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Research Article

EVALUATION OF CULTURE MEDIA, LIGHT REGIMES AND NATURAL HOST SEGMENTS ON GROWTH AND SPORULATION OF Pyricularia oryzae Cavara CAUSING BLAST DISEASE IN RICE

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Abstract- The fungus *Pyricularia oryzae* Cavara (sexual phase *Magnaporthe oryzae* B.C.Couch) is the causal agent of rice blast disease. Sporulation in *P. oryzae* is a process distinct from mycelial growth in many respects and also difficult to get sporulation in P. oryzae. Finger millet leaf extract (FLA) and Rice leaf extract Agar (RLA) culture medium proved as best media with the sporulation of 1.71×10^5 ml^{-1} and 1.65×10^5 ml^{-1}, respectively and no significant increase or decrease in sporulation was noticed under the three light regimes *i.e*., continuous fluorescent light, 12 hr dark + 12 hr light and continuous dark. Among all tested methods, the sporulation on *Brachiaria mutica* leaf and stem segments was found as best method with the sporulation of 7.61×10^5 ml^{-1}.

Keywords- *Pyricularia oryzae Cavara, sporulation, different media, light regimes and host segments*

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Introduction

Rice (*Oryza sativa L*.) is the most important human food crop in the world, directly feeding more people than any other crop. The fungus *Pyricularia oryzae* Cavara (sexual phase *Magnaporthe oryzae* B.C.Couch) is the causal agent of blast disease on rice crop and poses a threat to world food security. Rice plants are most susceptible to *P. oryzae* in the seedling, early tillering and heading stages of the crop [1]. The disease results in yield loss as high as 70-80% [2]. The yield loss of 10 per cent is significant as it is sufficient to feed 60 million people for one year. Realizing the importance, natural resource institute of London gave first rank to rice blast disease in its study of pre harvest disease occurring in South Asia [3]. Several management strategies have been proposed and evaluated to minimize the blast disease incidence. Cultural practices, host plant resistance and the use of synthetic fungicides are the three strategies adopted to control rice blast [4]. In case of host plant resistance, experiments involving the screening of rice varieties to blast resistance, conidia were required in large quantity for use as inoculums but culturing of *P. oryzae* on normal media like Potato dextrose agar could not produce spores. Sporulation in *P. oryzae* is a process distinct from mycelial growth in many respects. A certain amount of vegetative growth is a pre-requisite to the formation of conidia but did not always lead to sporulation. Hence the present study was carried out to describe the effects of some factors upon sporulation namely, culture media, light regimes and natural host segments.

Material and Methods

In this study, the effect of three factors *i.e*. culture media, light regimes and host segments were evaluated for satisfactory amount of sporulation (1×10^5 ml^{-1}) in a sequential order. First of all, Nine different culture media *i.e*., Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), Oat Meal Agar (OMA), Oatmeal Sucrose Agar (OSA), Rice Leaf extract Sucrose Agar (RLA), Rice Panicle extract Sucrose Agar (RPA), Finger millet Leaf extract Sucrose Agar (FLA), Napier grass Leaf extract Sucrose Agar (NLA), V8 Juice Agar were tested. In the second experiment, the best media from the first experiment *i.e*. RLA (Rice Leaf Extract Agar) along with PDA (Potato Dextrose Agar), OMA (Oat Meal Agar), PDA + R (PDA + Rice leaf extract) and OMA + R (OMA + Rice leaf extract) were further evaluated under three light conditions, *i.e*., 24 hrs fluorescent light, 12 hr dark/12 hr fluorescent light and 24 hr darkness. In both experiments (1st and 2nd), radial growth and sporulation was recorded on 14 days after incubation. The amount of sporulation was determined in the culture by adding 20 ml of sterile distilled water to dislodge the spores. In the third experiment, the stem and leaf bits of natural hosts of *P. oryzae viz*., Rice, Finger millet, and weed species namely *Pennisetum purpureum, Paspalum* sp., *Brachiaria mutica, Digitaria sanguinalis, Echinochloa colonum, Panicum repens* were collected and cut into small pieces of 1-5 cm length. A cotton swab was placed in 150 ml Erlenmyer flasks to maintain humidity. Thereafter fifteen gm of each host bits were placed on cotton swab and sterilized at 121°C for 20 min. At 15 lb. pressure. Each flask was inoculated with two 5 mm diameter mycelial discs of *P. oryzae* and incubated for 15 days at room temperature. To prepare the spore suspension, each species stem and leaf bits were crushed in 50 ml sterile distilled water using sterile pestle and mortar. The spore count was assessed at 10 DAI (Days after incubation) and 15 DAI using a haemocytometer and data obtained was represented in conidia/ml.

