agar medium was poured into a sterilized petri plate and solidified at room temperature under laminar air flow chamber. Mycelial disc of *Pyricularia* was inoculated at the centre of the plate from actively growing culture plate and incubated at different temperature levels in a BOD incubator. Each treatment was replicated four times. The mycelial growth in each treatment was recorded at 7 days after inoculation and presented in the table.

Nitrogen utilization

For nitrogen requirement study, Czapek's dox broth was taken as base media. Different nitrogen sources viz. ammonium carbonate, ammonium oxalate, sodium nitrate, potassium nitrate, sodium nitrite, calcium nitrate were used for the study. In every treatment only the source of nitrogen was changed instead of the original one. The quantity of nitrogen compound was determined on the basis of their molecular weight so as to provide an equivalent amount of nitrogen as that of sodium nitrate present in the base medium. Each treatment was replicated four times. Conical flasks containing different sources of nitrogen were inoculated with

the pathogen under aseptic conditions and incubated at 25 ± 1 °C. After 15 days, dry mycelial weight of the pathogen was recorded and data was analyzed statistically.

Results and Discussion

Growth behavior of *Pyricularia grisea* isolates in different growth media

All the three *Pyricularia grisea* isolates were grown in nine different media. The growth of *Pyricularia grisea* from rice was highest (83.33mm) in oat meal agar followed by potato dextrose agar (70.17mm) [Table-1]. Lowest growth was found in Richard's agar medium (10.00mm). PDA supported highest radial growth (84.00mm) for *Pyricularia* isolate from *Panicum repens* and finger millet (82.50mm). Mijan Hossain (2000) [4] reported PDA medium with highest radial growth (85.00 mm) followed by host extract (80.33 mm) for blast pathogen. Ravindramalviya (2014) [7] also reported highest growth of *Pyricularia grisea* in PDA followed by Richard's agar medium. In the present study, good growth of rice blast and finger millet blast were found in PDA medium which supported the studies conducted by above scientists. Sporulation of *Pyricularia grisea* was very sparse in PDA which confirmed the finding of Ravindramalviya (2014) [7]. Kulmitra *et al.* (2017) [3] also found oat meal agar medium to be the best for growth of *Pyricularia grisea* from rice.

Growth of *Pyricularia grisea* isolate from rice in different temperature ranges

Out of six temperature ranges taken for the growth of *Pyricularia grisea* isolated from rice, 30 °C was found to be the most conducive for growth with radial growth of 70.13 mm followed by growth in 25 °C (61.88 mm) [Table-2]. Teng (1994) [8] and Arunkumar and Singh (1995) [1] also found 25-30 °C to be the optimum for growth of *Pyricularia grisea* from rice. In the current study the growth increased from 10 °C upto 30 °C but then it was decreased at 35 °C (20.75 mm radial growth). This suggested that the growth of the test fungus was retarded at higher temperature. Rajput *et al.* (2017) [6] also observed good growth and sporulation upto temperature of 27 °C and decreased growth thereafter upto 32 °C.

Growth of *Pyricularia grisea* isolate from rice in different nitrogen sources

Six nitrogen sources were tested to study the growth of *Pyricularia grisea* isolated from rice. Sodium nitrate was found to be the most effective source and produced highest growth of mycelial dry weight (12079.0 mg) followed by ammonium oxalate (4971.0 mg). Sodium nitrite supported lowest growth of rice blast pathogen (1324.0 mg). Mijan Hossain (2000) [4]; Gashaw *et al.* (2014) [2] and Pandey (2014) [5] also reported sodium nitrate to be the best source of nitrogen for the growth of *Pyricularia grisea*. The current study confirmed to their findings. The test fungus produced less mycelial dry weight when sodium nitrite (1324.0 mg) and potassium nitrate (1820.0 mg) were taken as nitrogen source even lower than without any nitrogen source i.e. control (2150.0 mg). This suggested that sodium nitrite and potassium nitrate could not supply nitrogen rather inhibited the growth of test fungus [Table-3].

Table 1: Growth of *Pyricularia grisea* isolates in different growth media

Treatments	Radial growth of the <i>Pyricularia grisea</i> isolates (mm)		
	RPg**	PPg	FPg
T ₁ *	70.17	84.00	82.50
T ₂	54.00	83.33	80.33

T ₃	50.17	80.33	61.17
T ₄	38.83	82.00	71.33
T ₅	10.00	80.00	54.00
T ₆	66.17	70.33	54.33
T ₇	54.17	81.50	57.50
T ₈	61.83	81.00	55.83
T ₉	83.33	75.33	55.00
SEm (±)	0.55	0.55	0.67
CD (5%)	1.64	1.64	2.01

* T₁ – Potato dextrose agar, T₂- Potato sucrose agar, T₃- Czapek's dox agar, T₄- Sabouraud's agar, T₅- Richard's agar, T₆- Potato carrot agar, T₇- *Panicum* leaf extract, T₈- Rice leaf extract, T₉- Oat meal agar

** RPg – *Pyricularia grisea* from Rice, PPg – *Pyricularia grisea* from *Panicum repens*, FPg – *Pyricularia grisea* from Finger millet.

Table 2: Effect of temperature on growth of *Pyricularia grisea* isolate from rice

Temperature	Radial growth of pathogen (mm)
10 °C	22.13
15 °C	36.88
20 °C	46.63
25 °C	61.88
30 °C	70.13
35 °C	20.75
SEm (±)	0.47
CD (5%)	1.40

Table 3: Growth of *Pyricularia grisea* isolate from rice in different nitrogen sources

Treatments	Nitrogen sources	Mycelial dry weight of pathogen (mg)
T ₁	Ammonium carbonate	2685.0
T ₂	Sodium nitrate	12079.0
T ₃	Potassium nitrate	1820.0
T ₄	Ammonium oxalate	4971.0
T ₅	Calcium nitrate	2395.0
T ₆	Sodium nitrite	1324.0
T ₀	Control (No Nitrogen)	2150.0
SEm (±)		75.33
CD (5%)		230.71

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