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Survival of *Magnaporthe grisea* on Rice Seeds from Artificially Inoculated Panicles of Selected Rice Lines

K.D. Puri, S.M. Shrestha[1] , K.D. Joshi[2] and G.B. Khatri[3]

Institute of Agriculture and Animal Science (IAAS)
Chitwan, Nepal

ABSTRACT. *Survival and infection of Magnaporthe grisea was studied on seeds produced from artificially inoculated panicles of thirty three rice lines. A significant correlation was found between the severity of panicle infection and survival of pathogen in seed. However, some of the infected panicles yielded pathogen free seeds while other non infected panicle yielded infected seeds. Study conducted in laboratory showed that rice lines namely Barkhe 3018, Barkhe 3004 had no seed infection while Masuli×MT4 P # 168, Masuli×MT4 P # 140, Barkhe 2044, Masuli×MT4 P # 137, Masuli×MT4 P # 11, Barkhe 3015, and Barkhe 1034 had got higher (38 - 43%) level of seed infection. Survival and sporulation of M. oryzae on different part of seeds varied and showed highest infection on sterile lemmas (39%), followed by lemma (31.5%), awn (29.5%) and palea (28.8%). Pathogen survival was found varied with level of panicle infection on different rice lines. Survival on different parts of seeds varied and serves as major sources of primary inoculum.*

INTRODUCTION

Blast, a cosmopolitan disease of rice (*Oryza sativa* L.), is caused by Ascomycete fungus *Magnaporthe grisea* (T.T. Hebert), (Anamorph *Pyricularia grisea* Sacc.) (Rossman *et al.,* 1990). Disease is widely distributed and destructive under favorable environmental conditions (Ou, 1985). *M. grisea* infects the leaf blades, leaf collar, nodes, neck branches and internodal parts of culms (Thurston, 1998). The disease may occur at seedling, tillering and heading stages of crop (Zeigler *et al.,* 1994) and can cause complete loss of seedling in seedbed (Chaudary *et al.,* 1994; Adhikari and Shrestha, 1986) and epidemic in the field (Teng *et al.,* 1991). In Nepal, 10 - 20% yield reduction on susceptible varieties is reported due to this disease, but in severe cases it goes upto to 80% yield reduction (Manandhar *et al.,* 1992).

Shrestha *et al.* (1977) reported the presence of *M. grisea*, *Cochliobolus miyabeanus*, and *Trichoconis padwicki* on rice seeds. Seed transmission of *M. oryzae* was first reported from Japan (Kuribayashi, 1928). The fungus can withstand winter in rice seed (Agrawal *et al.,* 1989; Mew *et al.,* 1988) and could be the primary source of inoculum

[1] Professor of Plant Pathology, IAAS, Chitwan, Nepal.

[2] South Asia Research Coordinator, University of Wales, Bangor UK, C/O CIMMYT, Nepal.

[3] Assistant Professor of Plant Pathology, IAAS, Chitwan, Nepal.

(Manandhar, 1996; Honda and Nemoto, 1985) and plays important role in disease cycle (Lee, 1994). Aulakh *et al.* (1974) showed positive correlation between level of seed infection and field incidence.

Mycelium and conidia of *M. grisea* reported from the glumes and caryopsis, thus enabling survival under unfavorable weather conditions (Bernaux, 1981). The fungus was found on the rachilla, pedicel, palea, lemma and pericarp layers, but not in the embryo of rice seeds (Chung and Lee, 1983). The conidia are typically air borne but its seed borne behavior is also well known. However, the varietal reaction and contribution of panicle infection for seed infection as well as survival of *M. grisea* in different parts of seed is less known. Thus the aim of this study was to examine seed borne infection of *M. oryzae* in different rice lines and its survival on different part of seeds.

MATERIALS AND METHODS

The experiment was conducted at the laboratory of the Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan between March to April 2006. Seeds were collected from thirty one different rice lines harvested from all the rice line that were tested for the panicle infection in green house by inoculating panicles artificially. All rice lines were developed by CAZS Natural Resources (CAZS-NR), University of Wales, UK, and Local Initiatives for Biodiversity and Research and Development (LI-BIRD). Masuli (Mayang Ebos 80*2 × Taichung 65, from Malaysia) was used as susceptible check (Manandhar, 1987) and Sona Masuli (Sona×Mahsuri) as semi resistant check (Rathaiah *et al.,* 1988). Rice lines were of different growth habitat *i.e.* lowland, midland and upland situation.

