International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 11, 2018, pp.-1400-1405.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

IN VITRO EVALUATION OF DIFFERENT MEDIA AGAINST GROWTH AND SPORULATION OF *PYRICULARIA GRISEA* ISOLATES CAUSING RICE BLAST

CH VARAPRASADA RAO[1*] AND ANIL KUMAR P.[2]
[1]ICAR-Krishi Vigyan Kendra, Darsi, Prakasam, 523247, Acharya N. G. Ranga Agricultural University, Lam, Guntur, 522034, Andhra Pradesh, India
[2]Department of Plant Pathology, Agricultural College, Bapatla, 522101, Acharya N. G. Ranga Agricultural University, Lam, Guntur, 522034, Andhra Pradesh, India
*Corresponding Author: Email - varaprasadakvk@gmail.com

Received: November 01, 2018; Revised: November 26, 2018; Accepted: November 27, 2018; Published: November 30, 2018

Abstract- Five different solid nutrient media, *viz*., Potato Dextrose Agar, Czapek Dox Agar, Oat Meal Agar, Rice Polish Agar and Rice Leaf Extract Agar medium evaluated against mycelia growth and sporulation of *Pyricularia grisea* isolates collected from Prakasam *viz*., PKM 1 to PKM 6 and Sri Potti Sreeramulu Nellore districts *viz*., NLR 1 to NLR 6 of Andhra Pradesh. Among the solid media, Oat Meal Agar (79.7mm) followed by Potato Dextrose Agar (79.5mm) supported maximum colony diameter of all isolates while Czapek Dox Agar supported least mycelial growth of *P. grisea* isolates (65.9mm). The maximum no. of conidia was observed on Oat Meal Agar ($3.8X10^6$ conidia/cm^2) followed by Rice Polish Agar ($3.7X10^6$ conidia/cm^2) and no sporulation was observed in Czapek Dox Agar. Among different liquid media *viz*.,potato dextrose medium, oat meal medium, Czapek Dox medium, rice polish medium and rice leaf extract medium, maximum dry mycelia weight was observed in Potato Dextrose Medium (208.8 mg) followed by Oat Meal Medium (207.0 mg) and least growth was observed on Czapek Dox Medium (84.7 mg).

Keywords- *Pyricularia grisea, Nutrient medium, Colony diameter, Dry mycelial weight*

Citation: Ch Varaprasada Rao and Anil Kumar P. (2018) *In vitro* Evaluation of Different Media Against Growth and Sporulation of *Pyricularia grisea* Isolates Causing Rice Blast. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 11, pp.-1400-1405.

Introduction

Rice (*Oryza sativa* L.) is an important food crop in supplying approximately 23% per capita energy for Six billion people worldwide. Rice blast caused by *Pyricularia grisea* Sacc. is an important biotic constraint for rice production across the world where paddy is being cultivated [1]. The fungus has ability to overcome the resistance within a short time after release and spread of a resistant cultivar [2]. In recent times, rice blast has become one of the prevalent and major disease on rice in Prakasam and Sri Potti Sreeramulu Nellore districts of Andhra Pradesh, causing heavy losses to the rice growing farmers. The objective of the present investigation was to study the growth conditions and sporulation on different solid and liquid media *in vitro* for further characterization and variability among *Pyricularia grisea* isolates.

Material and Methods:

Glassware of Borosil® make were used. The glassware consisted of Petri dishes, conical flasks, test tubes, screw caps, measuring cylinders and watch glasses. The glassware was first washed with detergent powder followed by thorough cleaning with tap water. Later, the glassware were kept overnight in cleaning solution containing 75 g potassium dichromate, 500 ml of water and 1000 ml distilled water and again washed with tap water followed by rinsing with distilled water. Analytical laboratory grades of M/s SD Fine Chemicals and M/s Hi Media chemicals were used in the present investigation.

Composition and preparation of solid media

Potato dextrose agar (PDA)

Potato	: 200.0 g
Dextrose	: 20.0 g
Agar – agar	: 20.0 g
Distilled water	: 1000 ml

Potato extract was prepared by boiling 200 g of peeled potato pieces in 500 ml of distilled water. To another 500 ml; agar was added and melted by boiling. The potato extract was filtered through muslin cloth and the filtrate was collected and added to the melted agar. Finally the volume was made up to 1000 ml with distilled water after adding dextrose to it. The pH (6.5) of the medium was adjusted using sodium hydroxide (NaOH) or lactic acid before sterilization of the media in an autoclave.

