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On-farm evaluation of fungicide spray options to control potato late blight in South-western Uganda

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Abstract

Production of potato (*Solanum tuberosum* L) in the highlands of Eastern Africa is increasingly being threatened by devastating epidemics of late blight (*Phytophthora infestans* (Mont.) de Bary). Use of fungicide sprays is a feasible control strategy against this epidemic but appropriate spray intervals during crop growth have not been determined. An on-farm experiment was therefore, conducted to establish the appropriate spray interval for a contact (Dithane M45) fungicide in Uganda. Results showed that during the blight-favourable seasons yields were higher with 7-day spray interval than 14 and 21 day but economic analysis revealed that 2 and 3 sprays of Dithane M45 on tolerant and susceptible cultivars, respectively resulted into optimal yields. Overall, monitoring disease and then spraying (2 sprays) contained late blight severity to levels that yielded the most economic benefit.

Key words: Economic returns, host resistance, Mancozeb, *Phytophthora infestans*, *Solanum tuberosum*

Introduction

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is the most important constraint to potato production in the highlands of Eastern Africa (Hakiza, 1999; Olanya *et al.*, 2000). During the last 20 years, this disease has increased globally (Fry and Goodwin, 1997). Yield losses due to late blight damage are estimated at 30-40% in years of intermediate disease severity but up to 80% in the years of an epiphytotic (Anoshenko, 1999). In Uganda late blight is considered the most important biotic constraint to potato production, and total crop failure is not uncommon (Adipala, 1999). The situation is aggravated by the fact that the farmers are resource-poor, with limited capacity to control the disease. Moreover, there is continuous supply of inoculum due to all year round cultivation of the crop coupled with favorable weather (Kankwatsa *et al.*, 2002). Also, production in many of these areas is characterised by dependence on local cultivars, absence of elite late blight resistant clones, and lack of information on more economic late blight management practices. As such, some farmers attempt to control the disease with numerous fungicide applications, but this increases cost of production and has environmental and health hazards. Thus, development of an economically viable and sustainable integrated disease management (IDM) option is considered key to management of late blight in sub-Saharan Africa (Olanya *et al.*, 2000). Also, although host resistance is central to disease management, fungicide application is needed to enhance host resistance and achieve high economic returns. Determination of an appropriate spray schedule can aid to achieve more efficient use of fungicides (Mackenzie, 1981; Niklaus and Fry, 1999). Thus, the objectives of the study was to identify appropriate fungicide spray schedules for potato cultivars with different levels of tolerance to late blight.

net benefit (i.e., the change in net benefits) divided by the marginal cost (i.e., the change in costs) expressed as a percentage was computed to evaluate changes from one technology to another by expressing the relationship of total variable costs against the net benefits.

Results

Mean monthly rainfall averaged 9.9 mm, 3.5 mm and 5.4 mm for the 1999B; 2000A and 2000B seasons, respectively. Minimum and maximum temperatures averaged 10.4° C and 21.3° C, respectively, while relative humidity averaged 87%, 86% and 85% for the 1999B, 2000A and 2000B seasons. These conditions coupled with the different levels of fungicide application resulted in varied levels of late blight.

Effect of fungicide treatments on late blight severity

Late blight attacked all cultivars irrespective of the fungicide application regime. There were significant (P = 0.05) 2-way interactive effects of cultivar and fungicide spray regimes, indicating that the level of late blight control depended on both cultivar tolerance to late blight and spray regime (Fig. 1). The sprayed plots had less disease severity than the unsprayed, and weekly (high spray regime) had the lowest disease severity compared to 14 day, 21 day and monitored (2) spray options on all cultivars. Also disease progress on the unsprayed Kisoro and Victoria (susceptible) were significantly higher than on Rutuku and Nakpot 3 (tolerant) cultivars. The unsprayed potato clearly show that there