Results and Discussion

Evaluation of Different Solid Media on Radial Growth and Sporulation of *P. oryzae*

Colony colour

Greyish black colored colony was observed on the media which were prepared with host's leaf extract *viz*., Rice Leaf extract Agar (RLA), Finger millet Leaf extract Agar (FLA) and Napier grass Leaf extract Agar (NLA). Transparent growth was documented on Rice Panicles extract Agar (RPA).

Table-1 Evaluation of different media for growth and sporulation of *P. oryzae*

S	Media	Colony color	Average Radial growth(mm)	Average growth rate/day	Sporulation x 10^5/ml)
1	PDA	Whitish grey	73.17±0.60^{c}	4.88	0.21±0.141^{e}
2	PSA	Grey	75.25±2.52^{c}	5.02	0.64±0.092^{bcd}
3	OMA	Greyish white	86.00±0.58^{a}	5.73	0.48±0.092^{de}
4	OSA	Blackish grey	75.00±1.16^{c}	5	0.91±0.141^{b}
5	RLA	Greyish black	86.33±0.88^{a}	5.76	1.65±0.141^{a}
6	RPA	Transparent	69.00±0.58^{d}	4.6	0.53±0.141^{cde}
7	FLA	Greyish black	83.33±0.88^{ab}	5.56	1.71±0.053^{a}
8	NLA	Greyish black	80.00±0.58^{b}	5.33	0.85±0.107^{bc}
9	V8 Juice Agar	Grey	53.17±1.20^{e}	3.54	0.37±0.053^{de}
	C.D.		3.46		0.34
	C.V.		2.648		23.81

Table-2 Evaluation of different light regimes for radial growth and sporulation of *P. oryzae*

Media (A)	Radial Growth (mm) (B)				Sporulation (×10^5/ml) (B)			
	Light	Dark	Dark+Light	Average (A)	Light	Dark	Dark+Light	Average (A)
PDA	88	87.33	85	86.78^{b}	0.67	0.33	0.67	0.56^{a}
OMA	85.67	88.67	84	86.11^{b}	0.67	0.5	0.67	0.61^{a}
RLA	89.33	90	89.67	89.66^{a}	1.83	1	1.33	1.39
PDA+RLA	88.67	87.33	88.67	88.22^{a}	0.5	0.83	0.33	0.56^{a}
OMA+RLA	89	88.67	89	88.88^{a}	0.83	0.83	0.5	0.67^{a}
Average (B)	88.13	88.4	87.27		0.90^{a}	0.70^{a}	0.70^{a}	

Table-3 Sporulation of *P. oryzae* on leaf and stem bits of different hosts

S	Substrate (A)	Sporulation (×10^5/ml)@10 DAI (B)	Sporulation (×10^5/ml)@15 DAI (B)	Mean (A)
1	*Pennisetum purpureum*	5.89	6.08	5.99^{a}
2	*Paspalum sp.*	5.41	5.54	5.48^{b}
3	*Brachiaria mutica*	7.57	7.66	7.61
4	*Digitaria sanguinalis*	5.87	5.94	5.90^{a}
5	*Echinochloa colonum*	5.17	5.2	5.18^{c}
6	*Panicum repens*	0.99	1.32	1.16
7	*Oryza sativa*	5.18	5.3	5.24^{bc}
8	*Eluesine coracana*	2.27	2.52	2.4
	Mean (B)	4.26^{A}	4.40^{A}	

**Mean of three replications*

Greyish colony was observed on Potato Sucrose Agar (PSA) and V8 agar. Whitish grey and Greyish white colony were observed on Potato Dextrose Agar (PDA) and Oat meal Agar (OMA), respectively.