Czapek Dox agar medium (CDA)

Agar-agar	: 20.0 g
Dipotassium dihydrogen phosphahte ($K_2H_2PO_4$)	: 1.0 g
Ferrous sulphate ($FeSO_4.7H_2O$)	: 0.01 g
Magnesium sulphate ($MgSO_4.7H_2O$)	: 0.5 g
Potassium chloride (KCl)	: 0.5 g
Sodium nitrate ($NaNO_3$)	: 3.0 g
Sucrose	: 30.0 g
Distilled water	:1000 ml

The above chemicals except sucrose were added to 500 ml of distilled water contained in a 1000 ml flask which was heated in water bath for 15 min. After cooling the contents, sucrose was added to the flask. Agar agar (20 g) was melted in 500 ml of distilled water and added to the above nutrient solution. Final volume was made up to 1000 ml with the help of distilled water before strerilization of the medium in an autoclave.

Oat meal agar (OMA)

Oat flakes	: 30.0 g
Agar – agar	: 20.0 g
Distilled water	: 1000 ml

Oat meal agar medium was prepared by boiling 20 g of oats in 500 ml of distilled water for about 30 minutes in a conical flask. Agar-agar (20 g) was melted in 500 ml of water with distilled water in another conical flask and added to the oat meal broth.

Rice polish agar (RPA)

Rice bran	: 20.0 g
Dextrose	: 10.0 g
Agar	: 20.0 g
Distilled water	: 1000 ml

Twenty grams of rice polish (rice bran) was mixed with 500 ml of water and boiled for 15 min. The suspension was blended for several minutes and mixed with 500 ml of water containing 30 g of Agar agar. Distilled water was added to make up the volume to 1000 ml of rice polish agar medium.

Rice leaf extract agar (RLEA)

Host plant material	: 200.0 g
Dextrose	: 10.0 g
Agar- agar	: 20.0 g
Distilled water	: 1000 ml

Rice leaves collected from healthy plants were cut into small pieces (200 g) and then boiled in 500 ml of distilled water until the pieces became soft. The leaf extract thus obtained was filtered through muslin cloth. Dextrose (20 g) was added to the extract. Agar-agar (20 g) was melted in 500 ml of distilled water and added to the leaf extract. Final volume was made up to 1000 ml. by adding distilled water. pH of the medium was adjusted to 6.5 before sterilization.

Composition and preparation of liquid media

Potato dextrose medium

Peeled potatoes	: 200.0 g
Dextrose	: 20.0 g
Distilled water	: 1000 ml

Czapek Dox medium

Dipotassium dihydrogen phosphate (K2HPO4)	: 1.0 g
Ferrous sulphate (FeSO4 7H2O)	: 0.01 g
Magnesium sulphate (MgSO4 7H2O)	: 0.5 g
Potassium chloride (KCl)	: 0.5 g
Sodium nitrate (NaNO3)	: 3.0 g
Sucrose	:30.0 g
Distilled water	:1000 ml

Oat meal medium

Oat flakes	: 30.0 g
Distilled water	: 1000 ml

Rice polish medium

Rice bran	: 20.0 g
Dextrose	: 10.0 g
Distilled water	: 1000 ml

Rice leaf extract medium

Host plant material	: 200.0 g
Dextrose	: 10.0 g
Distilled water	: 1000 ml

Composition and preparation of liquid media was same as that of solid media but agar-agar was not added to the media. Flasks containing respective medium were inoculated with 5 mm mycelial disc obtained from the actively growing colony of *Pyricularia grisea* and the inoculated flasks were incubated at 27 ± 2ºC. The composition and procedures for preparation of the media used in this experiment were followed as explained by Ainsworth (1971) and Tuite (1969) [3,4]. Variability of *P. grisea* isolates was studied by collecting infected rice leaves from the twelve rice fields of Prakasam and SPSR Nellore districts (6 each) [Table-1] and isolating the casual organism *P. grisea* on oat meal agar medium. Five different solid nutrient media, *i.e.,* potato dextrose agar, Czapek Dox agar, oat meal agar, rice polish agar and rice leaf extract agar medium were used for studying the growth of *P. grisea*. Different solid media were poured separately into sterilized Petri dishes aseptically at the rate of 20 ml of the medium per dish. The media in Petri dishes were allowed to cool and solidify. Each medium contained in the Petri dish was inoculated with *P. grisea* by transferring the mycelial discs of 5 mm size with the help of a sterilized cork borer from 15 days old culture of the fungus. The inoculated Petri dishes were incubated at 27±1ºC. The growth of the fungus in different solid nutrient media was estimated by measuring the diameter of the mycelial colony 15 days after incubation. For each solid nutrient medium, four replications were maintained and the data was analysed using complete randomized design (CRD). Five different liquid nutrient media, *i.e*., potato dextrose medium, oat meal medium, Czapek Dox medium, rice polish medium and rice leaf extract medium were used to study the dry weight of mycelial growth of *P. grisea*. The sterilized liquid media (20 ml) prepared as described earlier were poured into sterilized 100 ml conical flasks aseptically and allow to cool and solidify. The liquid media in conical flasks were inoculated with *P. grisea* by transferring the mycelial discs of 5 mm size into each flask with the help of a sterilized cork borer from 15days old culture of *P. grisea*. The inoculated conical flasks were incubated for 15 days at 27±1ºC. The growth of the fungus was estimated by harvesting mycelial mat of *P. grisea* onto Whatman No.1 filter paper from each medium and measuring the dry weight of mycelial mat. For each medium, four replications were maintained

