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Efficacy of manual seed sorting methods in reduction of transmission of rice and cowpea seed-borne diseases

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Abstract The production of rice and cowpea in Uganda is increasing in acreage rather than productivity. This is attributed to a number of factors, mainly seed-borne diseases. The farmers in Uganda mainly use farm saved seeds for planting. These seed are infected with a wide range of seed-borne pathogens, which result into diseases in the field. The objective of this study was to evaluate the effectiveness of sorting rice and cowpea seed by 15% salt solution density and by hand in reduction of transmission of seed-borne diseases, improvement of germination and yield in the field. Seed dressing with mancozeb (2%) and unsorted seed were included as control. Results indicate that the treatment methods used significantly (P<0.05) improved rice and cowpea seed germination, with the highest germination recorded in seed dressed with fungicides and sorted by 15% salt solution density than in those which are hand sorted. Rice blast and brown leaf spot were recorded on rice, while on cowpea, Cercospora leaf spots (*Cercospora cruenta* and *C. canescens)*, septoria leaf spots (*S. vignae* and *S. vignicola*), brown blotch and sphaceloma scab (*Elsinoe phaseoli*) were recorded. The lowest disease incidences were recorded in plots planted with seeds sorted by salt solution (15%) density and seeds dressed with mancozeb than hand sorted seeds. Similarly, rice and cowpea yield was improved by seed dressing and 15% salt solution density, compared to hand sorted and unsorted seed. Therefore, 15% salt solution density can be used as an alternative to seed dressing in reducing transmission of rice and cowpea seed-borne diseases and improving rice germination and yield. This should be popularized among the farmers to improve rice yield in the farm.

Key word: Germination, seed-borne disease, sorting, transmission

Résumé La production de riz et de petit pois en Ouganda est en augmentation sur le plan de la surface cultivée plus qu'en termes de productivité. Ceci est attribué à un certain nombre de critères, notamment les maladies provenant des semences. Le planteur en Ouganda utilise généralement des semences récupérées et gardées au niveau de la ferme pour une nouvelle saison culturale. Ces semences sont infectées par une gamme variée d'agents pathogènes de la semence, résultant en diverses maladies au niveau du champ. L'objectif de cette étude était d'évaluer l'efficacité de la sélection de riz et petit pois par solution salée à 15% de densité et à la main, par rapport à la réduction de la transmission de maladies provenant de la semence, l'amélioration de la germination et du rendement dans le champ. Les semences par traitement au mancozeb (2%) et semences non sélectionnées étaient incluses dans le contrôle. Les résultats ont indiqué que les méthodes utilisées amélioraient significativement (P<0.05) la germination des semences de riz et petit pois, avec la germination la plus élevée étant celle avec des semences traitées aux fongicides et sélectionnées par solution salée à 15% de densité plutôt que celles qui sont formées à la main. Rice blast et brown leaf spot étaient remarqués sur le riz pendant que sur le petit-pois, Cercospora leaf spot (*Cercospora cruenta* et *C. canescens*), septoria leaf spots (*S. vigna* et *S. vignicola*), brown blotch et spatcheloma scab (*Elsinoe phaseoli*) étaient remarqués. Les incidences de maladie les plus faibles étaient enregistrées au niveau des parcelles plantées avec des semences sélectionnées par solution salée (densité 15%) et semences traitées au mancozeb par rapport aux semences sélectionnées à la main. De manière similaire, le rendement de riz et petit pois était amélioré par traitement de semence et solution salée à densité 15%, en comparaison aux semences sélectionnées à la main et non sélectionnées. Par conséquent, la solution salée à densité 15% peut être utilisée comme alternative dans le traitement de semences en vue de la réduction de la transmission des maladies provenant des semences et l'amélioration de la germination et du rendement de riz et du petit pois. Ceci devrait être rendu populaire au milieu des cultivateurs pour pouvoir améliorer le rendement de riz au niveau de la ferme.