Plate-1 Radial growth and colony colour of *P.oryzae* on different culture media 1: PDA,2: PSA,3: OMA,4: OSA,5: RLA, 6: RPA, 7: FSA, 8: NLA, 9: V8 Juice agar

Radial growth and Sporulation

Radial growth was recorded on 14 days after incubation. Among the nine media used, RLA medium has recorded the maximum growth with a colony diameter of 86.33 mm followed by OMA (86.00 mm) and FLA (83.33 mm) and no significant difference was found among them. 75.25 mm, 75.00 mm and 73.17 mm radial growth was recorded on PSA, OSA and PDA, respectively and found on par with each other. RPA medium was found with 69.00 mm and V8 agar medium has shown the minimal growth with 53.17 mm [Table-1] [Plate-1]. Good amount of sporulation was observed in case of FLA medium with 1.71×10^5 ml^{-1} followed by RLA medium with 1.65×10^5 ml^{-1} and found on par with each other. Next to these media, OSA, NLA and PSA were reported with the sporulation of 0.91×10^5 ml^{-1} , 0.85×10^5 ml^{-1} and 0.64×10^5 ml^{-1} , respectively. Least sporulation was recorded in PDA medium (0.21×10^5 ml^{-1}) with insignificant difference from V8 agar (0.37×10^5 ml^{-1}), OMA (0.48×10^5 ml^{-1}) and RPA medium (0.53×10^5 ml^{-1}) [Table-1].

Evaluation of Different Light Regimes on Radial Growth and Sporulation of *P. oryzae*

The maximum mean radial growth 89.66 mm was recorded on RLA medium with insignificant difference under three light conditions *i.e.,* light (89.33 mm), dark (90.00 mm) and dark + light (89.67 mm). This was followed by OMA+RLA (88.88 mm) and PDA+RLA (88.22 mm) medium and both were found with no significant difference under three light regimes. PDA medium recorded with the growth of 88.00 mm, 87.33 mm and 85.00 mm under light, dark and dark + light regimes, respectively. On OMA medium, significant difference was noticed among the three light conditions and found maximum growth under dark (88.67 mm) followed by light (85.67 mm) and dark + light (84.00 mm). In over all, with respect to the light conditions, no significant difference was found among the three light conditions *i.e.* light (88.13 mm), dark (88.40 mm) and dark + light (87.27 mm). With respect to the media, RLA (89.67 mm), OMA + RLA (88.88 mm) and PDA + RLA (88.22 mm) have shown significant difference with PDA (86.78 mm) and OMA (86.11 mm) medium [Table-2] [Plate-2]. High amount of significant mean sporulation (1.39×10^5 ml^{-1}) was recorded on RLA medium, but no significant increased or decreased sporulation was observed among three light regimes (1.33×10^5 ml^{-1} - 24hrs light; 1.83×10^5 ml^{-1}-24hrs dark and 1×10^5 ml^{-1}-12 hrs light & 12 hrs dark). This was followed by OMA+RLA (0.67×10^5 ml^{-1}) and OMA (0.61×10^5 ml^{-1}). The lowest amount of mean sporulation (0.56×10^5 ml^{-1}) was found in PDA and PDA+RLA. No significant difference in sporulation was found among the all media except RLA.

Over the media, no significant difference was observed among three light regimes *i.e*., light (0.90 ×10^5 ml^{-1}), dark (0.70 ×10^5 ml^{-1}) and dark + light (0.70 ×10^5 ml^{-1}) [Table-2]. In overall, the experiment revealed that no significant difference in radial growth and sporulation was documented under three light regimes.

Plate-2 Radial growth and colony colour of *P.oryzae* under different light regimes

Plate-3 Growth of *P.oryzae* on different hosts.