Sporulation

To assess number of conidia produced per unit area (cm^2) the isolates of *P. griseaconidia* were harvested from 15 day old culture plates (90 cm diameter) into 5 ml sterile distilled water. Conidial count was taken with the help of Naubauer haemocytometer. The observations were made for each isolate.

Results and Discussion:

Colony characters of isolates of *P. grisea*

Isolates under study showed variation in colony colour, growth and sporulation when cultured on different solid culture media [Table-2].

Colony colour

Colony colour was found to be buff, greyish black, black or white in different isolates. Growth of the colony was categorized as good (>80 mm radial growth), medium (70-80 mm radial growth) and poor (<70 mm radial growth) after 15 days of inoculation. On Potato Dextrose Agar (PDA), four isolates, *viz*., PKM 1, PKM 6, NLR 2 and NLR 5 produced buff coloured colonies where as PKM 2 and PKM 5 produced greyish black colonies. The black coloured colonies were formed by PKM 3, NLR 6, NLR 1 and NRL 3. The isolates PKM 4 and NLR 4 produced olive green coloured colonies. On Oat Meal Agar (OMA), isolates PKM 1, PKM 2, PKM 4, PKM 6, NLR 3, NLR 5 and NLR 6 formed greyish black colonies. The buff coloured colonies were formed by the isolates PKM 3, PKM 4, NLR 1 and NLR 4. Isolate NLR 2 produced black coloured colonies. On Czapek Dox Agar (CDA), black coloured colonies were formed by the isolates PKM 2, PKM 3, PKM 5, PKM 6, NLR 1, NLR 2 and NLR 5. Isolates PKM 4, NLR 3 and NLR 4 showed greyish coloured colonies. Buff coloured colonies were produced by the isolates PKM 1 and NLR 6. On Rice Polish Agar (RPA) greyish black coloured colonies were observed with PKM 1, PKM 2, PKM 5, NLR 1, NLR 2, NLR 4 and NLR 5. The black coloured growth was formed by PKM 3, PKM 4, PKM 6, NLR 3 and NLR 6.
The isolates PKM 2, PKM 5, NLR 1, NLR 2, NLR 3 and NLR 5 produced black coloured colonies on Rice Leaf Extract Agar (RLEA). The isolates PKM 3, PKM 4, PKM 6, NLR 4 and NLR 6 produced greyish black coloured colonies. The buff coloured colonies were produced by PKM 1 with good growth. From the results it is evident that in majority of isolates, the colour of the colony varied in general from buff or greyish black to black on different culture media. Only two isolates PKM 4 and NLR 4 produced white coloured colonies that to only on PDA. The results obtained in the present investigation revealing variation in colony colour of *P. grisea* was in accordance with the reports published earlier. Consolo *et al*. (2008) stated that the coloration of isolates of *P. grisea* was variable showing different colours as white, brownish yellow, pale brown, grey and blackish grey [5].

They also reported that in Czapek Dox medium *P. grisea* produced different coloured aerial mycelium. Partridge and Chandra (2011) reported that vegetative mycelial growth of *P. grisea* was highest using PDA, OMA and V8 agar media [6]. Huyly *et al*. (2012) reported that the colony produced by P. oryzae on potato dextrose agar was whitish grey [8] and similar findings were reported by Soytong and Quimio (1989) [9]

Table-1 Description of isolates of *P. grisea*

SN	Location	District	Isolate Designation
1	Darsi	Prakasam	PKM 1
2	Giddaluru	Prakasam	PKM 2
3	Mopadu	Prakasam	PKM 3
4	Chimakurthy	Prakasam	PKM 4
5	Pulipadu	Prakasam	PKM 5
6	Kandukur	Prakasam	PKM 6
7	Nellore	SPSR Nellore	NLR 1
8	Podalakuru	SPSR Nellore	NLR 2
9	Kavali	SPSR Nellore	NLR 3
10	Kovuru	SPSR Nellore	NLR 4
11	Atmakuru	SPSR Nellore	NLR 5
12	Bitragunta	SPSR Nellore	NLR 6

