Rice Seedborne Fungi and Their Effect on Seed Germination

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ABSTRACT

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Seeds from nine upland and seven lowland cultivars of rice (*Oryza sativa* L.) were examined for qualitative and quantitative estimates of seedborne fungi. The observed fungi were *Helminthosporium oryzae, Fusarium moniliforme, Penicillium* sp., *Curvularia lunata, Aspergillus* sp., *Rhizopus arrhizus, Geotrichum* sp., and *Alternaria* sp. High negative significant correlation was obtained between seed infestation by all isolated microflora and seed germination in the laboratory for all cultivars tested ($r = -0.79$), upland cultivars ($r = -0.84$), and lowland cultivars ($r = -0.74$).

The importance of disease-free, viable seeds in crop production cannot be overemphasized. Infected seeds germinate poorly and could be a major source of inoculum for new crops raised from them. For example, most pathogens causing abnormal seedlings of rice are seedborne (6). Moreover, infected seeds are often discolored (2,4,12), an indication of poor grain quality. Under epidemic conditions, total loss of a rice plantation to the pathogen may ensue. E. D. Imolehin (*unpublished*) recently indicated an epidemic outbreak of glume and kernel discoloration of rice caused by *Curvularia lunata*, a pathogen previously considered economically unimportant in Nigerian rice culture.

Many fungi, ranging from major pathogens to nonpathogens of rice, have been recorded as seedborne on rice. For example, Zainum et al (15) in Malaysia recorded 33 fungal isolates on 23 cultivars of rice obtained from 11 locations, of which *Trichoccnis* (*Alternaria*) *padwickii* was most common. In India, Majumdar et al (8) reported rice seeds infected with *Helminthosporium oryzae* (*Cochlobolus miyabeanus*), *Curvularia lunata, Cochlobolus lunatus, Alternaria tenuis*, and *Epicoccum* sp.

H. oryzae, a seedborne fungus, is a major pathogen of cultivated rice, causing brown leaf spot (1,2,7) and sometimes seed infection (1–3,11,15). Infection of rice seed by *H. oryzae* has also been considered a major cause of poor seed emergence (9,10), and *C. lunata* isolated from rice has also been reported to reduce seed germination and/or infect rice seedlings (8,14). Other seedborne fungi have been isolated from rice seed but have not been shown pathogenic on rice as a result of their presence on seeds. There is no adequate information, however, on the seedborne fungi of rice in Nigeria and their role in rice production in that country. This paper reports the incidence of seedborne microflora on rice seeds from various rice locations relative to seed germination.

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MATERIALS AND METHODS

Isolation of seedborne fungi. Sixteen rice cultivars, including nine upland cultivars (TOX 494-10, IR 2035-180-2, M1/336/1/2, 949m/9/2, TOX 718-1, TOX 475-1-1, IR 2043-104-3, TOX 718/2, and TOS 2583) and seven lowland cultivars (GB 90-2, ADNY 11, BG 902/TETEP-2, TOM 1-3, BG 6850, TOX 154-16-101-1, and IR 2035-120-3) obtained from rice breeders of the National Cereals Research Institute, Moor Plantation, Ibadan, Nigeria, were used for these studies. Rice seeds were dried and kept in small bags made of baft cloth and stored in a field laboratory for about 9 mo before they were studied.

Occurrence of seedborne microflora on seeds was determined by the modified blotter method. Twenty seeds of each cultivar selected at random were spaced on damp 9-cm Whatman No. 1 filter paper in plastic petri dishes and each was replicated five times. The experiments were repeated four times. The plates were incubated in 12-hr light and 12-hr darkness at 25 ± 2 C for 8 days.

After the 8-day incubation period, conidia and hyphae of fungi growing on the seeds were picked off each infected seed with fine forceps, mounted on a slide, and examined with a compound microscope. Each fungus was identified on the basis of its conidia and/or hyphae characteristics and frequency of isolation was tabulated.

Seed infestation relative to seed germination. To determine the effect of seed infestation on seed germination, 20 seeds from each of the cultivars were placed on damp 20-cm filter paper and covered by another filter paper. The base was folded in and the whole rolled up and secured with elastic bands. Each treatment was replicated five times and the experiment was repeated three times. Treatments were incubated upright in plastic trays at 25 ± 2 C for 8 days, then examined for germination. The number of germinated seeds was recorded. The relationship between seed infestation and seedling emergence was statistically determined using correlation coefficients.