1: *Pennisetum purpureum,* 2: *Paspalum* sp. 3: *Brachiaria mutica,* 4: *Digitaria sanguinalis,* 5: *Echinochloa colonum,* 6: *Panicum repens* 7: *Oryza sativa,* 8: *Eluesine coracana*

Evaluation of stem and leaf bits of different hosts for sporulation in *P.oryzae*

The sporulation in different hosts was assessed at 10 and 15 Days After Inoculation (DAI). With respect to the incubation time, the mean amount of sporulation was higher at 15 DAI (4.40 ×10^5 ml^{-1}) than at 10 DAI (4.26×10^5 ml^{-1}) in all the hosts, sporulation increased with increase in time but that increase from to 15 DAI (4.40 ×10^5 ml^{-1}) was found with no significant difference. High amount of sporulation was recorded in *Brachiaria mutica* leaf and stem bits with 7.61×10^5 ml^{-1} and significance difference was observed from all the remaining hosts. It was followed by *Pennisetum purpureum* (5.99×10^5 ml^{-1}) and *Digitaria sanguinalis* (5.90×10^5 ml^{-1}). *Paspalum* sp. and *Oryza sativa* (rice) were recorded as on par with the sporulation of 5.48 × 10^5 ml^{-1} and 5.24 × 10^5 ml^{-1}, respectively. In *Echinochloa colonum*, 5.18 × 10^5 ml^{-1} sprorulation was observed and it has shown no significant difference with rice. Least amount of sporulation was obtained in *Panicum repens* (1.15×10^5 ml^{-1}) followed by *Eluesine coracana* (2.39×10^5 ml^{-1}). [Table-3] [Plate-3]. Sporulation of *P. oryzae* is a process influenced by several factors. Some of the factors basically necessary for the production of vegetative mycelium might be listed as necessary for sporulation, *e.g*., pH of the medium and temperature. Other factors such as specific substrates *etc.* may be requirements for vegetative growth but possibly unsuitable for sporulation. In most previous studies, sporulation has been simply examined on different culture media coupled with different pH, temperature and light conditions. But in the present study along with media, different host's leaf and stem bits were attempted for inducing sporulation in *P. oryzae*. When compared with all methods evaluated, sporulation in host's leaf and stem bits was superior to other methods *viz*., different media and different media coupled with light regimes. It was ascertained from high amount of sporulation in *Brachiaria mutica* that it has succulent leaves and trichomes on both leaves and stems, which maintains continuous moisture in the flask results in high sporulation. Maintenance of humidity in the flask by placing cotton swab and nutrient compositon of host might have a major role in induction of sporulation in *P. oryzae*. The present results on investigation of different methods for sporulation in *P. oryzae* were confirmatory with the findings of several researchers. Ramkrishnan, 1948 [5] reported Rice leaf agar medium as the best medium for mycelial growth and sporulation of *P. oryzae* and Sun *et al*., 1989 [6] reported Corn meal and Rice straw agar media as supporting medium for sporulation of *P. oryzae*. In the present study, no light effect was found on radial growth and sporulation in *P. oryzae* and this finding agrees with the results of Chakrabarti and Wilcoxan, 1973 [7], who concluded that light was not necessary for sporulation in *P. oryzae*. High amount of sporulation on hosts leaf and stem bits was supported by the findings of Manjunath *et al*., 2013 [8], who reported high sporulation (1983 conidia ml^{-1}) on maize stem bits.

Conclusion

Finger millet leaf extract (FLA) and Rice leaf extract Agar (RLA) culture medium proved as best media with the sporulation of 1.71×10^5 ml^{-1} and 1.65×10^5 ml^{-1}, respectively in 20 ml spore suspension and no significant increase or decrease in sporulation was noticed under the three light regimes *i.e*., continuous fluorescent light, 12 hr dark + 12 hr light and continuous dark on all tested media. Whereas the sporulation on host leaf and stem segments, *Brachiaria mutica* was found with the sporulation of 7.61×10^5 ml^{-1} in 50 ml spore suspension. Thus the information on this method of sporulation is likely to be useful to produce sufficient spores for large scale inoculation experiments and also to understand the disease epidemics and control.

Application of research: This research is applicable to a mycologist who works on *Pyricularia oryzae* Cavara

Research Category: Mycology and Plant Pathology

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