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Study on Differential Response of *Pyricularia grisea* Isolates from Rice, Finger Millet and *Panicum* sp. with Local and Alien Media, and Their Host Range

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Abstract
Blast (*Pyricularia grisea*) is an economically important disease of rice and finger millet in Nepal. Isolates of the fungus from different hosts differed in their response in media for mycelial growth and sporulation. Radial mycelial growth (RMG) and days of sporulation (DOS) of *P. grisea* were studied by culturing three fungal isolates from rice, finger millet and *Panicum* sp. on six different media: prune agar (PA), oat meal agar (OMA), potato dextrose agar (PDA), finger millet leaf decoction agar (FLDA), finger millet polish agar (FPA) and finger millet meal agar (FMA). The highest RMG was found in the isolates from finger millet and the lowest in the isolates from rice. The shortest DOS (1 week) was found in the isolate from rice and the longest (>2 weeks) in the isolate from finger millet. Among the different media used, PA and OMA were found to be the best for mycelial growth and sporulation of the isolates both from rice and finger millet. The shape, color and compactness of the fungal colonies varied with the media and isolates used. Cross inoculation studies showed that the fungus isolates from rice were able to infect all the plant species (rice, finger millet, *Panicum* sp., *Eleusine indica* and *Setaria* sp.) while isolates from finger millet were only able to infect three plant species (*E. coracana*, *Setaria* sp. and *E. indica*). This shows that the weed management is more important in finger millet fields than in rice field to manage the blast disease; and growing of rice adjacent to finger millet field is dangerous for blast epidemics in finger millet since rice serves as the source of inoculums.

Key words: inoculation, radial mycelial growths, porulation

Introduction
Blast disease caused by *Pyricularia grisea* is an economically important disease of rice and finger millet which causes significant losse in yield. Various weed hosts growing near cultivated plants might serve as potential sources of inoculums for the disease and thus provide alternate means of survival for the fungus. Since collateral and alternate hosts are the most important sources of inoculums which are present throughout the year. Study of the host range has become an important aspect of the disease management.

The blast fungus *Magnaporthe grisea* (anamorph, *Pyricularia grisea*) has a wide host range and is known to infect almost 40 species of *Gramineae* (Asuyama 1965). Previous studies have given discrepant results concerning the host range of this pathogen (Ou 1985). Several workers have reported that the pathogenicity of the blast fungus is largely restricted to its host species of origin (Ramakrishnan 1948, Todman *et.al.* 1994), although successful infection of a host by an isolate from a different species has been reported under experimental conditions. Isolate from finger millet is pathogenic to *Setaria* and wheat, but not to rice, *Panicum ripens*, or crab grass. According to Thomas (1940), the fungus from finger millet infected its own host, wheat, oat, barley and corn, but the fungus from rice attacked finger millet. Thompson (1941) showed *Pyricularia* from finger millet failed to infect rice in

cross inoculation tests. According to Thompson (1941) and Ramkrishnan (1948), *Pyricularia* from finger millet and the fungus from *Setaria italica* were capable of infecting both of their own hosts when they were cross inoculated. The findings of pathogenicity tests done by Viji *et al.* (2000) in India showed that the blast fungus from the two hosts, rice and finger millet did not cross-infect, nor did the two forms cross in the laboratory. The results confirm that the rice and millet-infecting *M. grisea* populations in India were distinct. It has also been found that the pathogenicity of the fungus and cross inoculation reaction among different species may be changed according to the time and condition because of the genetic modification and crossing between the different isolates (Kato 1978).

Nishikado (1927) obtained good growth of *Pyricularia oryzae* isolated from *Oryza sativa* L. on decoction of their host material. Kumar and Singh (1995) studied *Pyricularia grisea* (*M. grisea*) from rice on different solid culture media. They found that, maximum colony diameter of rice isolate occurred on malt extract agar and Leonin agar. Xinfa *et al*.(1995) stated that, *Pyricularia* isolates from hosts including rice and common weeds in paddy fields sporulated abundantly on sterilized barley or sorghum grains. Hossain (2000) observed that among the non synthetic media, potato dextrose agar supported maximum radial growth (85.00 mm), next was host extract + 2 per cent sucrose agar medium (80.33 mm) followed by oat meal agar (75.00 mm). To study the different aspect of disease development, determination of nutritional and physiological conditions of the growth and sporulation of the fungus is necessary. Isolates of the fungus especially from different hosts differ in their response in media on mycelium growth and sporulation. Previous investigation showed that the artificial growth of any pathogen isolates was widely supported by their host extracts. Therefore the study was conducted to identify the proper media for growth and sporulation of *P. grisea* isolates from rice, finger millet and other weed species.