Growth of isolates of *P. grisea* on different solid culture media

Isolates of *P. grisea* were assessed for their growth on five different solid culture media, *viz*., PDA, OMA, CDA, RPA and RLEA. Observations on radial growth of individual isolate were recorded in terms of diameter of the colony, analysed and presented in [Table-3]. Mean radial growth of *P. grisea* isolates over all the culture media indicated that NLR 2 had least growth (54.9 mm) that differed significantly with all other test isolates followed by NLR 3 (60.3 mm) and NLR 4 (60.9 mm) with insignificant difference between them. Maximum mean growth was recorded with PKM 1 (85.4 mm) which differed insignificantly with PKM 2 (84.7 mm). Among SPSR Nellore district isolates NLR 6 (79.2 mm) and NLR 1 (77.3 mm) had maximum growth while NLR 2 had least growth (54.9 mm). Among the Prakasam district isolates PKM 1 (85.4 mm) and PKM 2 (84.7 mm) and PKM 3 (76.3 mm) had maximum growth while PKM 5 (74.3 mm) and PKM 6 (74.2 mm) had least growth. Mean colony diameter over all the isolates tested indicated least growth on CDA (65.9 mm) while maximum growth with OMA (79.7 mm) and PDA (79.5 mm). This result indicated variability existed in the mean growth of individual isolates depending on the media used.

Potato dextrose agar

Among all the isolates NLR 2 produced least growth (54.4 mm) and NLR 5 had highest growth (88.6 mm). Among the isolates of Prakasam district PKM 4 had least growth (75.3 mm) followed by PKM 3 (78.9 mm) and PKM 5 (80.6 mm) while highest growth was observed with PKM 1 (88.6 mm) followed by PKM 6 and PKM 2 (86.4 mm). Among the isolates of SPSR Nellore district, NLR 2 had least growth (54.4 mm) followed by NLR 3 (71.4 mm). Highest growth was recorded with NLR 5 (88.6 mm) followed by NLR 6 (85.3 mm).

Oat meal agar

Among all the isolates NLR 4 produced least growth (52.1 mm) followed by NLR 2(61.3 mm) while NLR 6 and PKM 2 had highest growth (90.0 mm). Among the isolates of Prakasam district PKM 6 had least growth (79.2 mm) followed by PKM 5 (83.2 mm) and PKM 4 (82.3 mm) while highest growth was observed with PKM 2 (90.0 mm) followed by PKM 3 (88.5 mm) and PKM 1 (85.5 mm). Among the isolates of SPSR Nellore district NLR 4 produced least growth (52.1 mm) followed by NLR 2 (61.3 mm) and highest growth was observed with NLR 6 (90.0 mm) followed by NLR 5 (83.2 mm).

Czapek Dox agar

Among all the isolates NLR 3 produced least growth (48.3 mm) followed byNLR 2 (50.2 mm) and NLR 4 (51.2 mm) while PKM 1 (82.1 mm) and PKM 2 (80.2 mm) had highest growth. Among the isolates of Prakasam district PKM 3 had least growth (63.2 mm) followed by PKM 4 (65.4 mm) and highest growth was observed with PKM 1 (82.1 mm) and PKM 2 (80.2 mm) followed by PKM 6 (72.4 mm). Among the isolates of SPSR Nellore district NLR 3 produced least growth (48.3 mm) followed by NLR 2 (50.2 mm) and NLR 4 (51.2 mm). The highest growth was observed with NLR 1 (75.4 mm) followed by NLR 6 (72.3 mm).

Rice polish agar

Among all the isolates NLR 3 produced least growth (51.1 mm) followed by NLR 2(58.5 mm) while PKM 4 had highest growth (89.4 mm) followed by PKM 1 (89.1 mm). Among the isolates of Prakasam district PKM 6 had least growth (60.8 mm) followed by PKM 5 (68.3 mm) and highest growth was observed with PKM 4 (89.4 mm) and PKM 1 (89.1 mm) followed by PKM 3 (88.2 mm). Among the isolates of SPSR Nellore district NLR 3 produced least growth (51.1 mm) followed by NLR 2 (58.5 mm). The highest growth was observed with NLR 5 (80.1 mm) followed by NLR 6 (76.4 mm).

