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Seed-borne fungi associated with farmer saved rice seed in Uganda and their effect on germination

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Abstract

Seventy seed samples from three districts of Uganda, namely, Bugiri, Pallisa, and Lira were examined by the blotter method for fungal infection at Makerere University. Twenty different seed-borne fungi were identified and their incidence and infection levels varied significantly ($P<0.01$) with respect to location and within districts. *Bipolaris oryzae* was the most prevalent pathogen with an infection range of 3-94%. *Phoma oryzae, Pyricularia oryzae, Alternaria padwickii, Fusarium moniliforme, Curvularia lunnata, Nigrospora oryzae, Verticilium cinabarium and Alternaria alternata* were also present in all the districts. However, some pathogens were localised. For example, *Exserohilum rostratum,* and *Curvularia eragrostidis* were only encountered in Lira and Bugiri districts, respectively. Pallisa district had the least number of fungal species while Bugiri registered the greatest diversity. Mean infections were also low in Pallisa as opposed to Bugiri and Lira districts. Nine categories of seedling abnormality were recorded. Seedlings with shoot and root decay were encountered frequently. *B. oryzae, Phoma oryzae, Pyricularia oryzae, A. padwickii, F. moniliforme, C. lunnata, Melanospora zaminae* and *Alternaria alternata* were found associated with 100%, 55.6%, 44.4%, 66.7%, 77.8%, 11.1% and 11.1% of the different abnormalities, respectively. Results of the present study have revealed the occurrence of a wide range of pathogenic fungi and their association with seedling abnormalities.

Key words: *Oryzae sativa,* seedling abnormality, seed infection, seed health

Introduction

Rice (*Oryzae sativa)* is the third most important cereal in Uganda after maize and sorghum. The importance of rice as a commercial crop in the country has recently soared and production is rapidly expanding (FAO, 2000). However, rice production is constrained by a wide range of diseases, most of which are seed-borne. Although the range of organisms infecting rice seed is extensive in Uganda (Richardson, 1990), very little is known about the role and importance of seed-borne inoculum in the dissemination of rice diseases. Seed-borne diseases have in the past caused heavy losses to field crops all over the world (Khanzada and Jamil Khan, 1987). Thus, the role of seed in disseminating diseases cannot be under estimated particularly in developing countries where routine chemical treatment is expensive and the majority of farmers rely on farm saved seed and purchases from local markets.

Nearly all the major fungal diseases of rice are seed-borne (Mew, 1998). No studies on seed-borne diseases of rice have been reported in Uganda yet rice has been grown for a long time. Consequently, only few rice diseases in Uganda have been formally recorded. Several major rice pathogens endemic elsewhere have also been reported in other parts of Eastern Africa (Asura *et al*, 1999) and are likely to be present in Uganda. Given the paucity of information on the diversity of seed-borne fungi of rice, it was necessary to identify them, investigate their incidence and distribution. This study was therefore initiated with the objectives of identifying the different seed-borne fungi present on rice seed, their incidences and distribution in Uganda and also establish the different seedling abnormalities associated with the different seed-borne fungi.

Material and methods

Seventy rice seed samples were collected from farmer saved seed in the districts of Lira, Bugiri and Pallisa in January 2003. Seed samples were taken to the laboratory at Makerere University for fungal identification and germination tests. The data obtained were subjected to analysis of variance using Genstat computer package (Lawes Agricultural Trust Rothamsted Experimental Station, 1993) and means of significant effects separated using Fisher's Protected Least Significant Difference (LSD) test at 5% probability level.

Fungal identification

Fungal detection was done using the blotter method (ISTA, 1999). Three layers of blotting papers moistened with distilled water were placed inside plastic petri-dishes (9 cm diameter). From a random sample of 400 seeds, 25 seeds were placed in each petri-dish and incubated at 22°C under a 12 h NUV light and 12 h dark cycle for 7 days. Thereafter, they were examined for fungal growth under a stereomicroscope with magnification ranging from 6 to 50 times. Fungal identification was based on cultural growth characteristics, spore shapes and type of fruiting bodies as viewed under a compound microscope (Agarwal *et al.*, 1989; Burgess *et al.*, 1994; Mathur and Kongsdal, 1994). Infection levels were recorded as the percentage infected seeds in each sample.