RESULTS

Isolation and identification of seedborne fungi. Frequency of isolation of each fungus is presented in Table 1. *H. oryzae* was the most frequently isolated pathogen, closely followed by *F. moniliforme, Aspergillus* sp., and *R. arrhizus* for upland cultivars and by *R. arrhizus* for lowland cultivars. *F. moniliforme, Penicillium* sp., and *Aspergillus* sp. were infrequently isolated from lowland cultivars. Three fungus genera (*Geotricum, Alternaria*, and *Curvularia*) were infrequently isolated. Infection of rice seeds varied both within and between the cultures from the two sources. For example, *H. oryzae, F. moniliforme*, and *Aspergillus* sp. represented 50, 16, and 9% of the total fungi identified on the upland grain, respectively, and 80, 3, and 0%, respectively, in the lowland cultivars.

Figure 1A,B shows the effect of seed infestation on seed germination. The figure shows that seed germination is decreased by seed infestation regardless of the rice cultivars tested. Seed infestation by seedborne fungi had a high statistical negative correlation with seed germination ($r = -0.79$).

Table 2 shows correlations between each seedborne fungus and seed germination. Data indicated that *H. oryzae* was significantly correlated with poor germination of rice seeds regardless of their source. Although infestation by *F. moniliforme* had significant negative correlation with germination of lowland cultivars and all cultivars combined, *Aspergillus* sp. was only correlated with germination of all the cultivars tested. Seed infestation by *R. arrhizus* had significant negative correlation with germination of upland rice cultivars and significant positive correlation with germination of lowland cultivars and all

Table 1. Frequency of isolation of seedborne fungi[a] from some cultivars of rice

Cultivar	*Helminthosporium oryzae*	*Fusarium moniliforme*	*Penicillium* sp.	*Curvularia lunata*	*Aspergillus* sp.	*Rhizopus arrhizus*	*Geotrichum* sp.	*Alternaria* sp.
Upland								
TOX 494-10	15	5	3	2	2	...	...	...
IR 2035-180-2	41	1	...	...	6	...	...	...
M1/336/1/2	9	2	1	1	4	5	...	...
949m/9/2	8	2	1	...	11	4	...	...
TOX 718-1	7	5	...	...	1	2	...	...
TOX 475-1-1	5	1	...	...	10	4	...	...
IR 2043-104-3	10	5	...	...	...	3	...	...
TOX 718/2	5	4	1	...	11	4	...	...
TOS 2583	24	11	4	...	4	...	...	...
Lowland								
BG 90-2	21	4	1	...	...	2	...	...
IR 2035-120-3	56	2	...	2	...	5	...	3
TOX 154-16-101-1	35	...	2	...	...	3	...	...
TOM 1-3	16	1	...	1	1	4	...	...
ADNY 11	18	...	...	...	...	3	...	...
BG 902/TETEP-2	6	...	...	2	...	1	1	...
BG 6850	18	1	...	0	...	...	1	...

[a] Fungi isolated from seeds examined were not mutually exclusive.

the rice cultivars tested. Infestation of rice seeds by all the mycoflora was highly correlated with poor seed germination.

DISCUSSION

The most frequently isolated fungi from rice seed may vary from minor pathogens (15) to a major pathogen of economic importance to the rice industry (1,3,5,11,13,15). In these studies, *H. oryzae* was the most frequently isolated seedborne fungus regardless of the source of the rice cultivars tested. *H. oryzae* has been shown to reduce rice yield in Nigeria (2) as in most other rice-producing countries. This pathogen has been reported to reduce seed germination (9,10) and has also been shown to cause seedling blight (6). The high frequency of isolation of *H. oryzae* from rice seeds may explain in part the high incidence of brown leaf spot in most rice fields in Nigeria (*personal observation*,1). These findings thus indicate that this pathogen may pose a serious threat to the rice industry in Nigeria if adequate control measures are not implemented.

In contrast, *Pyricularia oryzae*, a very important seedborne pathogen of rice (1,2), was not isolated from any of the rice cultivars. Aluko (1) reported that 81% of the rice seed samples tested were infected by *H. oryzae* compared with 8.4% infection by *P. oryzae*, indicating the scarcity of *P. oryzae* on rice seeds in Nigeria. This may indicate that blast caused by *P. oryzae* will be less frequent on rice raised on newly cultivated land compared with the brown spot disease caused by *H. oryzae*.