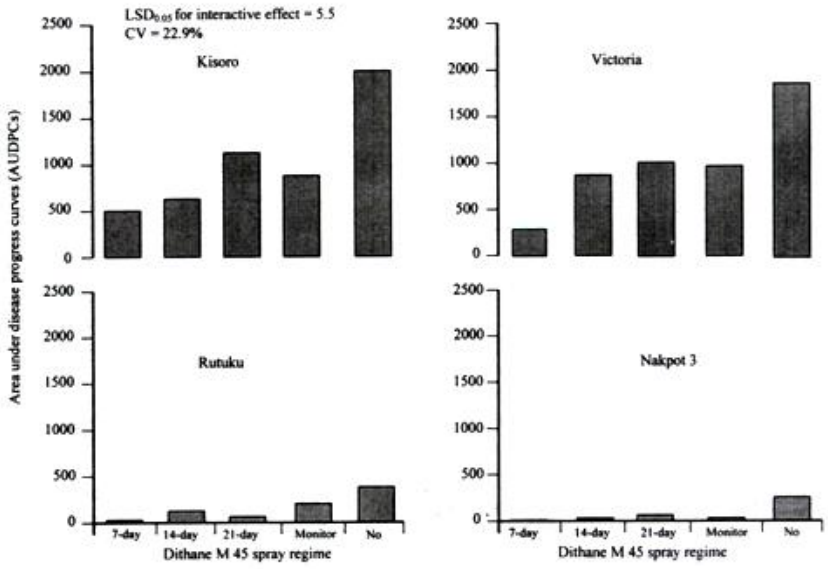

Figure 1. Late blight severity (AUDPCs) on four cultivars with varying levels of resistance under different fungicide spray schedules. Only two sprays were applied in the monitored treatment, the first and second at 10 and 31 days after crop emergence; the 7, 14 and 21- day spray intervals resulted in 12, 6 and 3 sprays, respectively.

was significant difference between tolerant and susceptible cultivars, with mean AUDPCs of 303 and 151 on Rutuku and Nakpot 3, compared to 1990 and 1877 on Kisoro and Victoria respectively.

Effects of fungicide spray interval on tuber yield

Table 1 shows that all the 7-day fungicide spray interval treatments resulted in the highest tuber yield as compared to yields accruing from 14 day, 21 day and monitored (2) spray schedules. The results also indicate that there were significant (P = 0.05) differences in yield losses between sprayed and unsprayed plots but the extent of loss depended on fungicide-spray interval and varietal susceptibility. Yields obtained from the 21-day and monitored spray schedules (both sprayed twice) were similar. Although higher tuber yields were obtained from tolerant cultivars (Rutuku and Nakpot 3), fungicide application increased their yields considerably. Also, although the unsprayed plots of resistant cultivars Rutuku and Nakpot 3 recorded relatively high yields, the magnitude of yield addition due to increase in the number of fungicide spray applications was lower than that of susceptible cultivars such as Victoria. Furthermore, without fungicide usage, a farmer recorded over 90% tuber loss on a susceptible (Kisoro) compared to only 40% on a tolerant (Rutuku) cultivar (values computed from Table 1).

Table 1. Effect of fungicide application on tuber yield of four cultivars with varying levels of tolerance to late blight in four on-farm sites.

Variety	Treatment	Tuber yield (mt ha^{-1})				
		Nyamiyaga	Butego	Rusyekye	Kataraga	Mean t ha^{-1}
Kisoro	7 day	13.8	24.0	17.7	18.4	18.5
	14 day	14.4	18.0	12.7	15.3	15.1
	21 day	9.3	11.0	12.3	21.0	13.4
	Monitoring	9.7	14.0	13.0	12.0	12.2
	No spray	3.8	0.7	2.0	1.9	2.2
Victoria	7 day	14.9	32.3	21.0	22.6	22.7
	14 day	13.7	18.5	18.4	17.0	16.9
	21 day	7.4	13.9	14.5	11.8	11.9
	Monitoring	7.7	14.8	17.8	13.3	13.4
	No spray	3.1	1.7	3.0	2.6	2.6
Rutuku	7 day	17.4	30.4	12.1	18.1	19.5
	14 day	16.1	29.2	9.8	20.1	18.8
	21 day	15.9	25.0	8.9	13.6	16.6
	Monitoring	15.3	25.3	11.9	17.5	17.5
	No spray	7.2	9.6	9.0	5.4	8.3
Nakpot 3	7 day	10.7	22.7	16.7	14.7	16.2
	14 day	9.7	16.9	15.2	15.8	14.4
	21 day	12.5	21.1	11.8	14.6	15.0
	Monitoring	11.0	21.0	15.5	15.7	15.8
	No spray	9.4	10.6	8.5	10.7	9.8
LSD0.05		3.9	6.1	6.4	5.6	5.5
CV=%		20.5	18.2	30.1	19.5	22.9

Pooled data for three seasons.