Mots clés: Germination, maladies provenant de la semence, sélection, transmission

Introduction

Rice is a staple food for about a half of the world population (Purseglove, 1988). In Africa, it is ranked the fourth most important cereal crop after maize, sorghum and millet. Its consumption in Uganda is increasing, especially in urban areas. Its production is also increasing throughout the country. This increment is mainly attributed to expansion in area of cultivation (State of the Environment Report for Uganda, 1994) rather than increase in its productivity. The low productivity is attributed to a number of factors, important of which is seed-borne diseases, causing up to 50-80% yield losses depending on the crop susceptibility, disease severity and agro ecology (Raymundo, 1980).

Similarly cowpea is one of the most important food crops of the developing worlds, supporting farmers needs from the early seedling stages up to harvesting time. This is because it is consumed at different stages in its development, providing high quality nutrition to the farmers (IITA, 1984). In Uganda, cowpea is increasingly becoming an important cash crop (Sabiti, *et al*, 1994). However, its yield has remained low at the farmers' field (between 150-400kg/ha) compared to the potential of 2500kg/ha. This is partly due to diseases (Edema, 1995; Edema and Adipala, 1996) some of which are seed-borne. Many of these diseases are of fungal origin (Nsemwe and Wolfhechel, 1999), transmitted mainly through use of infected seeds for planting. Early studies (Biruma *et al*, 2003; Kanobe *et al*., 2004) reported a wide range of seed-borne fungal pathogens on the farmers saved rice and cowpea seeds, which they mainly relay on to propagate the next generation of crop.

High germination percentage and seedling vigour are essential in crop establishment and in ensuring a good stand in the field. However, these are usually suppressed by seed-borne micro-organisms (Mathur *et al.*, 1972; Agarwal and Sinclair, 1987). Seed-borne pathogens are reported to cause seed and seedling rots (Islam *et al.*, 2000) as well as seedling abnormalities such as twisting and splitting of epicotyl and hypocotyls, lack of or malformed leaves, extensive browning of collar regions causing seedlings to break (Nakawuka, 1996). *Fusarium moniliforme* was reported to cause necrotic lesions on roots of rice seedling that die before or after transplanting (Misra *et al.*, 1994) while Islam *et al.*, (2000) reported the same pathogen in decayed seed and seedlings that had no roots. In Uganda, Nakawuka (1996) reported reduced germination of up to 40% in untreated seeds that was attributed to seed-borne pathogens while Nabakka, (1997) reported germination reduction of up to 59.3% as a result of *Macrophomina phaseolina* infection in cowpea seeds. Considerable loss in seed viability, seed germination (leading to sub optimal plant population per unit area), reduced vigour of the surviving plants (Roberts, 1972; Islam *et al*., 2000) and transmission of diseases to the field (Mia, 2001) prevail.

Control of rice seed-borne diseases has mainly been by use of chemical sprays in the field. However, its application is environmentally unfriendly, expensive and requires technical expertise which is lacking in most of the Ugandan farmers. Therefore, it is imperative that before planting, seed is sorted to minimize the level of seed-borne pathogens carried to the field. Sorting rice and cowpea seed by salt solution density has been proved in the laboratory to be a better method than hand sorting in reducing the incidence of seed-borne fungal pathogens in the seed (Kanobe *et al*., 2004). However, since not all infected seeds give rice to infected seedlings in the field (Richardson, 1990), there is a need to evaluate these methods in the field. Therefore, the objective of this study was to evaluate the effectiveness of sorting seed by salt solution density and by hand in reducing transmission of seed-borne diseases to the field, increase in germination and yield.

Methodology

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 WAB450 and Ebelat were used for rice and cowpea, respectively. Seed samples (for each crop) collected from each district were bulked to form a composite sample, from which four working samples of 400g were drawn, using random sampling method (ISTA, 1999). These were subjected to the following treatments; 1) seed sorting by hand where the diseased, shriveled, broken and mould seeds (presumed diseased seeds) were removed, leaving the healthy looking ones 2) seed sorting by salt solution density (15% levels); where seeds were put in a beaker containing 15% salt solution. The seeds that sunk were presumed to be healthy, hence used in the treatment, while those that floated were regarded to be infected, hence were not used in the study. Seeds dressed with mancozeb (2% ai/kg) and unsorted seed samples were included as positive and negative controls, respectively.