Germination test

The germination test was done using the paper roll method. Two sheets of square blotters (AGF 725-230 x 265 mm) were wetted using distilled water, leaving an adequate margin. One-hundred seeds were placed evenly on the wet blotter and rolled. For each sample, a total of 400 seeds were used for the test. The rolls were placed upright inside a plastic bag to avoid drying and were incubated at 28°C, with alternating 12 h NUV light and 12 h darkness for fourteen days. On the 14^{th} day, normal, abnormal, diseased seedling and dead seeds were counted and placed in separate plastic petri- dishes. Fungal species occurrence on abnormal seedlings and decayed seeds was subsequently assessed and their infection levels recorded.

Results and discussions

Occurrence of different fungal species

The different fungal species detected and their corresponding infection levels are presented in Table 1. Twenty different fungal species were identified, but their incidence and infection levels varied among seed samples. *Bipolaris oryzae* was the most common pathogen encountered (100%) followed by *Phoma oryzae* (94.1%). The level of seed infection by *B. oryzae* ranged from 3 – 94% while that of *Phoma oryzae* ranged from 2-57%. Results of the present study suggests that *B. oryzae* is the most widely distributed fungi followed by *Phoma oryzae*.

The least encountered species were *Microchium oryzae, Cercospora janseana, Curvularia trifolli, Phaetrichoconis crotalariae* and *Curvularia eragrostidis* (Table1). Although *Fusarium moniliforme* was encountered in 85% of the samples, its range of infection was relatively low compared to *Curvularia lunnata* which was encountered in 73.5% of the samples assayed with up to 48% seed infection level (Table 1). Similar observations were made on cowpea *(Vigna unguiculata)* (Nabaka, 1997) and Sesame *(Sesamum indicum)* (Mudingotto, 2002). These findings suggest that *Fusarium moniliforme* is widely adapted to a range of environments as opposed to *Curvularia lunnata* but less devastating than the latter. Besides the pathogenic fungi detected on the seed samples, a number of saprophytes were also detected. The most common saprophytes were *Aspergillus* sp., *Cladosporium* sp., and *Rhizopus* sp. Seed samples collected from Pallisa district had the highest incidence of storage fungi, but interestingly with the least incidence of seed-borne fungi.

Geographical distribution of the fungal species

The distribution of the different fungal species encountered and their mean percentage incidences are presented in Table 2. Significant variations (P<0.01) with respect to pathogenic infection and fungal diversity were observed in different districts (Table 2). *Bipolaris oryzae, Phoma oryzae, Pyricularia oryzae, Alternaria padwickii, Fusarium moniliforme, Curvularia lunnata, Nigrospora oryzae, Verticilium cinabarium* and *Alternaria alternata* were present in all the districts. *Exserohilum rostratum* and *Curvularia eragrostidis* were only encountered in Lira and Bugiri districts, respectively while *Microdochium oryzae* was encountered in Lira and Bugiri districts but not in Pallisa. Pallisa district had the least number of fungal species detected while Bugiri registered the greatest fungal diversity. Mean infections were likewise low in Pallisa as opposed to Bugiri and Lira districts (Table 2).

The high *B. oryzae* incidence could be attributed to poor soils used for rice production since the pathogen is prevalent in crop produced in nutrient deficient or unfavorable soil conditions. Brown spot caused by *B. oryzae* serves as an index of poor soil conditions for rice production, hence, the name 'poor man's' disease (Ou, 1985; Webster and Gunnell, 1992). Variations in the distribution of the fungal species in seed samples among districts and within districts probably indicates the importance of these pathogens in these areas. The difference in their distribution probably suggests differences in climatic and cropping systems. Rice diseases increase as the scale of production increases. This may account for the greater diversity and high incidences of fungal species in Bugiri and Lira as opposed to Pallisa which has a short history of rice cultivation.

