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Seed-borne fungi associated with cowpea and rice seed and their possible control by seed sorting

C. Kanobe, G. Kawube, M. Biruma, P. J. Mudingotto, R. Edema, P. Okori, G. Tusiime, S. B. Mathur† and E. Adipala
Crop Science Department, Makerere University, P.O. Box 7062, Kampala, Uganda
†Danish Government Institute of Seed Pathology for Developing Countries (DGISP), Freeriksberg, DK-1871, Copenhagen, Denmark

Abstract

The objective of the study was to examine the effectiveness of different seed sorting techniques for improving seed health of cowpea and rice seed. Two separate trials were conducted. In each trial, a sample of 1,400 seeds was used from which sets of 200 seeds were each sorted by hand, floated in water (as a standard), floated in 10% and 15% salt solutions (to separate floated and sunken seeds), dressed with Benlate (1g ai/kg), Mancozeb (2g ai/kg) (positive control) and the last set was an unsorted control. In comparison to the unsorted seeds (control), all the cowpea treatments significantly reduced the incidence of *Fusarium moniliforme, Cladosporium* sp., *Fusarium poae, Nigrospora* sp., *Phoma* sp., and *Phomopsis vexans*. Seeds floated in 15% salt solutions had significantly less fungal incidence than hand sorted seed and did not differ from the seed dressed treatments. Seeds skimmed off the three salt solutions as floated seed were all infected with *F. moniliforme* and *F.semitectum* and majority got rotten upon blotting. For rice, *Bipolaris oryzae*, *Phoma* spp, and *Pyricularia oryzae* were the predominant rice seed-borne pathogens. The lowest incidence of the seed-borne fungal pathogens was recorded in fungicide seed dressed samples followed by 15% and 10% salt floated samples. Hand sorted samples had similar incidence of fungal flora as the unsorted samples.

Key words: *Oryzae sativa*, seed-borne diseases, seed health, *Vigna unguiculata*, Uganda

Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an important legume in the tropics and sub-tropics (Purseglove, 1988). But despite its importance, cowpea yields are disappointingly low. For example, while potential cowpea yields in Uganda are estimated at about 3,000 kg ha^{-1} (Rusoke and Rubaihayo, 1992), only 400 kg ha^{-1} is obtained at farm level (Adipala *et al.*, 1997). These low yields are attributed to a complex of constraints (Adipala *et al.*, 1997), including a range of seed-borne diseases (Nakawuka *et al.*, 1997).

Similarly, diseases are the major constraint to rice (*Oryzae sativa*) production, causing up to 50–80% losses depending on the crop susceptibility, disease severity and agroecology (Raymundo, 1980). Nsemwa and Wolfhechel (1999) noted that many of the major diseases attacking rice are seed-borne and of fungal origin. These pathogens do not only lower the seed quality but also the germinability, seed emergency (Rath, 1974) and weight of the rice seed. For both cowpea and rice, majority of farmers use seeds saved from previous seasons for planting. Such seeds have impurities and are often infected with pathogens (Fujisaka *et al.*, 1993), ensuring an early parasitic association, and as such, disease outbreaks are common.

Control strategies for most crop diseases have been directed to the field grown crop, where copious quantities of chemicals and resistant cultivars have been widely used (Manandhar *et al.*, 1998; Veena *et al.*, 2000). Chemical treatment in form of seed dressing with fungicides has also been used, although less so in subsistence agriculture. Rahman and Mia (2000) found hand sorting to be effective in separating infected from healthy rice seeds, while Quazi (2001) used salt density to separate infected from healthy eggplant and tomato seeds. These techniques have not been tested for improving seed health and germinability of cowpea and rice seeds in Uganda. Therefore, the objective of this study was to evaluate different seed sorting techniques for separating diseased from healthy seeds of cowpea and rice.