The harvested seeds of different rice lines were stored separately at room temperature (18 - 20^{o}C) for one month. Presence of pathogen was studied in laboratory by standard blotter test method (Misra *et al.,* 1994). Seeds were kept in three layers of moistened white blotting paper, equidistantly in the petri dishes at 25 seeds/dish in 16 plates. Both filled and unfilled grains were randomly drawn. The plates were incubated at 25 ± 2^{o}C for 36 - 72 hrs under 12 hrs NUV light and 12 hrs darkness. Each seed was examined microscopically to detect sporulation of *M. grisea* and total number of infected seed was counted. Total seed infection or seed borne inoculum was calculated by using the following formula.

$$\text{Percentage seed infection} = \frac{\text{No of infected seed} \times 100}{\text{Total number of seed observed}}$$

All the panicles of individual rice lines were collected and panicle blast incidence was calculated using formula:

$$\text{Percentage panicle blast disease incidence} = \frac{\text{No. of infected panicle} \times 100}{\text{Total number of panicle counted for each line}}$$

Panicle infections ranging from 9.09% to 93.6% were used in study to calculate the correlation between visual panicle symptoms and seed infection. Four hundred seeds from each of the rice lines were examined for the presence of *M. grisea.* Survival of *M. grisea* in different parts of seed was identified by observing different parts of seed microscopically. The number of infected seeds were counted for each part *viz.* sterile lemmas, lemmas, palea and awn.

Percentage seed infection, percent neck infection were tested for analysis of variance (ANOVA) to see difference between various rice lines. Similarly correlation coefficient analysis was done. Mean comparisons were done using paired Duncan's Multiple Range Tests (DMRT). Percentage data were transformed into log(x+1) and Arcsine√percentage (Gomez and Gomez, 1984). Microsoft© Excel (2000), and MSTATC (1986), and SIGMAPLOT (2000) were used for data analysis.

RESULTS AND DISCUSSION

Seed infection: Reaction of rice lines to neck blast and its transmission to seed was shown in Table 1. There was linear relation between neck blast incidence and seed infection by *M. grisea* (Table 1) with positive correlation and highly significant (p=0.01). Seeds from high neck blast infection had significantly higher degree of infection than seeds from lower level of infection However; some rice lines had no seed infection even though they got high neck infection.

The highest survival of *M. grisea* on seed was recorded from Barkhe 1034 (43.0%) and Barkhe 3015 (42.3%), followed by Masuli×MT4 P # 11, Masuli×MT4 P # 140, Barkhe 2044, Masuli×MT4 P # 137 and Masuli×MT4 P # 168 (Table 1). Survival of pathogen on seed was even higher than susceptible check cultivar Masuli (31.75%). Less infection was found on Barkhe 3004 and no or lowest survival on recorded on Barkhe 3018, and Super 3004 however Barkhe 3018 had higher neck infection (32.4%) (Table 1).

a. Survival on upland rice lines: Among the upland rice lines, highest seed infection was recorded on Barkhe 1034 followed by Masuli (31.75%), Sona Masuli (30.25%), and Barkhe 1006 (30%) (Figure 1a). But very less infection was recorded on Barkhe 1035 (1.5%) (Table 1).

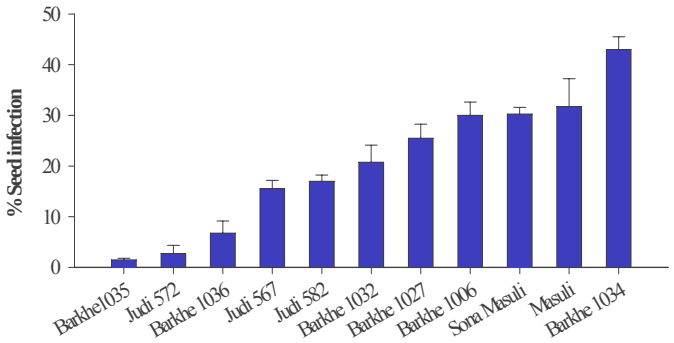
b. Survival on lowland rice lines: In low land rice lines, highest infection was in Barkhe 3015 (42.3%) higher than Masuli (31.8%), followed by Barkhe 3017 (24.5%) and Sugandha 2002 (9.8%). However, in Barkhe 3019 and Super 3004 had only around 1% infection (Figure 3b). Moreover, Barkhe 3004 and Barkhe 3018 had no infection although these lines had a substantial level of neck infection (Table 1).