Experimental setup. Land preparation was done using a disc plough followed by hand hoe to reduce the big clods and leveling the field. Rice seeds were row planted in a plot measuring 4m x 4 m. The spacing adopted was 30 cm x 5 cm as described by Imanywoha (2001). One seed was planted per hole using a small hand hoe. The experimental design was a Randomised Complete Block Design (RCBD) with three replicates. No pesticides were applied to the rice fields.

While for cowpea, seeds (as per treatment) were planted in the plots of size 4 m x 5 m in a randomized complete block design (RCBD) and replicated five times. The spacing adopted was 20 cm x 90 cm as described by (Orawu, *et al.*, 2001). Two seeds were planted per hole to ensure an equal number of seeds planted per plot. Field pests were controlled using Cypermethrin (Ambush) at a rate of 30ml per 20 liters of water, applied at budding, flowering and podding stages (Opolot, 2003). The fields were hand weeded three times during the growth period to ensure weed free conditions.

Data collection and analysis. Data collected included germination, disease incidences and grain yield.

Germination. Seed germination was assessed at one month after planting (MAP) for rice, while for cowpea it was assessed eight days after planting. This was done to take care of the irregularity in the rate of germination of the seeds. Germination percentage (%) was assessed by counting the number of seedlings on the two inner rows of each plot expressed as percentage of the total seedlings expected in the two rows.

Disease incidences. Disease assessment was carried out on all the plants that had symptoms in the two rows that were earlier on used germination test. This was done at four different times corresponding to the following rice plant stages, namely one MAP, at the beginning of flowering, 50%, flowering and physiological maturity. This was done to capture seedling and adult plant diseases. While for cowpea, seed-borne disease incidence was determined at physiological maturity (3 months after planting). In cases where the disease symptoms were not clear, the infected parts were plucked and taken for laboratory analysis where they were cut into pieces and put on moist blotters (in a clean Petri dishes) and incubated for three days under alternating cycles of darkness and near ultraviolet light before observing under stereomicroscope for fungal growth characteristics.

Yield. An area of 12 m^2 was marked out in the field (excluding boundary lines) from which the yield was harvested. The harvested rice and cowpea grains per plot were put in the polythene bags and taken for sun drying. This was followed by threshed to separate the grains from trash. At the end of the season all the rice and cowpea grain yield from each plot was put separately, weighed and recorded in grams. The results were then extrapolated to tones per hectare.

Data analysis. All data collected were subjected to analysis of variance (ANOVA), to test for the significance of the treatment methods using Genstat computer package. Where ANOVA showed significant differences, the means were separated by Fishers Protected Least Significant Difference (LSD) at 5% probability levels.

Results

Effect of different seed sorting methods on germination of rice and cowpea seed. Generally treatment methods significantly ($P<0.05$) influenced the germination of rice and cowpea seed, with the highest percentage recorded in plots planted with seeds dressed with mancozeb, followed by those sorted by 15% salt solution density, while the least germination was recorded in unsorted seeds, which was significantly different from that achieved by hand sorting for rice (Table 1), unlike cowpea (Table 2). Similarly the germination achieved by seed dressing and seed sorting of 15% was not significantly different from each other.

Table 1. Effect of seed sorting methods and dressing on field germination and yield of rice.

Treatment	Germination (%)	Yield (t/ha)
Unsorted	45.3	0.406
Hand	68.4	0.499
15% salt	81.3	0.743
Mancozeb	82.9	0.493
$LSD_{0.05}$	19.5	0.046

Table 2. Effect of seed sorting methods and dressing on field germination and yield of cowpea.