Materials and methods

Seed samples used in the study were collected from farmer saved seeds from the districts of Bugiri, Pallisa and Lira (rice) and Kumi (cowpea). Varieties *Supa* and WAB 450 were used for rice while for cowpea varieties MU 93 and *Ebelat* were used. Seeds of the same variety collected from each district were bulked to form a composite sample, from which seven working samples of 400g were drawn, using the random sampling method (ISTA, 1999) for laboratory testing.

Seed Health Testing (Blotter Method) (ISTA, 1999)

Four working samples of each crop were sorted by hand (visual sorting), floatation in water, floatation in 10 and 15% salt (*Kyoga* iodized table salt) solutions and dressed with two fungicides, respectively. A non-sorted control sample was included. It was assumed that floated seeds were diseased while sunken seeds were relatively disease-free. For seed dressing, Dithane M45 (Mancozeb) and Benlate (Ridomil) were used at a rate of 2g and 1g ai. per kilogram of seed, respectively as recommended in earlier studies (Nakawuka *et al.*, 1997). From each working sample, 200 seeds were randomly selected and plated on three layers of pre-moistened blotting papers in petri-dishes and incubated for seven days under alternating cycles of 12 hours of darkness and 12 hours of near ultraviolet (NUV) light at room temperature, i.e. 23-25°C (ISTA,1999). This was done separately for floated and sunken seeds. For cowpea, ten seeds were plated while for rice twenty five seeds were plated per dish. On the eighth day, the petri-dishes were examined under a stereo followed by a compound microscope for presence of fungal growth and their identification, respectively. Fungal identification was based on "habit characteristics" and confirmation on morphological characteristics of their fruiting bodies, i.e., conidia and spores as recommended by Mathur and Kongsdal (2000). The different fungi observed were recorded and the percentage of seeds infected with the different seed-borne pathogens determined for the different sorting methods.

The data generated were subjected to analysis of variance (ANOVA) and means separated using the Least Significant Difference (LSD) test at 5% probability level (Steel *et al.*, 1997).

Results

Incidence of fungal pathogens

The most common cowpea seed-borne pathogens identified were *Fusarium moniliforme, Fusarium semitectum, Phoma lingam, Nigrospora* sp. and *Phomopsis vexans*. Other seed-borne pathogens recorded with moderate occurrence and low incidence included *Colletotrichum* sp., *Curvularia lunata, Cercospora vignicola, Macrophomina phaseolina, Hainesia lythri, Dreschleria catenaria, Pestalotia* sp., *Rhizoctonia solani, Epicoccum* sp. and *Stemphilium* sp. Besides the seed-borne pathogens, storage fungi identified included *Aspergillus flavus, Penicillim* sp., *Alternaria alternata, Aspergillus niger, Rhizopus* sp. and *Alternaria sesame* (Table 1). On rice seeds, the most encountered pathogens were *Bipolaris oryzae, Phoma* spp., *Pyricularia oryzae, Alternaria padwickii, Curvularia lunnata* and *Fusarium moniliforme*, but the pathogen incidences varied with variety. The variety *Supa* had a higher pathogen load than WAB450 (Table 2).

Effect of different seed sorting methods on seed health of cowpea and rice seed

The highest incidences of cowpea and rice seed-borne pathogens were recorded in unsorted and hand sorted seed samples as well as in samples that floated on water and on the salt solutions (Tables 3 and 4). *Fusarium moniliforme* had the highest incidence of 37.2% recorded in the unsorted cowpea samples. For most cowpea seed pathogens, 15% salt solution significantly ($P<0.05$) reduced their

Table 1. Incidence (%) of the different fungal pathogens observed on seeds of two cowpea varieties.