Rice leaf extract agar

Among all the isolates, NLR 2 produced least growth (56.4 mm) followed by PKM 5(68.4 mm) while PKM 3 had highest growth (89.4 mm) followed by PKM 2(88.4 mm). Among the isolates of Prakasam district PKM 5 had least growth (68.4 mm) followed by PKM 6 (71.6 mm) and highest growth was observed with PKM 3 (89.4 mm) and PKM 2 (88.4 mm) followed by PKM 1 (82.1 mm). Among the isolates of SPSR Nellore district NLR 2 produced least growth (56.4 mm) followed by NLR 3 (72.8 mm). The highest growth was observed with NLR 6 (81.6 mm) and NLR 4 (80.4 mm) followed by NLR 5 (78.5 mm). From the above results it was observed that significant differences were present in the growth of the isolates on different solid culture media and even on the same culture medium. Of the twelve isolates the isolates PKM 2 (90.0 mm) and NLR 6 (90.0 mm) showed significantly the highest mycelial growth on oat meal agar medium. Significantly lowest mycelial growth was recorded with NLR 3 (48.2 mm) followed by NLR 2(50.2 mm) on Czapek Dox Agar. All the isolates were categorized based on the radial growth on different solid culture media. Isolates with <70 mm radial growth were considered as 'poor', 70-80 mm as 'medium' and > 80 mm as having 'good' growth. Isolates NLR 2 on PDA, NLR 2 and NLR 4 on OMA, NLR 2, NLR 3, NLR 4, NLR 5, PKM 3 and PKM 4 on CDA, NLR 2, PKM 5 and PKM 6 on RPA and NLR 1and NLR 5 on RLEA had poor growth. Isolates NLR 3, NLR 4, PKM 5 and PKM 4on PDA, PKM 6 on OMA, NLR 1, NLR 6, PKM 5 and PKM 6 on CDA, NLR1, NLR 3, NLR 5 and NLR 6 on RPA and NLR 2, NLR 3, NLR 5, PKM 4 and PKM 6 on RLEA were found to have medium growth. Isolates NLR 1, NLR 3, NLR 5, NLR 6, PKM 1, PKM 2, PKM 3, PKM 4 and PKM 5 on OMA, PKM 1 and PKM 2 on CDA, PKM 1, PKM 2, PKM 3 and PKM 4 on RPA and NLR 4, NLR 6, PKM 1, PKM 2 and PKM 3 on RLEA were found to have good growth. In all 58.3% of isolates on PDA 75% of isolates on OMA, 16.75% of isolates on CDA, 41.7% of isolates on RPA and 41.7% of isolates on RLEA were found to have good growth. The present investigation revealed that OMA followed by PDA supported good growth of isolates while CDA did not supported the growth of *P. grisea* well. Okeke *et al*. (1992) tested malt extract and rice extract media on growth and sporulation of P. oryzae and stated that the two culture media OMA and PDA encouraged good growth of the fungus with very low or without sporulation [10]. Arun Kumar and Singh (1995) observed that host extract and oat meal agar supported good growth and sporulation of P. oryzae [7]. Patridge and Chandra (2011) reported higher growth with V8 agar, oat meal agar and potato dextrose agar [6]. Consolo et al. (2008) stated that variability was observed within the isolates of *P. grisea* when they were cultured in different media [5]. Media rich in salts were not favourable for growth of *P. grisea* isolates, while media rich in organic compounds than the salts favoured mycelial growth and sporulation. Tripathi (2006) reported that *P. grisea* grew well on potato dextrose agar and carrot agar and required optimum temperature of 25±2ºC and pH 7 for the growth and sporulation [11]. The results obtained in the present investigation were in accordance with these reports. Sporulation of *P. grisea* isolates on different solid media Isolates of *P. grisea* were assessed for their sporulation on five different solid culture media, *viz*., PDA, OMA, CDA, RPA and RLEA. Observations on sporulation of individual isolate were recorded as number of conidia per cm^2 [Table-4]. Mean conidiation per cm^2 of the medium over all the isolates indicated that Czapek Dox Agar gave least support to *P. grisea* conidia with no conidia formed followed by potato dextrose agar with $1.8x10^6$ conidia/cm^2.

Table-2 Colony colour of *P. grisea* isolates on different solid nutrient media

Isolate	Colony colour of *Pyricularia grisea* *				
	Potato Dextrose Agar	Oatmeal Agar	Czapek Dox Agar	Rice Polish Agar	Rice Leaf Extract Agar
PKM 1	Buff colour	Greyish black	Buff colour	Greyish black	Buff colour
PKM 2	Greyish black	Greyish black	Black colour	Greyish black	Black colour
PKM 3	Black colour	Buff colour	Black colour	Black colour	Greyish black
PKM 4	Olive green	Greyish black	Greyish black	Black colour	Greyish black
PKM 5	Greyish black	Buff colour	Black colour	Greyish black	Black colour
PKM 6	Buff colour	Greyish	Black	Black colour	Dull black
NLR 1	Black colour	Buff colour	Black colour	Greyish black	Black colour
NLR 2	Buff colour	Black colour	Black colour	Greyish black	Black colour
NLR 3	Black colour	Greyish black	Greyish white	Black colour	Black colour
NLR 4	Olive green	Buff colour	Black colour	Greyish black	Grayish black
NLR 5	Buff colour	Greyish black	Black colour	Greyish black	Black colour
NLR 6	Black colour	Greyish black	Buff colour	Black colour	Black colour