Treatment	Germination (%)	Yield (t/ha)
Unsorted	52.9	0.70
Hand	58.95	0.85
15% salt	72.25	1.25
Mancozeb	75.3	1.6
$LSD_{0.05}$	8.45	0.3

Effect of different seed sorting methods on the disease incidence on rice and cowpea. There were two and four seed-borne diseases recorded on rice and cowpea fields, respectively. The seed-borne diseases observed on rice plants included rice blast and brown spot, with the former having the highest incidence. Cercospora leaf spots (*Cercospora cruenta* and *C. canescens)*, septoria leaf spots (*S. vignae* and *S. vignicola*), brown blotch and sphaceloma scab (*Elsinoe phaseoli*) were recorded on cowpea with their incidence in a descending order. The treatments significantly ($P<0.05$) influenced the occurrence of the different seed-borne diseases on both crops except for sphaceloma scab.

The highest incidence of the rice diseases (rice blast and brown spot) was recorded from plots planted with unsorted rice seeds, followed by plots planted with rice seeds sorted by hand, while the least disease incidences were recorded in plots planted with seeds dressed with mancozeb, and seeds sorted by salt solution density of 15% (Table 3).

For cowpea, the highest disease incidence were cersospora leaf spots, septoria leaf spots and brown blotch, recorded in the plots planted with unsorted seed while the highest scab incidence was recorded in both plots planted with the unsorted (control) and hand sorted seed samples. These were followed by hand sorted and 15% salt solution floated treatments while mancozeb treated sample plots had the least disease incidences (Table 4).

Cercospora leaf spot incidence on the unsorted sample treatments were significantly different from hand sorted, 15% salt solution density and chemical treatments, while

Table 3. Effect of seed sorting methods and dressing on seed-borne disease incidence of rice.

Treatment	Blast	Brown spot
Unsorted	78.9	38.9
Hand sorting	51.1	32.2
15% salt	25.5	13.3
Mancozeb	32.2	11.1
$LSD_{0.05}$	14.5	16.8

Table 4. Effect of seed sorting methods and dressing on seed-borne disease incidence of cowpea.

Treatment	Field disease incidence (%)			
	Cercos*	Sept*.	Bb*	Scab
Unsorted	9.0	6.9	5.6	0.8
Hand	5.8	4.7	4.4	0.8
15% salt	2.8	2.9	2.0	0.6
Mancozeb	2.0	1.5	1.3	0.6
$LSD_{0.05}$	2.7	1.4	1.8	0.7

*Cercos= Cercospora leaf spots., Sept= Septoria leaf spot., Bb= Brown blotch.

those of 15% salt solution density treatment were not significantly different from the chemically treated sample. Brown blotch disease incidence in the unsorted and hand sorted treatments were not significantly different from each other but were significantly different from 15% salt solution density and chemical treatment. However, the later were not significantly different from each other. Generally, sphaceloma scab recorded the least disease incidence among all the treatments. This incidence was not significantly different for all the treatments (Table 4).

Effect of different seed sorting methods on rice and cowpea yield. There were significant differences (P<0.05) in yield among the treatments. The highest rice grain yield was obtained from plots planted with seed samples sorted by 15% salt solution density, while the lowest was obtained from plots planted with unsorted rice seed samples (Table 1). The rice grain yield recorded from plots planted with seeds sorted by hand and that dressed with mancozed (contact fungicide) were intermediate, and not significantly higher than that obtained from plots planted with unsorted seed samples.

For cowpea, plots planted wih mancozeb treated seed samples recorded the highest yield, followed by those planted with seeds sorted by 15% salt soluition density and hand sorted seeds. the lowest yield was recorded from plots planted with unsorted cowpea seed (Table 2). Yield from plots planted with unsorted seed samples were not significantly lower than that from plots planted with hand sorted seed sample, but significantly lower than that from plots planted with seeds sorted by 15% salt solution density. Yield from plots planted with mancozeb dressed seed was significantly higher than that obtained from plots planted with seed samples sorted by 15% salt solution density (Table 2).