c. Survival on midland rice lines: Among midland rice lines highest seed infection was recorded on Masuli×MT4 P # 11 (40.75%), followed by Masuli×MT4 P # 137 (40.5%), Barkhe 2044 (40.5%), Masuli×MT4 P # 140 (40.5%) and Masuli×MT4 P # 168 (38.0%) (Figure 1c) higher than Masuli (31.75%), Masuli×MT4 P # 86 (31.5%), and Sona Masuli (30.25%). thus the most of midland rice lines from Masuli×MT4 parentage had both high level of neck and seed infection (Table 1).

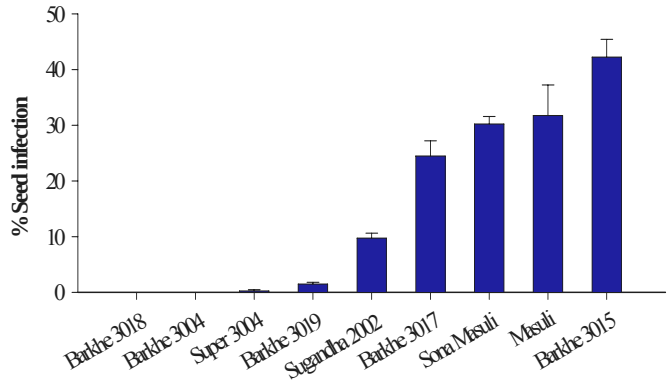
Table1 1. Percent neck age blast in screen house and seed infection by *M. grisea* as estimated in laboratory.

Rice lines	Cross	% neck infection	% seed infection
Barkhe 1035	KIII/Azucena//Sugandha 1	9.09	1.5^{kl} $(6.9)^{P}$
Barkhe 1006	IPB	11.4	30.0^{b-d} (33.2)
Barkhe 3004	K III/IR 64	11.5	0.0^{m} (0.3)
Super 3004	K III/IR 64	13.9	$0.3l^{m}$ (1.6)
Barkhe 1032	Judi 582/Barkhe 2027	14.8	20.7^{e-g} (26.8)
Judi 572	Radha 32/ K III	18.5	2.8^{kl} (6.9)
Barkhe 2014	K III/IR 64	25.4	4.0^{jk} (10.9)
Barkhe 3019	K III/IR 64	27.7	1.5^{kl} (6.9)
Barkhe 3015	K III/IR 64	29.8	42.3^{a} (40.5)
Barkhe 1027	K III/IR 64	31.9	25.5^{ef} (30.2)
Barkhe 3018	K III/IR 64	32.4	0.0^{m} (0.3)
Sugandha 2002	Irradiated Pusa Basmati (IPB)	32.4	9.7^{h} (18.2)
Barkhe 2001	IPB	33.1	11.3^{h} (19.5)
Masuli×MT4 P # 140	Masuli/MT4	34.7	40.5^{ab} (39.4)
Judi 567	K III/IR 64	35.4	15.5^{gh} (23.1)
Barkhe 2045	Masuli/MT4	38.6	3.5^{jk} (10.5)
Masuli×MT4 P # 143	Masuli/MT4	40.3	17.3^{f-h} (24.5)
Barkhe 1036	KIII/Azucena//Sugandha 1	40.9	6.7^{ij} (13.1)
Sona Masuli	Sona×Mahsuri	41.2	30.3^{b-d} (33.4)
Judi 582	Radha 32/ K III	41.4	17.0^{f-h} (24.3)
Barkhe 2044	IPB	41.8	40.5^{ab} (39.5)
Masuli×MT4 P # 109	Masuli/MT4	42.4	10.3^{hi} (18.6)
Barkhe 2024	IPB	46.4	10.0^{hi} (18.3)
Barkhe 1034	KIII/Azucena//Sugandha 1	46.5	43.0^{a} (40.9)
Masuli×MT4 P # 193	Masuli/MT4	48.7	22.3^{d-g}(28.0)
Masuli×MT4 P # 69	Masuli/MT4	51.7	28.0^{c-e}(31.7)
Barkhe 3017	Masuli/Laxmi	56.3	24.5^{d-f}(29.6)
Masuli×MT4 P # 137	Masuli/MT4	56.3	40.5^{ab} (39.5)
Masuli×MT4 P # 168	Masuli/MT4	57.9	38^{a-c} (37.9)
Masuli×MT4 P # 182	Masuli/MT4	62.7	10.5^{hi} (18.5)
Masuli×MT4 P # 86	Masuli/MT4	62.9	31.5^{b-d}(34.1)
Masuli×MT4 P # 11	Masuli/MT4	71.7	40.7^{b} (39.6)
Masuli (SC)	Mayang Ebos 80*2 × Taichung 65	93.6	31.7^{b-d}(34.0)
SE			1.976
$LSD_{0.05}$			5.543
CV%			17.20