**Observations from fifteen days old culture plates*

Table-3 Radial growth of *Pyricularia grisea* isolates on different solid nutrient media

Isolate	Colony diameter (mm) *					
	Potato Dextrose agar	Oat meal agar medium	Czapek Dox agar	Rice polish agar	Rice leaf extract agar	Mean
PKM 1	88.2	85.5	82.1	89.1	82.1	85.4
PKM 2	86.4	90	80.2	86.7	88.4	84.7
PKM 3	78.9	88.5	63.2	88.2	89.4	76.3
PKM 4	75.3	82.3	65.4	89.4	74.3	75.5
PKM 5	80.6	82	70.4	68.3	68.4	74.3
PKM 6	86.4	79.2	72.3	60.8	71.6	74.2
NLR 1	82.7	80.3	75.4	72.3	76.4	77.3
NLR 2	54.4	61.3	50.2	58.5	56.4	54.9
NLR 3	71.4	82.3	48.3	51.1	72.8	60.3
NLR 4	75.2	52.1	51.2	75.2	80.4	60.9
NLR 5	88.6	83.2	60.1	80.1	78.5	74.4
NLR 6	85.3	90	72.2	76.4	81.6	79.2
Mean	79.5	79.7	65.9	74.7	76.7	

	Isolates	Media	Isolates × Media
S. Em±	0.7	0.5	0.4
C.D (0.01)	2.3	1.5	1.2
C.V	1.1	1.5	1.8

**Observations from fifteen days old culture plates*

Table- 4 Variability in the sporulation of *Pyricularia grisea* isolates

Isolate	No. of conidia/cm^2 (X 10^6)*					
	Potato Dextrose Agar	Oat Meal Agar	Czapek Dox Agar	Rice Polish Agar	Rice Leaf Extract Agar	Mean**
PKM 1	1.6	3.4	0	6.5	5	2.8
PKM 2	3.7	2.8	0	4.7	3.1	2.4
PKM 3	0.9	6.5	0	3.4	4.3	2.5
PKM 4	2.5	4.3	0	2.8	1.9	1.9
PKM 5	0	1.9	0	6.5	2.5	1.8
PKM 6	4.3	6.2	0	3.4	1.6	2.6
PKM Mean	2.2	4.2	0	4.6	3.1	2.3
NLR 1	0	0.9	0	1.9	0	0.5
NLR 2	0.9	3.4	0	4	3.4	2
NLR 3	2.5	4	0	3.1	1.6	1.9
NLR 4	1.6	2.2	0	2.2	0.9	1.2
NLR 5	4	6.8	0	2.5	3.7	2.8
NLR 6	0	2.8	0	3.7	5.3	2
NLR Mean	1.5	3.4	0	2.9	2.5	1.7
Over all Mean	1.8	3.8	0	3.7	2.8	2

**Spore count was taken from 15 days old culture plates, ** Observations were average of 50 counts*

Maximum no. of conidia was observed on oat meal agar (3.8X10^6 conidia/cm^2) followed by rice polish agar (3.7X10^6 conidia/cm^2). Over all the media and isolates, isolates of Prakasam district had higher conidiation (2.3X10^6 conidia/cm^2) compared to isolates from SPSR Nellore district isolates (1.7X10^6 conidia/cm^2). Among Prakasam district isolates, over all the media, PKM 1 had maximum conidiation (2.8X10^6 conidia/cm^2) while PKM 5 had least conidiation (1.8X10^6 conidia/cm^2). Among SPSR Nellore district isolates, over all the media, NLR 5 had maximum conidiation (2.8X10^6 conidia/cm^2) while NLR 1 had least conidiation (0.5X10^6 conidia/cm^2). On Potato Dextrose Agar, the isolate PKM 6 produced higher number of conidia (4.3X10^6 conidia/cm^2) followed by NLR 5 (4.0 X106 conidia/cm2) and PKM 2 (3.7 X10^6 conidia/cm^2). The isolate NLR 3 and PKM 4 produced (2.5 X10^6 conidia/cm^2) followed by PKM 1and NLR 4 (1.6 X10^6 conidia/cm^2), PKM 3 and NLR 2 (0.9 X10^6 conidia/cm^2). NLR 1, PKM 5 and NLR 6 isolates did not produce any conidia on PDA medium. On Oat Meal AgarThe isolate NLR 5 produced maximum number of conidia (6.8 X10^6 conidia/cm^2) followed by PKM 3 (6.5 X10^6 conidia/cm^2) and by PKM 6 (6.2 X10^6 conidia/cm^2). The isolate PKM 4 produced 4.3 X10^6 conidia/cm^2 followed by NLR 3 (4.0 X10^6 conidia/cm^2), PKM 1 and NLR 2 (3.4 X10^6 conidia/cm^2). The isolates PKM 2 and NLR 6 produced 2.8 X106 conidia/cm2 followed by NLR 3 (8.0 X10^6 conidia/cm^2). The isolate NLR 1 produced significantly lowest number of conidia (0.9 X10^6 conidia/cm^2). On Rice polish Agar the isolates PKM 1 and PKM 5 produced maximum number of conidia (6.5 X10^6 conidia/cm^2) followed by PKM 2 (4.7 X10^6