Economics of fungicide sprays on potato

The cost of each spray varied with the stage of crop growth, i.e., during the early (emergence to vegetative), middle (premodia to flowering) and late (pre- senescence) crop growth stages. The number of sprays averaged 6, 3, 2, and zero for the weekly, fortnightly, monitored and unsprayed treatment, respectively. During the late blight favourable seasons the average variable cost of these spray regimes were US$ 62.3, 62.3, 56.8 and 0 for the weekly, fortnightly, monitored and host resistance (no spray) schedules, respectively .

To determine the economic viability of these spray regimes, an economic assessment was conducted. This evaluation was done using the farm gate prices of 100-kg bag, which is the standard measure of marketing ware potato at farm level in the study area. There was variation in price according to variety, Kisoro, Victoria and Nakpot 3 were sold at an average price of Ug.Sh.100,000 (US$ 56), 388575.5 at Ug.Sh.108,000 (US$ 60) and Rutuku at Ug.Sh.110,000 (US$ 61) per tonne (10 x 100 kg bag). The cost of individual spray varied according to a particular growth stage, i.e., Ug.Sh.79,560 (US$ 44,2), Ug.Sh.124,920 (US$ 69.4) and Ug.Sh.131,940 (US$ 73.3) for early, flowering and post-flowering stages, respectively. Thus, the total average cost of late blight control using a contact fungicide in a late blight favourable season was Ug.Sh. 672,840 (US$ 373.8), Ug.Sh.336,420 (US$ 186.9), Ug.Sh.204,480 (US$ 113.6) for the 7-day (6 sprays), 14-day (3 sprays) and monitored (2 sprays), respectively.

Partial budgets

Tables 2 and 3 show that it is profitable to spray against late blight but benefit depends on the potato variety (level of resistance). Partial budgets analyses indicate that the spray regimes (number of fungicide sprays) affected the value of inputs associated with the experimental variables with weekly spray option resulting in the highest total variable costs, in comparison to the fortnightly, tri-weekly and monitored sprays, respectively, and no variable costs for the unsprayed plots. Even though the tri-weekly and monitored spray schedules resulted in 2 sprays each, spraying when necessary (monitoring) resulted in lower total variable costs and higher net benefits. Although the gross benefits for varieties Kisoro, Rutuku and Nakpot 3 were higher when the crop was sprayed weekly, the net benefits were lower when the crop was sprayed following the fortnightly and monitored spray schedules. Whereas net benefits continued to increase after 2 sprays on susceptible cultivars such as Victoria, in the cases of tolerant varieties e.g., Rutuku, beyond 2 sprays the net benefits declined. The low net benefit from no spray control confirmed that use of host resistance alone may not be adequate. Surprisingly, the net benefits accruing from the unsprayed resistant Nakpot 3 cultivar were higher than the weekly sprayed plots.

Dominance analysis showed that sprays involving more than 2 sprays of a contact fungicide were dominated on resistant cultivars such as Rutuku and Nakpot 3 under fortnightly and weekly options. But, the same control options were not dominated on susceptible cultivars such Victoria, that is, the farmer could spray more than twice on a susceptible cultivar. Therefore, it was not economical to spray the resistant cultivars more than twice.

Net benefit and marginal analysis

Table 3 shows that spraying twice under monitored sprays resulted in the highest marginal rate of return for all cultivars and it was the only economic fungicide spray option for resistant cultivars (Rutuku and Nakpot 3). For every (US$) dollar invested in fungicide purchase and its application, farmers recovered the original dollar and approximately an additional US$ 2.5 for susceptible cultivars (Kisoro and Victoria), but only US$ 1 for resistant cultivars when the crop was sprayed following the monitoring schedule. However, when the farmer sprays susceptible cultivars thrice (fortnightly), the additional

Table 2. Partial budget for integration of host resistance and minimum fungicide use for management of late blight on four cultivars.