Methodology

Cross inoculation test

Five different plant species under the family Gramineae *O. sativa, E. coracana, E. indica, Panicum* sp., *and Setaria* sp. were tested with two *P. grisea* isolates, one isolated from rice and the other from finger millet. The study was conducted in a screen house at the Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan during August to October, 2008. Host species, rice and finger millet were grown from seed, while *E. indica, Panicum* sp., *and Setaria* sp. were grown from clumps collected from disease free area in the vicinity of IAAS, Rampur.

Pyricularia grisea from rice, finger millet and a weed species (*Eleusine indica)* were isolated and cultured using single spore isolation technique to maintain purity in culture stock. These isolates were cross inoculated to rice, finger millet and three weed species (*E. indica, Panicum* sp. *Setaria* sp.*)* which were planted or prepared for inoculation in tin trays with three replications in a greenhouse.

Each of the two isolates of the pathogen were inoculated to each 5 species of plants in separate iron cages so as to protect the air drift of blast spores and, covered with wet jute bags. At 18 days after sowing in case of rice and finger millet whereas 7 days after transplanting in case of other three plant species were inoculated with the spore suspension ($10^5 ml^{-1}$) with a hand sprayer at late afternoon (4 PM). Seedlings were then covered with wet jute bags for 48 hours. After this the jute bags were removed and the plants were covered with a white transparent polythene sheet during day and open during the night until scoring to maintain the humidity which is reduced during the day. Plants were monitored at 6 hours intervals to watch disease appearance till five days after inoculation. Infection of the plants was determined by microscopic observation of the fungal spores in the suspected leaves.

Cultural behavior

Radial mycelial growth (RMG) and days of sporulation (DOS) of *P. grisea* were studied by culturing 3 fungal isolates obtained from *O. sativa*, *E. coracana* and *Panicum* sp. on six different media; prune agar (PA), oat meal agar (OMA), potato dextrose agar (PDA), inger millet leaf decoction agar (FLDA), finger millet polish agar (FPA) and finger millet meal agar (FMA) during August to October, 2008 at the central laboratory of the IAAS, Chitwan, Nepal.

Pure cultures of the isolates were prepared and fungal blocks of 5 mm diameter were cut from the pure culture

and were placed at the center of petridishes in all six types of media. Cross lines with semi-permanent board markers were drawn on the undersurface of the lower plate along with the centre of the fungal block. Each plate was wrapped with parafilm tape to protect from contamination and they were incubated in the central laboratory at ambient temperature.

Experimental design and observation

The experiment was conducted in a completely randomized design with three replications. The RMG of the colonies was recorded at nine days after incubation. The diameters of the fungal colonies were measured with a measuring scale. The inoculated petridishes were incubated under continuous fluorescent light for fungal sporulation. Production of spore was detected by microscopic observation from five days after incubation at the interval of two days up to 30 days after inoculation.

Data analysis was done with Microsoft Excel (2000) and MSTAT-C (1986). Data were subjected to analysis of variance (ANOVA). When differences were found, means were separated using Duncan's Multiple Range Test (DMRT) .

Results and Discussion

Cross inoculation test

Five plant species grown in tin trays in a shade house were cross inoculated with two *P. grisea* isolates, one from rice and the other from finger millet to find out their host range. All the isolates from rice were pathogenic to *O. sativa, E. coracana, Setaria* sp., *Panicum* sp. and *E. indica*. The isolates from finger millet were only able to infect *E. coracana, Setaria* sp. and *E. indica* but not *O. sativa* and *Panicum* sp. (Table 1). Infection by *P. grisea* to different plant species in cross inoculation test are presented in figure 1.

Table 1. Disease developed by two isolates of *P. grisea* on 5 plant species in shade house at Rampur.