Table-5 Mycelial dry weight of *Pyricularia grisea* isolates on different liquid media

Mycelial dry weight on liquid media (mg)*						
Isolates	Potato Dextrose medium	Oat meal medium	Czapek Dox medium	Rice polish medium	Rice leaf extract medium	Mean
PKM 1	228.3	223.1	65.9	141.5	132.7	158.3
PKM 2	155.6	209.2	88.7	199.6	119.5	154.5
PKM 3	149.8	158.6	69.5	201.6	212.5	158.4
PKM 4	215.3	119.8	101.2	192.2	282.3	182.1
PKM 5	168.9	206.2	98.2	119.5	229.9	164.5
PKM 6	192.9	223.3	79.9	158.3	261.4	183.1
NLR 1	213.4	257.4	88.7	221.8	198.9	196
NLR 2	258.2	198.9	65.2	238.4	228.3	197.8
NLR 3	277.6	205.3	55.3	212.5	115.2	173.2
NLR 4	229.5	228.1	99.8	215.5	142.6	183.1
NLR 5	198.6	255.6	104.2	99.9	201.3	171.9
NLR 6	217.5	198.9	99.9	148.5	228.4	178.6
Mean	208.8	207	84.7	179.1	196.1	145.9

	Isolates	Media	Isolates × Media
S. Em±	0.87	0.1	1.4
C.D (0.01)	1.7	2.1	2.3
C.V	1.3	0.9	1.2

**Observations from fifteen days old culture plates*

conidia/cm^2), NLR 2 (4.0 X10^6 conidia/cm^2) and by NLR 6 (3.7 X10^6 conidia/cm^2). PKM 3 and PKM 6 produced 3.4 X10^6 conidia/cm^2 followed by NLR 3 (3.1 X 10^6 conidia/cm^2), PKM 4 (2.8 X10^6 conidia/cm^2) and NLR 5 (2.5 X10^6 conidia/cm^2). The lowest conidial production was observed with NLR 4(2.2 X10^6 conidia/cm^2). On Rice Leaf Extract Agar the highest number of conidia (5.3 X10^6 conidia/cm^2) was produced by the isolate NLR 6 followed by PKM 1 (5.0 X10^6 conidia/cm^2). The isolate PKM 3 produced 4.3 X10^6 conidia/cm^2 followed by the isolate NLR 5 (3.7 X10^6 conidia/cm^2), NLR 2 (3.4 X10^6 conidia/cm^2), PKM 2 (3.1 X10^6 conidia/cm^2), PKM 4 (1.9 X10^6 conidia/cm^2) and PKM 6 and NLR 3 (1.6 X106 conidia/cm^2). The lowest conidial production was recorded with NLR 4 (0.9 X10^6 conidia/cm^2). NLR 1 did not produce any conidia on the medium.None of the twelve test isolates of *P. grisea* produced conidia on Czapek Dox Agar. Thus the present investigation revealed variation in individual isolates to sporulate on different media and also variation in different isolates on the same medium. Patridge and Chandra (2011) reported higher conidial production of *P. grisea* on St. Augustene grass agar (3.3×10^5 conidia/cm^2) followed by V8 agar (1.9×10^5 conidia/cm^2) and oatmeal agar (1.4×10^5 conidia/cm^2) [6].