Cultivar	Fungicide spray regime				
	Weekly (7 sprays)	Fortnightly (3 sprays)	Tri-weekly (2 sprays)	Monitoring (2 sprays)	No spray (0 spray)
Kisoro (susceptible)					
	18.5	15.1	13.4	12.2	2.2
	16.7	14.0	12.1	11.0	2.2
	56	56	56	56	56
	44.9	44.9	44.9	44.9	44.9
	2.8	2.8	2.8	2.8	2.8
	8.3	8.3	8.3	8.3	8.3
	102	51	31	31	-
	113.2	56.6	34.4	34.4	-
	56.6	28.3	17.2	17.2	-
	102	51	31	31	-
	373.8	186.9	113.6	113.6	-
	749.8	628.6	343.3	493.9	98.8
	376	441.7	229.7	380.3	98.8
Victoria (susceptible)					
	22.7	16.9	11.9	13.4	2.6
	20.4	15.2	10.7	12.1	2.6
	56	56	56	56	56
	44.9	44.9	44.9	44.9	44.9
	2.8	2.8	2.8	2.8	2.8
	8.3	8.3	8.3	8.3	8.3
	102	51	31	31	-
	113.2	56.6	34.4	34.4	-
	56.6	28.3	17.2	17.2	-
	102	51	31	31	-
	373.8	186.9	113.6	113.6	-
	916	682.5	480.4	543.3	116.7
	542.2	495.6	366.8	429.7	116.7
Rutuku (resistant)					
	19.5	18.8	16.6	17.5	8.3
	17.6	16.9	14.9	15.6	8.3
	61	61	61	61	61
	49.9	49.9	49.9	49.9	49.9
	2.8	2.8	2.8	2.8	2.8
	8.3	8.3	8.3	8.3	8.3
	102	51	31	31	-
	113.2	56.6	34.4	34.4	-
	56.6	28.3	17.2	17.2	-
	102	51	31	31	-
	373.8	186.9	113.6	113.6	-
	878.2	843.3	743.5	778.4	414.2
	504.4	656.4	629.9	664.8	414.2
Napkoi 3 (resistant)					
	16.6	14.4	15.0	15.8	9.8
	14.4	13.0	13.5	14.2	9.8
	56	56	56	56	56
	44.9	44.9	44.9	44.9	44.9
	2.8	2.8	2.8	2.8	2.8
	8.3	8.3	8.3	8.3	8.3
	102	51	31	31	-
	113.2	56.6	34.4	34.4	-
	56.6	28.3	17.2	17.2	-
	102	51	31	31	-
	373.8	186.9	113.6	113.6	-
	745.3	583.7	606.2	737.6	440.0
	371.5	396.8	492.6	624	440

return obtained for every dollar drops to about US$1. Whereas the weekly spray schedule on Victoria was not eliminated in the dominance of the contact fungicide (Dithane M45) analysis, the 25% marginal rate of return shows that it was not viable either to apply 6 sprays on susceptible varieties since the marginal rate of return below 50% is considered uneconomical (CIMMYT, 1988).

Discussion

The presence of late blight in all the seasons confirms Walter (1993) assertion that the climate of Eastern Africa favours the disease all the year round. The appearance of late blight symptoms in the first 10-12 days leads to severe epidemics and 80-100% foliage damage on early maturing varieties and 70-80% for medium and late varieties (Anoshenko, 1999). Walter (1993) also observed that under favourable conditions, lesions may appear on leaves 3-5 days after infection. In this study, late blight lesions appeared on the leaves between 12 and 15 days after crop emergence on Victoria and Kabale (susceptible) and 21 to 24 days on Rutuku and Nakpot3 (tolerant) cultivars. And, 100% leaf damage was recorded on Victoria in 1999B (September to December) and 2000B (September to December) seasons. In these two seasons, rainfall averaged 9.9mm, temperature 15.6°C and relative humidity 87% in 1999B; and 5.4mm, 15.6°C and 84.3% in 2000B. These conditions were conducive for late blight epidemics.

Further, according to Harrison (1992) and Low (1997) severe late blight epidemics occur during heavy rains, presence of moisture on potato leaves extending for at least 8-10 hours for several consecutive days, cool temperatures (less than 20°C) and high relative humidity (more than 80%). Although season 2000A recorded similar average temperatures and relative humidity, rain period was short and average rainfall was less than 4 mm per day and was received mostly early in the season. Thus, the weather conditions were favourable for disease development in the early stage of plant growth but the lesions could not progress because of limited leaf wetness.

Table 3. Marginal analysis of four cultivars subjected to different fungicide spray treatments.