	Symptoms developed on five plant species				
Isolates from	*Oryza sativa*	*Eleusine coracana*	*Setaria* sp.	*Panicum* sp.	*Eleusine indica*
O. sativa	+	+	+	+	+
E. coracana	-	+	+	-	+

+ = symptoms developed, - = symptoms not developed

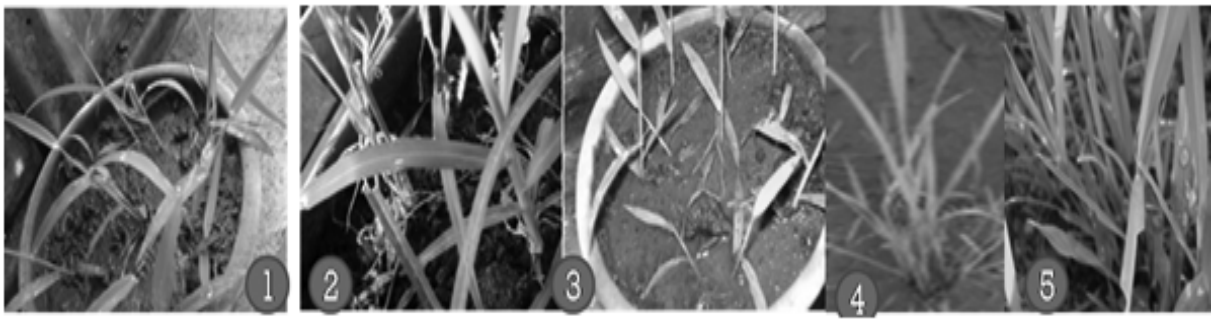

Fig. 1. Infection by *P. grisea* to different plant species in cross inoculation test 1. *Panicum* sp. 2. *E. indica* 3. *Setaria* sp. 4. *O. sativa and 5. E. coracana*

Mycelium growth of *Pyricularia* isolates *in vitro*

Mean radial mycelial growth (RMG) of different *P. grisea* isolates in different growing media in one week period was studied. The RMG was significantly different (P=0.00) among the *Pyricularia* isolates and the media (Table 2). Among the isolates, the RMG of the isolate from the finger millet was significantly higher than from the rice or the *Panicum* spp.

Among the media, OMA supported for the best mycelial growth of all *P. grisea* isolates, followed by PDA and PA. The growth was the lowest in FMPA and relatively better in FMA among the fingermillet-based media

Mean RMG of the isolate from finger millet was the highest (70 mm) in OMA followed by isolate from *Panicum* (65 mm) and isolate from rice (55 mm). The least RMG was obtained in FMLDA for isolates from rice (2 mm), *Panicum* (2.5 mm) and finger millet (3 mm) (Table 2).

Table 2. Mean mycelial growth of *P. grisea* isolates in different media.

S.N.	Isolates from	Mean growth
1	Finger millet	45.25[a]
2	Rice	27.67[b]
3	*Panicum*	24.77[b]
SEM		0.4718
	Media	Mean growth (mm)
1	PA	40.67[c]
2	OMA	63.58[a]
3	PDA	53.25[b]
4	FMLDA	14.92[e]
5	FMPA	3.208[f]
6	FMA	19.75[d]
SEM		0.6672
LSD		3.276

The colonies had an'upside' shape (inverted U shaped) in all the isolates, but the color of the colonies differed with the isolates. It was bluish green for *Panicum* isolate, grey for rice isolate and black grey for the finger millet isolate. The compactness of mycelia also differed with media. It was floppy in PDA and OMA, and compact in PA and FMA. Among the plant sources the isolate from *Panicum* showed the most floppy growth. Conidia of the isolate from finger millet were elongated and pointed, and that from rice were more globose, shorter and wider in comparison to the isolate from finger millet.