Fungal pathogen	Cultivar : Ebelat		Cultivar: MU 93	
	Mean Infection level (%)	Range of infection level (%)	Mean Infection level (%)	Range of infection level (%)
Fusarium moniliforme	47.5	0-95	0.0	0
Fusarium semitectum	40.5	0-81	27.0	6-54
Cladosporium sp.	34.0	0-68	16.5	0-33
Aspergillus flavus	14.0	0-28	24.5	0-60
Penicillium	12.5	0-25	64.0	0-100
Phoma lingam	10.0	0-20	3.0	0-8
Fusarium poae	7.0	0-14	4.5	0-9
Nigrospora	6.5	0-13	3.0	0-9
Phomopsis vexans	4.5	0-9	2.0	0-7
Alternaria alternata	4.0	0-8	2.5	0-37
Colletotrichum sp.	3.0	0-6	2.0	0-4
Curvularia lunata	2.5	2-5	21.0	0-42
Cercospora vignicola	2.5	0-5	0.0	0
Aspergillus niger	2.0	0-4	11.5	0-26
Rhizopus	1.5	0-3	11.0	0-22
Macrophomina phaseolina	1.5	0-3	0.0	0
Hainesia lythri	1.5	0-3	0.0	0
Dreschleria catenaria	1.0	0-2	1.0	0-2
Pestalotia	0.5	0-1	0.5	0-1
Alternaria sesami	0.0	0	0.5	0-1
Rhizoctonia solani	0.0	0	1.0	0-2
Epicoccum	0.0	0	1.5	0-1
Stemphilium	0.0	0	0.5	0-1

Table 2. Incidence (%) of different fungal pathogens observed on seeds of two rice varieties.

Pathogen	Cultivar: WAB450		Cultivar: *Supa*	
	Mean Infection level (%)	Range of infection level (%)	Mean Infection level (%)	Range of infection level (%)
Bipolaris oryzae	9.13	2.0 - 20.0	20.12	0.0 - 56.0
Phoma spp	10.75	1.0 - 32.0	19.37	0.0 - 43.0
Pyricularia oryazae	6.0	0.0 - 17.0	6.75	2.0 - 19.0
Alternaria padwickii	3.12	0.0 - 12.0	2.50	0.0 - 6.0
Curvularia lunnata	5.37	1.0 - 15.0	4.25	0.0 - 16.0
Fusarium moniliforme	2.12	0.0 - 8.0	2.62	0.0 - 6.0
Nigrospora oryzae	0.00		0.88	0.0 - 4.0
Melanospora zaminae	0.00		1.00	0.0 - 5.0
Verticilium cinabarium	0.00		0.13	0.0 - 1.0
Phaetrichoconis crotalariae	0.00		0.16	0.0 - 1.25

incidence; resulting in similar incidence levels to those from chemically treated samples except for *Fusarium semitectum* and *Curvularia lunata* that persisted even after chemical treatments. Up to 74.6 % of the cowpea seeds that floated on the different salt solutions got rotten on blotting to the extent that no pathogen could be recovered from them. However, the few that did not get disintegrated were found to be infected with *Fusarium moniliforme* and *Fusarium semitectum* (Table 5).

Hand sorting did not significantly reduce the incidence of rice seed-borne pathogens except for *Bipolaris oryzae*. But separating rice seeds using salt solutions significantly reduced their incidence. On the contrary, floatation in water increased the incidence of *Phoma* spp. in the sunken seeds (37.5%), but lowered the incidence of *Bipolaris oryzae*, *Pyricularia oryzae*, *Curvularia lunnata*, *Alternaria padwickii* and *Fusarium moniliforme*. With the exception of *Bipolaris oryzae*, 10% salt solution

Table 3. Effect of salt floatation, manual sorting and seed treatment with Benlate and Mancozeb on incidence of seed-borne fungal infections of cowpea.*