Variety	Treatment	TVC	NB	Change in TVC	Change in NB	MRR
Kisoro	No spray	-	98.8	-	-	-
	Monitoring	113.6	380.3	113.6	281.5	248
	Fortnightly	186.9	441.7	73.3	61.4	84
	-Weekly					
Victoria	No spray	-	193.1	-	-	-
	Monitoring	113.6	429.7	113.6	313	276
	Fortnightly	186.9	495.6	73.3	65.9	90
	Weekly	373.8	542.2	186.9	46.6	25
Rutuku	No spray	-	414.2	-	-	-
	Monitoring	113.6	664.8	113.6	250.6	220
	- Fortnightly					
	- Weekly					
Nakpot 3	No spray	-	440.0	-	-	-
	Monitoring	113.6	624.0	113.6	184.0	162
	- Fortnightly					
	- Weekly					

- Dominated values not included; TVC = Total variable costs; NB = Net benefits; MRR = Marginal Rate of Return i.e. Change in Net Benefits/ Change in Total Variable Costs x 100%.

The results, however, show that during seasons of high late blight severity, plots provided with adequate fungicide yielded well. Yield losses averaged 14.1m t ha^{-1} (51%) on Rutuku, a tolerant cultivar and 18.5 mt ha^{-1} (77%) on Kabale, a susceptible cultivar (Table 1). The yield differences between sprayed and unsprayed plots justify the necessity of fungicide application in the control of late blight. For example, the yield of Kabale (susceptible) increased from less than 5 t ha^{-1} to about 18 t ha^{-1} when sprayed weekly for six times. The importance of host resistance in controlling late blight severity was also demonstrated in the unsprayed plots. For example, the yield of unsprayed Kabale (susceptible) averaged 5 t ha^{-1} in late blight favourable seasons of 1999B and 2000B, while in case of Nakpot 3 (resistant) the yield averaged 11 t ha^{-1}. The corresponding yields for the two cultivars when sprayed twice under monitored regime were 13.5 and 15 t ha^{-1}, respectively. Thus, there was markedly reduced yield loss in resistant cultivars, as compared to the susceptible cultivar. These results justify the need to incorporate resistant varieties in late blight management. This was because resistant varieties delayed the disease development and therefore the need for first fungicide spray. After 2 monitored sprays, disease severity was controlled at about 5% as compared to more than 15% for unsprayed Rutuku (resistant) and Victoria (susceptible), respectively. However, the results also indicate that sole use of fungicide or host resistance (no spray) may not be adequate for control of late blight; greater benefit accrued where both factors were combined, which agreed with Parry (1990) finding that management of late blight requires an integrated approach.

The unsprayed plots had lower net benefits than the sprayed plots which is consistant with Neiderhauser (1999) assertion that fungicides must be used to control late blight. Hakiza (1999) also argued that fungicide application is an integral part of potato late blight management. However, the high total variable costs as a result of weekly sprays indicate that such a spray schedule is prohibitively expensive but also environmentally unfriendly (Landeo, 1993). Higher net benefits from tolerant cultivars Rutuku and Nakpot 3 showed the importance of cultivar tolerance in late blight management. The lower net benefits resulting from weekly than fortnightly spray schedules (Table 3) indicate that even on a susceptible cultivar like Kisoro weekly fungicide application is not economically viable. Contrastingly, the higher net benefits under fortnightly than monitored sprays justified spraying a susceptible cultivar like Kisoro (Table 3) thrice. Table 3 also indicates that 2 monitored sprays on a tolerant cultivar such as Nakpot 3 result in the highest net benefits which agrees with Clayton and Shattock (1995) that resistant cultivars provide an opportunity to reduce amounts of fungicide needed to control potato late blight. Fry and Shtienberg (1990) also reported that host resistance and forecasting considerably reduce the production costs. Furthemore, Niklaus and Fry (1999) have suggested that monitoring for disease occurrence and then spraying as opposed to calendar sprays, result in higher benefits from fungicide applications.