Table 3. Interaction on mycelium growth between three isolates from three plant species of *P. grisea* and six artificial growing media *in vitro*

SN	Media†	Isolate from		
		Finger millet	Rice	*Panicum* sp.
1	PA	45.25[e]	45.00[e]	31.75 [f]
2	OMA	71.25[a]	65.00[b]	54.50[c]
3	PDA	72.25[a]	45.00[e]	42.50[e]
4	FMLDA	30.50[f]	4.25h[i]	10.00[g]
5	FMPA	3.50h[i]	2.750[i]	3.37[hi]
6	FMA	48.75[d]	4.00h[i]	6.50[h]
SEm		1.156		
Grand sum		2344.500		
mean		32.563		
$LSD_{0.05}$		3.276		
C.V.		7.10%		

†: PA = Prune agar, OMA = Oat meal agar, PDA = Potato dextrose agar, FMLDA = Finger millet leaf Decoction agar, FMPA = Finger millet polish agar, FMA = Finger millet meal agar.

Time taken to sporulate in vitro

The same isolates of *P. grisea* from three plant species and six media were used for the study of mycelia growth, were further incubated for observation of sporulation in vitro. Average days of sporulation (DOS) was calculated from all the replications of each treatment. Average shortest DOS of isolates from the rice and the finger millet were 7.75 and 17, from PA and FMLDA respectively (Table 4). The DOS were not significantly different among the media. The isolates from

Table 4. Mean days required for sporulation for three isolates of *P. grisea* obtained from finger millet, rice and *Panicum* sp. isolates in different media

Media	Days of sporulation		
	Isolates from		
	Finger millet	Rice	*Panicum* sp.
PA	20	7.75	NS
OMA	21.5	9	NS
PDA	NS	20	NS
FMLDA	17	NS	NS
FMPA	18.75	NS	NS
FMA	18	NS	NS

NS: No sporulation up to 29 days after inoculation.

the isolates from *Panicum* did not sporulate in any of the media used.

The isolates from finger millet took more than two weeks for sporulation. The shortest period was observed in FMLDA (17 days) and the longest in OMA (21.5 days). They sporulated in all media except PDA, where the highest bacterial contamination was also observed.

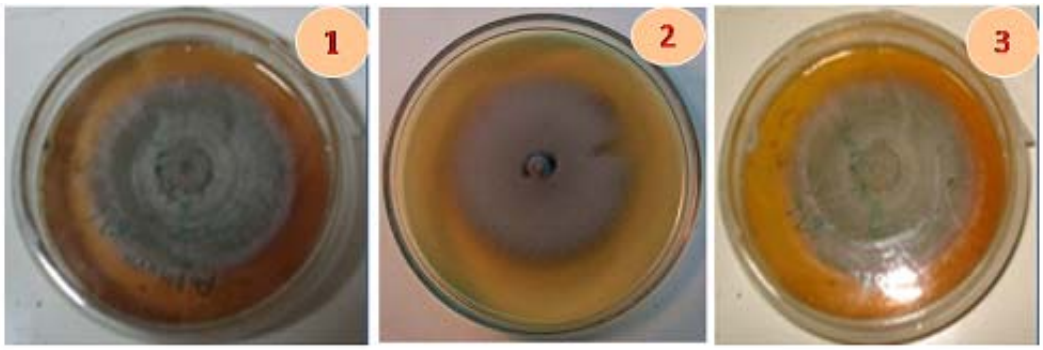

Plate No. 1 (1) Finger millet isolates (2) Rice isolates (3) *Panicum* isolates of pyricularia grisea grown on pruneagar media

The cross inoculation test showed that there was a difference between the isolates from the rice and the finger millet. All the isolates from rice were pathogenic to five plant species (*O. sativa, E. coracana, Setaria.* sp., *Panicum* sp. and *E. indica*) while the isolates from finger millet were only able to infect three plant species (*E. coracana, Setaria* sp. and *E. indica*). The isolates from finger millet failed to produce susceptible reaction on *O. sativa* and *Panicum* sp. Work in Uganda (Adipala 1989) supported this result indicating some isolates of blast from weeds also infected finger millet. The results suggest that the blast fungal isolates from rice and finger millet are close relatives but there is more genetic specialization for pathogenicity in rice and *Panicum* sp. than others. Similarly, it could be recommended that management of weed has more significant role on blast management in finger millet field than in rice field. Hence, it implicates the need for proper crop rotation and removal of alternate hosts.