Treatment	Incidence of fungal pathogens** (%)								
	Clad	F. m	F.s	F.p	Nig	P. l	P.v	Col. sp	Curv. l
Hand sorted	20.8	28.0	17.0	5.0	4.8	4.0	3.0	2.0	11.7
Water	14.0	29.8	15.7	3.8	1.5	2.8	2.8	1.8	5.0
10% Salt	6.5	32.7	15.3	4.0	1.3	1.8	0.8	1.0	3.7
15% Salt	8.5	17.5	12.3	1.8	0.8	4.0	1.3	1.25	3.2
Mancozeb	0.0	1.5	0.0	0.5	0.0	0.0	0.0	0	1.3
Benlate	0.0	2.0	0.7	0.0	0.0	0.0	0.0	0	3.3
Unsorted / untreated	23.5	37.2	20.3	2.3	4.3	7.0	4.0	2.5	9.5
LSD $_{(0.05)}$	11.4	14.8	17.9	3.3	2.3	3.5	2.4	2.4	8.7

*Data for the salt treatments are from the sunken seeds.
**Data presented are pooled means for the two varieties (Ebelat and MU 93).
Clad = *Cladosporium* sp., F.m = *Fusarium moniliforme*., F.s = *Fusarium semitectum*., F.p = *Fusarium poae*., Nig = *Nigrospora* sp., P.l = *Phoma lingum*. P.v = *Phomopsis vexans*. Col = *Colletotrichum* sp., Curv = *Curvularia lunata*.

Table 4. Effect of salt floatation, manual sorting and seed treatment with Benlate and Mancozeb on incidence of different seed-borne fungal infections on sunken rice seeds.*

Treatments	Incidence of fungal pathogens** (%)					
	Bipolaris oryzae	*Phoma* sp.	*Pyricularia oryzae*	*Alternaria padwickii*	*Curvularia lunnata*	*Fusarium moniliforme*
Unsorted/untreated	37.5	23.0	18.0	7.0	14.0	6.5
Hand sorted	25.0	25.5	11.5	8.0	11.0	5.5
water	22.5	37.5	6.5	0.0	6.0	4.0
10% salt	12.0	12.5	3.5	2.5	2.5	1.5
15% salt	8.0	5.0	2.0	2.5	1.0	0.0
20% salt	8.0	6.0	2.0	1.5	1.5	1.5
Mancozeb	2.0	6.5	5.0	1.0	1.0	0.0
Benlate	2.0	4.5	2.5	0.0	1.5	0.0
LSD $_{(0.05)}$	6.83	7.29	7.35	4.31	5.84	2.17

*Data for the salt treatments are from the sunken seeds.
**Data presented are pooled means for the two varieties (WAB450 and Supa).

significantly reduced the incidence of all common rice seed-borne pathogens to levels similar to those of chemically treated samples. Generally, sunken seeds had lower fungal pathogen incidence compared to the floated seeds (Table 6). None of the fungicides used for seed dressing eliminated all the seed-borne pathogens.

Discussion

The majority of the seed mycoflora observed in this study were previously reported on cowpea (Nakawuka *et al.*, 1997) and rice seed (Kamwezi *et al.*, 1997; Biruma *et al.*, 2003). Thus these pathogens may be endemic in the cowpea and rice growing areas of Uganda and hence, they keep reappearing season after season.

Seed sorting is a good practice to ensure good quality seeds for planting. The higher pathogen incidences in the presumed healthy seeds sorted by hand could have been due to inability to visually identify diseased seeds in the seed samples since the pathogens are microscopic, some living in the embryo as well as the endosperm of infected seeds (Suzuki, 1930). One of the effects of seed-borne pathogens on seed is weight reduction (Rath, 1974). Thus, seeds that are heavily infected usually weigh

Table 5. Incidence (%) of fungal seed-borne and storage fungi on sunken cowpea seeds.