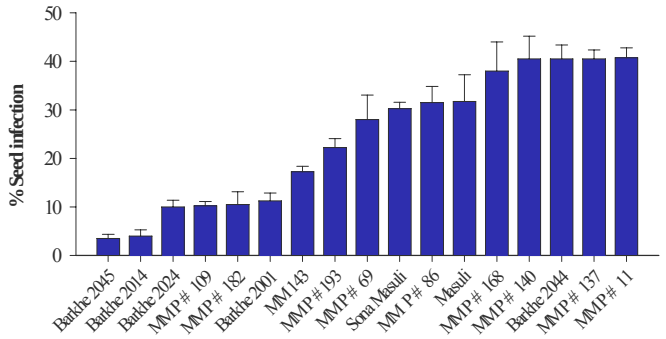
Note: In columns, figures followed by the same letter were not significantly different at P = 0.05 according to DMRT.
CV: Coefficient of Variation, SEM: Standard Error of Mean, LSD: Least Significant Difference.
[P] Figures on parenthesis were Arc sine transformed values: Arcsin√ percent.



(a) Upland rice line



(b) Lowland rice lines



(c) Midland rice lines

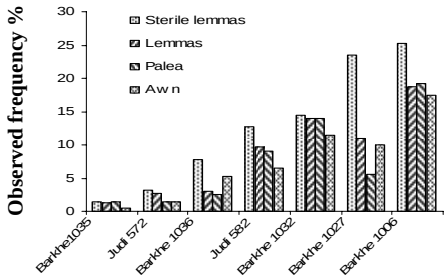
Figure 1. Mean infection of *M. grisea* on seed sample of upland (a) lowland (b) and mid land (c) rice lines obtained from the panicle infection in screen house in Rampur.

Note: Vertical bar represents the standard error of means.

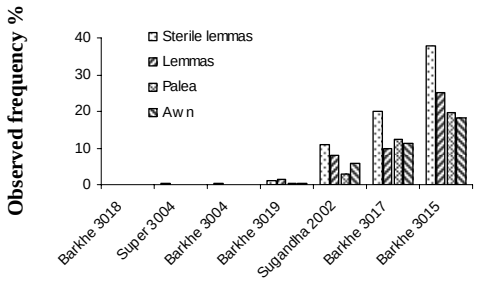
Distribution of pathogen on different part of seed

In most of rice lines, maximum survival of the pathogen was recorded from sterile lemmas having positive correlation with other parts. Among upland lines *e.g*. Barkhe 1006 and Barkhe 1027 got highest infection on sterile lemmas. In other rice lines such as Barkhe 1035, Judi 572, and Barkhe 1036 had low survival in all over seed (Figure 2a). Similar behavior reported from lowland lines too, where maximum survival was on Barkhe 3015, followed by Barkhe 3017 and Sugandha 2002. However, in Barkhe 3018, Barkhe 3004, Super 3008 had no survival and in Barkhe 1019 low frequency pathogen survival (Figure 2b). Relatively, midland rice lines had higher pathogen survival on sterile lemmas compared to other rice lines. Minimum survival was found in Barkhe 2014, Barkhe 2025, but Masuli×MT4 cross had relatively higher level of pathogen survival. Thus, survival of pathogen on different parts was directly correlated to both percentage seed infection and host susceptibility.