Regarding the host range of *Pyricularia* isolates, very contradictory results have been reported by different workers. The reason of contradictory result from cross inoculation test may be due to variation of the race/ strain of the pathogen, resistance in the hosts, methods of inoculations, environmental conditions and nutritional status of the soil in which the crop was grown (Ashuyama 1965, Ou 1985, Kumar & Singh 1995). Ou (1985) supported the results that *O. sativa* isolates of the fungus infected *E. coracana* but contrasting result were obtained by Ramkrishan (1963) in his study in which finger millet isolates infected *O. sativa* , *E. coracana* and *E. indica*. Result of the cross inoculation tests carried out with *P. grisea* isolates from *Eleusine* and *O. sativa* showed that the isolates were host-specific. This result agreed with those of Kato *et al.* (1977) and Todman *et al.* (1994), who also found that *Pyricularia* isolates of *E. coracana* failed to infect rice and *vice versa*. Kumar and Singh (1995) have reported contradictory results regarding the ability of the pathogens from rice and finger millet to cross-infect. The reasons for this variation might be due to environmental conditions provided during experimentation in addition to the nutritional status of soil (Asuyama 1965, Ou 1985). Contradictory reports might also be due to some variability in host range involving a small genetic diversity of *P. grisea* isolates within a population. The results of the cross-inoculation tests also supported the view that the populations of the blast fungus infecting rice and finger millet were distinct and host-specific. It is clear that the gene flow between the pathogen infecting rice and finger millet was restricted. Thus, though finger millet is cultivated often in fields adjacent to rice, it is unlikely to serve as inoculums to the rice crop. Our results support the conclusion of Hamer *et al.* (1989) and Valent *et al.* (1986) that the *P. grisea* populations are strongly delimited by host range although blast is found to infect a range of plant species.

All the *Pyricularia* isolates showed best performance in OMA for both RMG as well as sporulation. The medium was recommended by most of the workers in the past (Singh & Kumar 1995). However, better result

of sporulation and less contamination in PA than OMA established PA as an ideal media for the purpose. Among the locally made media, finger millet isolates showed best performance for all tested parameters in finger millet leaf decoction agar (FMLDA) than others. Medium prepared from young and immature leaves showed better result than from old and matured leaves. The earlier sporulation in FMLDA would be due to the presence of some special biochemical compounds required for finger millet isolates. Better result in immature leaf is supported by the finding of Roumen (1992) who explained the blast development process slowed down with leaf and plant age. Nishikado(1917), Suemato (1916), Tochinai & Nakano (1940) strongly supported the result they noticed *Pyriculria* isolates from rice could not grow and not sporulated in Agar-agar but able to grow and sproulate, on addition of hot water extract of rice straw in the medium. Finger millet meal agar did not give better result for all parameters as on OMA. This may be due to the scarce of available nutrients in finger millet meal as in oat meal and having a low glycemic index with high fiber content in finger millet which create undesirable environment (Hittalmani *et al.* 2004).

Finger millet isolates took long time for sporulation than rice isolates in all media, but *Panicum* isolates were unable to sproulate in any media. This might also be due to the absence of some specific biochemical compounds required for their sporulation (Tanaka & Katsuki 1952).

Present study showed that inter-host isolates of *Pyricularia* showed differential interaction with media and have different sporulating characters, which further indicates that isolates from rice and finger millet are closely related while isolates from *Panicum* is physiologically far apart from these isolates. The result is concretely supported by the result of Kumar and Singh (1995). They also concluded that isolates from rice and finger millet are closely related, but far apart from isolates of *Panicum* in the sense of physical factors they preferred for their growth and development. Similarly, the results were also supported by Lee and Wu (1967). They stated that different isolates of fungi differed in utilization of carbon sources.

The results of the present study suggest that the host range of the isolates of rice are much more wider than isolates of finger millet. Thus, cultivation of finger millet in fields adjacent to rice could serve as the source of inoculum to the finger millet crop. The importance of weeding and sanitation in finger millet is more precious than in rice for blast management. Similarly, it could also be concluded that the inter-host isolates of the fungus showed differential response with media. It further indicates that finger millet leaf decoction agar and prune agar are best culture media for finger millet blast fungus. Similarly it took comparatively longer duration for sporulation of *P. grisea* isolate isolated from finger millet than of those isolated from rice.

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