Table 1. Seed-borne fungi detected by the blotter method on rice seed samples from different regions of Uganda.

Fungi	% sample infection	Range of infection level (%)	Mean infection Level (%)
Bipolaris oryzae	100.0	3 - 94	26.1
Phoma oryzae	94.1	2 - 57	14.4
Pyricularia oryzae	76.5	1 - 41	9.6
Fusarium moniliforme	85.0	1 - 29	9.8
Curvularia lunnata	73.5	1 - 48	6.6
Alternaria padwickii	47.1	1 - 42	3.4
Nigrospora oryzae	35.0	1 - 9	0.9
Fusarium solani	11.8	1 - 3	0.2
Cercospora janseana	2.9	1 - 3	0.2
Verticilium cinabarium	11.8	1 - 6	0.9
Exserohilum rostratum	8.8	1 - 2	0.1
Melanospora zaminae	6.0	1 -25	0.5
Curvularia inaequalis	17.6	0 - 2	1.0
Sarocladium oryzae	6.0	0 - 2	0.1
Microdochium oryzae	2.9	0 - 2	0.6
Alternaria raphani	6.0	1 - 15	0.2
Alternaria alternata	6.0	1 - 27	2.4
Curvularia trifolli	2.9	1 - 8	0.1
Phaetrichoconis crotalariae	2.9	0 - 9	0.1
Curvularia eragrostidis	2.9	0 - 4	0.1

Occurrence of abnormal seedlings and associated fungal species

Nine different kinds of seedling abnormalities were observed (Table 3). The incidence of the associated fungi differed significantly with respect to the different abnormalities. Seedlings with decay in shoot and root were the most frequently encountered while those with short main root were least encountered. More than one fungal species were found in association with the different abnormalities with the exception of 'shoot roots' which was only associated with *B. oryzae* (Table 3). *Bipolaris oryzae* was associated with all abnormalities and was more prevalent in seeds that had decayed shoots and roots. *Fusarium moniliforme*, *Curvularia lunnata*, *Alternaria padwickii*, *Phoma oryzae*, *Pyricularia oryzae*, *Melanospora zaminae* and *Alternaria alternata* were associated with seven, six, six, five, four, one and one out of nine seedling abnormalities, respectively (Table 3). The association of *B. oryzae* with all abnormalities encountered demonstrates the importance of this pathogen on rice. Although Gaungly (1947) thought that the seed-borne infection of this fungus was of little importance, Singh *et al.* (1979), Herrrera and Seidel (1978) and Aluko (1970) observed up to 29%, 66% and 29% reduction in germination as a result of *B. oryzae*, respectively. Similar observations were made by Kulkarni *et al.* (1980) in India and Kulik (1977) in the U.S.A. *Bipolaris oryzae* manifests itself as seedling blight or as a foliar and glume disease of mature plants (Webster and Gunnell, 1992).

The highest percentage of decayed seed was caused by *Fusarium moniliforme* (Table 3). Similar observations have been reported elsewhere (Islam *et al.*, 2000). Other pathogens such as *Alternaria*

Table 2. Occurrence of seed-borne fungi and their mean infection levels on rice seed samples collected from different regions of Uganda.