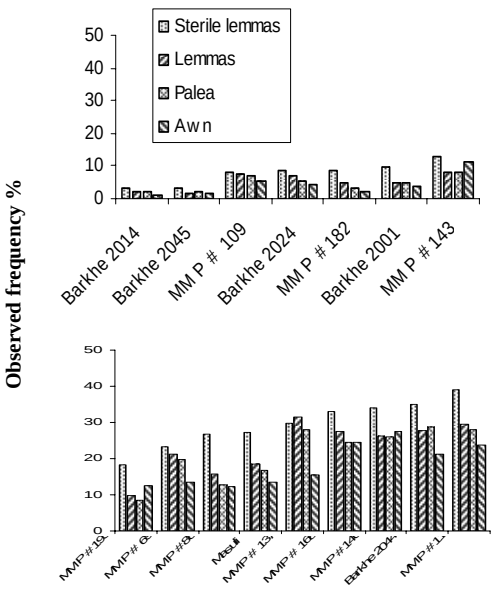
Presence of pathogen on different part of seed surface was significant ($p = 0.01$) and positively correlated. Pathogen presence on sterile lemmas and lemmas had high correlation ($r = 0.96$), similarly its correlation with palea was ($r = 0.93$), with awn was ($r = 0.95$), and partition between palea and lemma was ($r = 0.92$). The highest correlation was between Lemma and Palea ($r = 0.98$) infected seed serves as a primary source inoculum for the rice blast under field conditions (Agrawal *et al.,* 1989; Chung and Lee, 1983; Lamey, 1970). Manandhar *et al.* (1998) showed the correlation between neck infection and pathogen transmission to seed. Positive correlation between neck infection and its successive infecton to seed was also reported earlier (Aulakh *et al.,* 1974). Kato *et al.* (1970) showed the role of diseased spikelets as the secondary inoculum source in infection chain of blast. Until now, infection pattern of seeds either from infected panicles by systemic growth of fungus or by spreading conidia from sporulating lesions to seed was not clearly known. In this work healthy-looking panicles also yielded infected seeds and *vice versa*. So that presence or absence of disease symptoms in field does not clearly tell the survival and infection to seeds. Similar results were also found by Manandhar *et al.* (1998). Thus, this should be an important consideration in seed certification programme. Schilder and Bergstrom (1994) also reported positive correlation between the severity of tan spot caused by *Pyrenophora tritici-repentis* on the flag leaves of wheat and seed infection by this fungus. *M. grisea* was reported from all over the surface of seed and more severely on sterile lemmas. Reports showed that >90% frequency from the sterile lemmas, and relatively low *i.e.* about 10% from lemmas and/palea. Further, Agrawal *et al.* (1989) reported *M. grisea* even from the embryo, endosperm, bran layer, kernel, glumes, and between glumes of rice. Aulakh *et al.* (1974), showed positive correlation between seed infection in laboratory and its field incidence. In this work, the highest percentage of seed infection was reported from Barkhe 3015 (42.3%) and Barkhe 1034 (43.0%).



(a). Upland rice lines.



(b). Lowland rice lines.



(c). Midland rice lines.

Figure 2. Observed frequency of *M. grisea* on different part of seed on different rice lines Upland.

Note: (a.) Upland (b.) Lowland and (c.) Midland rice line
Seed were harvested from the infected panicle from screen house at Rampur, Chitwan, 2006 (MM refers to Masuli×MT4).

Seed serves as a source of primary inoculum and its nonsystemic aerial transmission from artificially infested grain to rice seedling initiate rice blast on young seedlings (Long *et al.,* 2001). Inoculum thresholds and survival were important parameters that function as primary inoculum of disease (Xu, and Ridout, 1998; Kuan, 1988; Gabrielson, 1988). In this study, conidiophore and conidia formation was dominantly confined to the embryonic end of seeds. In a few cases, sporulation occurred on the entire seed coat. These results were similar to those of Chung and Lee (1983) and others (Manandhar *et al.,* 1998; Lamey, 1970). Manandhar *et al.* (1998) further reported that sporulation can occur on all parts of the seed, especially if the seeds were not viable. Thus, use of pathogen-free seed is an important component of integrated rice blast management (Sattar and Savitri, 1999).

CONCLUSIONS

Pathogen showed the varying level of survival on different rice lines ranging from 0 - 93.4%. Namely, Barkhe 3018, Barkhe 3004, Barkhe 3018 had infection while on other genotypes, particularly the progenies of Masuli X MT4. Masuli×MT4 P# 168, Masuli×MT4 P# 140, Barkhe 2044, Masuli×MT4 P # 137, Masuli×MT4 P# 11, Barkhe 3015, and Barkhe 1034 had higher infection. The infection of pathogen was found highest on sterile lemmas followed by Lemma, Palea, and Awn. Seeds from higher neck infection had relatively high level of seed infection. Thus the seed treatment could be promising technique for the disease management.

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