Discussion

Chemically treated seed samples had germination percentage higher than all other treatments. This could have resulted from the ability of the chemical to eliminate most of the seed-borne pathogens from the seed. Similar findings were reported by Mundigotto *et al.* (2002) and Nakawuka (1996) on sesame and cowpea seeds, respectively.

One of the effects of seed-borne pathogens on seed is weight reduction (Rath, 1974). The high salt solution density used contributed to the high germination percentage of the salt-solution sorted samples by reducing the number of infected and presumably lighter seeds in the heavier and presumably healthy seed that sunk upon separation. From related studies, salt solution density sorting was reported to have improved the germination percentage of rice (Mabagala, 2001) in Tanzania as well as eggplant and tomato seeds (Quazi, 2001) in Bangladesh.

Seed-borne pathogens such as *Fusarium* sp. are associated with discolored, shriveled and moldy seeds (Nakawuka *et al.*, 1997). Therefore, seeds with these characteristics were easy to identify and pick out during hand sorting. There is also a tendency during hand sorting to pick out the small seeds, leaving only the larger ones. These large seeds are possibly well filled, with high content of food reserves such that even if the seed is infected with seed-borne pathogens, can grow vigorously and counteract the effect of the pathogens on the seedling. These factors could have caused higher germination percentage of hand sorted seed samples compared with the unsorted one. When working on rice, Islam *et al.,* (2000) reported that physically good-looking seeds had higher germination percentage and seedling vigour than the unsorted control and could therefore be used for better germination and seedling growth than unsorted ones. However, the level of infection cannot be easily determined by unaided eye since the pathogens are microscopic, with some infecting the inner parts of the seed. This explains why there was lower germination in hand sorted seed compared to those sorted by salt solution density and that dressed with chemicals.

The results also clearly indicate that seed-borne disease occurrence was very low compared to the infection levels of the seed reported by Biruma *et al*., 2003 and Kanobe *et al*., 2004. Two seed-borne diseases i.e. rice blast and brown leaf spot were recorded on rice while on on cowpea four seed-borne diseases i.e cersopora leaf spot, septoria leaf spot, brown blotch and sphaceloma scab were recorded. One of the major effects of seed-borne pathogens on seed germination is seed and seedling death (Sinha and Khare, 1977). Therefore, the low incidence of seed-borne diseases in the field could have resulted from failure of the infected seeds to geminate and some of the germinated ones die due to seedling blight.

High germination percentage and seedling vigour are essential in crop establishment and in ensuring a good stand in the field (Mathur *et al.*, 1972; Agarwal and Sinclair, 1987).and consequently higher yield. This could account for the higher yields obtained in the plots planted with seeds dressed with fungicide. The lower yield in mancozeb treated seed for rice, is probably because the rice seed sample used was severely infected before the treatments were applied to the extent that even with chemical dressing, weaker seedlings emerged, hence low yield. Unlike in cowpea where the infection could have been moderate, hence relatively stronger seedlings emerged that gave higher yields. The high growth vigour of the rice seed sorted by 15% salt solution density could have resulted into higher production of tillers, which might have translated into higher yield.

Conclusion

From the foregoing, seed sorting methods significantly influenced field germination, occurrence of the seed-borne diseases and yield of rice and cowpea in the field. The results have not only stressed further the importance of seed health in realizing the full potential of rice and cowpea in Uganda as earlier reported Nabakka, (1996), but also demonstrated the efficacy and prospect of simple seed sorting techniques such as 15% salt solution density sorting in controlling seed-borne infections of rice and cowpea and increasing yield. Although seed treatment offers the most effective options of preventing seed to field transmission of seed-borne diseases, use of seeds sorted by 15% salt solution density could be used as an alternative to the former since all parameters assessed for the two treatments were not significantly different on both crops, except for rice yield where 15% salt solution density resulted into the highest. In view of this, it is important to disseminate seed sorting by 15% salt solution density technology to the resource poor rice and cowpea farmers in Uganda to enable them increase their yields and consequently their incomes by controlling the seed-borne pathogens.

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