Fungi	Lira		Bugiri		Pallisa	
	% freq*	% mean infection	% freq*	% mean infection	% freq*	% mean* infection
Bipolaris oryzae	100.0	33.7	100.0	33.6	100.0	11.1
Phoma oryzae	100.0	16.0	90.9	22.4	75.0	4.7
Pyricularia oryzae	64.5	8.4	81.8	20.0	25.0	0.5
Fusarium moniliforme	71.0	6.1	72.7	7.8	87.5	15.4
Curvularia lunnata	54.8	6.9	81.8	12.8	12.5	0.1
Alternaria padwickii	48.4	2.7	63.6	6.5	18.2	1.0
Nigrospora oryzae	19.4	0.2	54.5	1.8	18.2	0.6
Fusarium solani	3.2	0.1	27.3	0.5	0.0	0.0
Cercospora janseana	3.2	0.1	27.3	0.6	0.0	0.0
Verticilium cinabarium	9.8	0.3	36.4	1.5	12.5	0.8
Exerohilium rostratum	9.8	0.3	0.0	0.0	0.0	0.0
Melanospora zaminae	0.0	0.0	18.2	1.4	0.0	0.0
Curvularia inaequalis	0.0	0.0	9.0	3.1	0.0	0.0
Sarocladium oryzae	3.2	0.1	0.0	0.0	0.0	0.0
Microdochium oryzae	3.2	0.1	0.0	0.0	25.0	1.8
Alternaria raphani	6.4	0.1	9.1	0.4	0.0	0.0
Alternaria alternata	32.3	1.1	36.4	5.3	27.3	0.9
Curvularia trifolli	0.0	0.0	9.1	0.2	0.0	0.0
Phaetrichoconis crotalariae	0.0	0.0	9.1	0.2	0.0	0.0
Curvularia eragrostidis	0.0	0.0	9.1	0.2	0.0	0.0
$LSD_{(0.05)}$		0.9		2.4		1.3

*Percentage samples infected with a particular fungus out of the total number of samples collected per district; Lira, n = 32, Bugiri, n = 11, Pallisa, n = 27.

Table 3. Mean incidence of the different fungi and associated seedling abnormalities.

Abnormality	Mean fungal infection (%)								
	Bip ory	Cuv lun	Fus mon	Pyr ory	Alt pad	Pho ory	Mel ory	Alt alt	LSD (0.05)
Decay in shoot and root	11.0	1.0	2.1	0.6	1.9	1.6	0.0	0.0	2.3
No roots	0.1	0.0	0.3	3.7	0.0	0.0	0.0	0.0	1.2
No root, decay in shoot	6.1	0.5	1.2	0.8	2.1	0.4	0.0	0.0	1.7
Coiled or twisted shoot	1.6	0.4	0.2	0.0	0.0	0.1	0.0	0.0	0.4
Short roots	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NS
No secondary roots	0.64	0.0	0.0	0.1	0.5	0.0	0.0	0.0	NS
Spindly or watery shoot	5.4	0.7	0.2	0.0	0.9	0.0	0.0	0.0	1.7
Decayed seed	2.5	0.7	5.8	0.0	0.3	1.9	0.3	0.2	1.9
Basal shoot decay	6.0	0.45	1.6	0.0	1.1	1.8	0.0	0.0	1.6

Bip ory; *Bipolaris oryzae*, Cuv lun; *Curvularia lunnata*, Fus mon; *Fusarium moniliforme*, Pyr ory; *Pyricularia oryzae*, Pho ory; *Phoma oryzae*, Mel ory; *Melanospora zaminae*, Alt alt; *Alternaria alternata:*

padwickii, Curvularia spp., *B. oryzae, A. alternata, Phoma oryzae* and *Melanospora zaminae* were also associated with seed decay (Table 3). Seed infection by Fusarium species reduces the percentages of the normal seedlings, the extent of damage depending on the degree of infection of the individual grains (Tempe, 1964). *Fusarium moniliforme* on the other hand, covers the entire seed causing its failure to germinate while infected seedlings develop necrotic lesions on roots and die before or after transplanting (Misra *et al.*, 1994). Similarly, Ou (1985) reported seedling death due to *Alternaria padwickii*.

The present investigation has shown that a wide range of seed-borne pathogenic fungal species are endemic in the major rice growing areas of Uganda with *Bipolaris oryzae* being the most prevalent. Occurrence of these pathogens varies with location. The occurrence of such a wide range of seed-borne fungi suggests that environmental conditions in the rice growing areas of Uganda favours the growth, survival and manifestation of these pathogens on rice. This seems to be excerbated by the use of infested seed season after season since majority of the farmers rely on their own saved seed. The study also established the association of different seedling abnormalities and seed decay with the different fungal pathogens. According to the study, *Bipolaris oryzae*, and *Fusarium moniliforme* are the two most devastating pathogens with respect to seedling abnormalities and seed decay.

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