Pathogen	Sunken seed		Floated seeds**	
	Mean infection level (%)	Range of Infection level (%)	Mean infection level (%)	Range of infection level (%)
Fusarium semitectum	14.4	12.3- 15.7	24.5	0-60.0
Fusarium moniliforme	26.7	17.5-32.7	24.7	5-50
Cplletotrichum sp.	1.3	1-1.8	-	-
Fusarium poae	3.2	1.8-4.0	-	-
Cladosporium sp.	9.6	8.5-14	-	-
Nigrospora sp.	1.2	0.8-1.5	-	-
Phoma lingum	2.8	1.8-4.0	-	-
Curvularia	4.0	3.2-5.0	-	-
Phomopsis vexans	1.58	0.8-2.8	-	-

*Data presented are pooled means for the floatation treatments and for two varieties (*Ebelat* and MU 93).
**Floated seed means presented were from the few recovered seeds that did not get rotten.
***Not detected.

Table 6. Incidence (%) of fungal seed-borne fungi on floated and sunken rice seeds.

Seed-borne fungi	Floated seed		Sunken seed	
	Mean infection level (%)	Range of infection level (%)	Mean infection level (%)	Range of infection level (%)
Bipolaris oryzae	22.63	18.5 – 25.5	12.63	8 – 22.5
Phoma spp	39.69	18.5 – 80.25	15.25	5.0 – 37.5
Pyricularia oryazae	9.63	7.0 – 14.0	3.40	2.0 – 6.5
Alternaria padwickii	6.63	4.0 – 9.5	1.63	0.0 – 2.5
Curvularia lunnata	13.75	5.5 – 32.5	2.75	1.0 – 6.0
Fusarium moniliforme	4.0	2.0 – 6.5	1.75	0.0 – 4.0
Nigrospora oryzae	0.13	0.0 – 0.5	0.0	0.0

*Data presented are pooled means for the floatation treatments.

less than those that are healthy or less infected and the latter will sink when immersed in a solution. The inability of water to reduce seed-borne pathogens in the sunken seeds could have been due to its low density that permitted even seeds with substantial infection to sink. The high incidence of pathogens such as *Phoma* spp. in rice, *Cladosporium* sp. and *Curvularia lunata* in cowpea seeds floated in water was probably because the water increased their spread to healthy seeds. However, floatation in the salt solutions (10 and 15%) significantly reduced incidence of seed-borne pathogens in both rice and cowpea, and gave comparable results with samples in which fungicides were used (Tables 3 and 4).

The results of the salt solution sorted samples indicate that as the salt concentration increases, the proportion of the contaminated seed moving to the floated fraction increases, leaving less and less contaminated seed in the fraction that sank. Seeds that are heavily infected with seed-borne pathogens are usually lighter than healthy seeds and hence can easily be separated by increased density of the salt solution. The present results indicate that salt solutions of 10% for rice and 15% for cowpea could be used as alternatives to seed dressing with fungicides. When working with tomato and eggplant seed, Quazi (2001) reported that floatation of seed in salt solution significantly improved the seed health and germination percentage of sedimented fractions by separating the diseased (light) seed from the healthy (heavy) seed. Similarly, Mabagala (2001) reported that this technique worked with rice seeds in Tanzania. On the contrary, Mudingotto *et al.* (2002) reported that the technique was not effective in separating diseased sesame seeds from the healthy ones. This may have been due to the low salt concentrations they used (2.5 and 5%) which were unable to effectively separate the infected from healthy seed. The high *F. semitectum* and *F. moniliforme* infection levels in the cowpea seeds that floated were probably responsible for the rotting of these seeds upon blotting. These pathogens were reported to be responsible for seed and seedling rots in cowpea (Nakawuka *et al.*, 1997).

Based on the results of this study, it is necessary to integrate management of seed-borne infections into field disease control programmes for these two crops. Our results indicate that seed sorting by floatation in 15 and 10% salt solutions could be used as an on-farm technology for cowpea and rice seed health improvement before planting. It could help farmers get rid of fungal infested and dead seed as well as seeds which produce abnormal seedlings, consequently improving field establishment and yield as long as field diseases are also controlled.

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