Cultural characters in liquid media

Mean mycelial dry weight of *P. grisea* isolates over all the liquid media indicated that PKM 2 had least mycelial dry weight (154.5 mg) followed by PKM 1 (158.3 mg). Maximum mean growth was recorded with NLR 2 (197.8 mg) followed by NLR 1 (196.0 mg) [Table-5]. Among SPSR Nellore district isolates NLR 2 (197.8 mg) and NLR 1 (196.0 mg) had maximum mycelial dry weight while NLR 3 had least mycelial dry weight (173.2 mg). Among the Prakasam district isolates PKM 6 had (183.1 mg) maximum mycelial dry weight followed by PKM 4 (182.1 mg) while PKM 2 (154.5 mg) and PKM 1 (158.3 mg) had least growth. Mean mycelial dry weight over all the isolates tested indicated least growth on CDA (84.7 mg) while maximum growth with PDA (208.8 mg) and OMA (207.0 mg). Variability occurred in the mycelial dry weight of individual isolates depending on the liquid media used. On potato dextrose broth the isolate PKM 3 showed significantly least dry mycelial weight of 149.8 mg followed by PKM 2 (155.6 mg). The isolate NLR 3 (277.6 mg) produced maximum mycelial dry weight followed by NLR 2 (258.2 mg). On Oat meal broth, the isolate PKM 4 produced significantly least mycelial dry weight (119.8 mg) followed by PKM 3 (158.6 mg). The highest mycelial dry weight was observed with NLR 1 (257.4 mg) followed by NLR 5 (255.6 mg) which differed insignificantly with each other. On Czapek Dox broth the isolate NLR 3 produced least mycelial dry weight (55.3 mg) followed by NLR 2 (65.2 mg). The highest mycelial dry weight was observed with NLR 5 (104.2 mg) followed by PKM 4 (101.2 mg). On Rice polish broth the isolate NLR 5 produced least mycelial dry weight (99.9 mg) followed by PKM 5 (119.5 mg) and the highest mycelial dry weight was observed with NLR 2 (238.4 mg) followed by NLR 1 (221.8 mg). On Rice leaf extract broth the isolate NLR 3 produced least mycelial dry weight (115.2 mg) followed by PKM 2 (119.5 mg) and the highest mycelial dry weight was observed with PKM 6 (261.4 mg) followed by PKM 5 (229.9 mg). From the above results it was observed that significant differences were present in the mycelial dry weight of the isolates on different liquid culture media and even on the same culture medium. Of the twelve isolates, the isolates PKM 4 (282.3 mg) and PKM 6 (261.4 mg) showed significantly higher mycelial growth on Rice leaf extract broth. Significantly lowest mycelial growth was produced by NLR 3 (55.3 mg) followed by NLR 2 (65.2 mm) on Czapek Dox broth. Among the different liquid nutrient media, potato dextrose broth supported significantly higher growth of all the isolates of *P. grisea* followed by oat meal broth. Between liquid broth having rice constituents, rice leaf extract supported higher mycelial dry weight compared to rice polish broth. The lowest growth of the isolates was recorded in Czapek Dox broth.

Application of research: Rice blast is a major disease in Andhra Pradesh where rice is growing in large area. The two districts Prakasam and Sri Potti Sreeramulu Nellore districts are facing severe rice blast problem in every year. In view of this the cultural characteristics and conditions for spore production was studied to take better source for growth and sporulation for further studies

Research Category: Nutrient media, sporulation

Abbreviations: PKM –Prakasam, NLR-Nellore

Acknowledgement / Funding: Authors are thankful to ICAR-Krishi Vigyan Kendra, Darsi, Prakasam, 523247, Acharya N. G. Ranga Agricultural University, Lam, Guntur, 522034, Andhra Pradesh, India.

***Research Guide or Chairperson of research: Professor P. Anil Kumar**
University: Acharya N. G. Ranga Agricultural University, Lam, Guntur, 522034
Research project name or number: PhD Thesis, 'Studies on blast disease of rice caused by *Pyricularia grisea* Pers. EX (Fr) Grove'

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript

Conflict of Interest: None declared
Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors

Sample Collection: Isolates collected from Prakasam *viz*., PKM 1 to PKM 6 and NLR 1 to NLR 6 from Sri Potti Sreeramulu Nellore districts

References

[1] Ratna Madhavi K., Srinivas Prasad N., Laha G.S., Madan Mohan K., Seshu Madhav M. and Viraktamath B.C. (2011) *Indian Journal of Plant Protection*,39(2),124-129.
[2] Mc Donald B.A., Mc Dermott J.M. and Godwin S.B. (1989) *Annual Review of Phytopathology.* 27:77-94.
[3] Ainsworth G.C. (1971) *Commonwealth Mycological Institute. Kew, UK*. 663.
[4] Tuite J. (1969) *Burges Publishing Company, USA*. 239
[5] Consolo V.F., Cordo C.A. and Salerno G.L. (2008) *Australian Plant Pathology*, 37,357-364.
[6] Patridge S. and Chandra A. (2011) *Cell and Plant Sciences*, 2(3),9-11.
[7] Arun Kumar and Singh R.A. (1995) *Indian Journal of Mycology and Plant Pathology*,25,238-242
[8] Huyly T., Chaiwat M., Aram U and Kasem S. (2012) *Journal of Agricultural Technology*,8 (1),289-903
[9] Soytong K. and Quimio (1989) *The Philippine Agriculturist*, 72(1),59-65
[10] Okeke B., Murandi F.S. and Steiman L.S. (1992) *Agronomie*, 3,325-329
[11] Tripathi S.K. (2006) *Annals of Plant Protection Science*, 14(1),6-10