F. moniliforme, the third most frequently isolated fungus from seeds, was associated with poor seed germination in these studies, confirming earlier reports on this pathogen (3). Infected seedlings often fail to emerge in cold, wet soil. This pathogen may therefore contribute in part to reduced rice stands in many rice plantations where infected seeds have been planted. Because *F. moniliforme* is also known to cause discoloration of rice seeds (12), its frequent isolation from seeds indicates that it may contribute to poor seed quality of Nigerian processed rice.

Table 2. Correlation of seedborne fungi on laboratory germination of rice seeds

Fungus genera	Lowland cultivars	Upland cultivars	All cultivars
Helminthosporium oryzae	−0.78*	−0.70*	−0.69**
Fusarium monilliforme	−0.36	0.73*	−0.52
Penicillum Sp.	−0.69	−0.13	−0.36
Curvularia lunata	−0.32	−0.54	−0.45
Aspergillus Sp.	−0.45	−0.79	−0.63**
Rhizopus arrhizus	−0.82*	−0.77*	−0.79**
All genera	−0.74	−0.84**	−0.79**

[a] Level of significance: * = P=0.5 and ** = P=0.01.
[b] Number of seed infected of 100 examined on modified blotter test.
[c] Three lots of 100 seeds per sample were germinated on modified blotter and number germinated after 8 days was recorded.

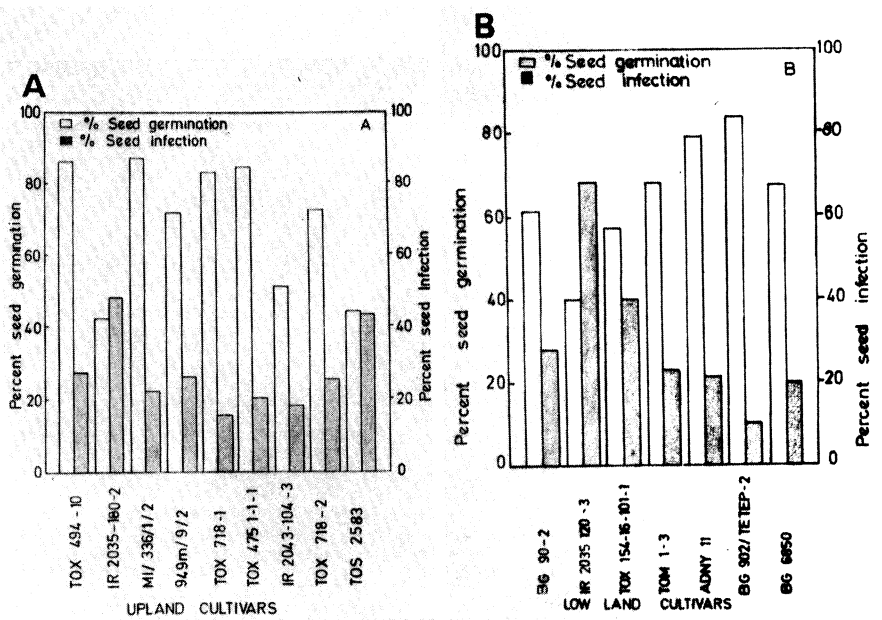

Fig. 1. Effects of seed infestation on germination of rice seeds. Effect of seedborne mycoflora on germination of **(A)** upland rice cultivars and **(B)** lowland rice cultivars. Seed infestation had significant negative correlation with seed germination for upland (r = −0.84) and lowland (r = −0.74) rice cultivars at P = 0.01 and P = 0.05, respectively.

Aspergillus sp. and *R. arrhizus*, although regarded as surface contaminants, were also frequently observed on rice seeds. Besides the fact that some *Aspergillus* spp. produce aflatoxins, they

have also been shown to deteriorate stored grains (14). These studies have also associated these fungi with reduced germination of seed in the laboratory. These fungi, like rice pathogens, are therefore of economic importance in Nigerian rice production.

Although other seedborne fungi were rarely isolated from the rice seeds tested, this does not indicate that they cannot cause serious diseases of rice under more favorable environmental conditions. For example, *C. lunata* was recently reported to cause glume and kernel discoloration on rice in Nigeria (E. D. Imolehin, *unpublished*).

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