According to Maniford and Norton (1987), a necessary condition for taking a control action is that in which the economic benefit is greater than the economic cost. Whereas the partial budget analysis (Table 2) showed that high net benefits were achieved under high (frequent) fungicide usage, the dominance analyses showed that use of more than 2 sprays with a contact fungicide was only economical on susceptible (Kisoro and Victoria) but not on resistant varieties like Rutuku and Nakpot 3. These results concur with those of Fry (1975) that growing resistant varieties reduce the need and amount of fungicide sprays. The marginal analysis also confirmed that beyond 2 sprays the marginal rate of return starts to decline and it became less than 50% when a susceptible cultivar like Victoria was sprayed 6 times. Although marginal analysis also showed that susceptible cultivars such as Victoria needed more than 2 sprays, as earlier suggested by Christine (1999), the marginal rate of return declined to 25% beyond 3 sprays which is below the recommended minimal marginal rate of return (CIMMYT, 1988). Nevertheless, though the high fungicide usage (6 sprays) was not economically viable, it agreed with CIMMYT (1988) that farmers choose less risky options to protect themselves against risks of loss.

The dominance of tolerant varieties after 2 sprays confirmed that two sprays for tolerant cultivars are the most economic, and that for susceptible varieties like Victoria the marginal rates of return

increasingly became uneconomical after 3 sprays. The results of this study strongly support the hypothesis that late blight monitoring can reduce the frequency and use of fungicide sprays in potato production.

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Effect of spacing and variety on potato seed tuber production in eastern Uganda

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Abstract

In the highlands of eastern Uganda, potato (*Solanum tuberosum*) productivity per unit area is constrained by limited availability of quality seed. This study evaluated the effect of plant spacing and variety on potato seed tuber production at three locations in eastern Uganda. Two varieties and four different spacings were used. Results for all locations and seasons showed that close plant spacing (20 x 20 cm) produced significantly (P = 0.05) highest potato seed tubers, total tubers and tuber yield per unit area while seed tubers were least at widest crop spacing of 70 x 30 cm. However, high plant density resulted in significantly (P = 0.05) reduced average tuber weight. Variety x spacing interactions were not significant for most variables, indicating that spacing was the most critical factor for determining tuber size number.

Key words: Quality seed, seed-size tubers, *Solanum tuberosum*

Introduction

Although the present trends in Uganda and elsewhere in Africa (except Egypt and South Africa) show an increase in potato output, this is attributable to expansion in area cultivated rather than increased productivity per unit area (Okigbo, 1997). In the highlands of eastern Uganda, potato (*Solanum tuberosum*) is an important food and the main cash crop (Adipala *et al*., 2000). However, it is becoming increasingly difficult to expand productivity in the region because of limited availability of quality seed (Alacho *et al.*, 2000), suitable arable land due to high population density and bacterial wilt (*Ralstonia solanacearum)* disease infestation. Because of land limitation, few potato growers adhere to recommended crop rotation which is a key component in bacterial wilt management (Tusiime and Adipala, 2000; Adipala *et al.*, 2001, 2002). Instead, farmers rely on purchased seed and/or recycled seed, which is expensive not only in terms of high costs but is a potential source of bacterial wilt disease spread through latently infected ware tubers used as seed tubers (Nyangeri *et al*., 1984; Adipala *et al*., 2002), thus limiting available land for seed potato production. Furthermore, due to limited availability of quality seed in the region, about 99% of the farmers grow unimproved potato seed, hence the low yields of <7 t ha^{-1} (Adipala *et al*., 2000). This compares poorly with the production potential of 25 t ha^{-1} for Uganda (Hakiza *et al.*, 1997). Therefore, development of good quality potato seed production system is fundamental to increased potato production, especially since seed quality and its availability plays a very critical role in plant growth and development.

In an effort to enhance potato seed availability to farmers, the International Potato Centre (CIP) has advocated for and encouraged informal potato seed production system (Adipala *et al*., 2001). With this system, success has been reported in Cameroon (Demo *et al*.; 2000), Ethiopia (Tesfaye Getachew and Awole Mela, 2000), southwestern Uganda (Alacho *et al.*, 2000; Tindimubona *et al.*, 2000) and Kenya (Kinyua *et al.*, 2001). However, in Uganda, due to limited finances, a farmer-based seed production scheme has so far only been promoted in southwestern Uganda and is currently lacking in eastern

Uganda, an important potato growing region. Consequently, these factors have impacted negatively on the transfer of potato production technologies and rates of adoption among farmers. Farmers who have appreciated use of disease-free seed have had to get it from Kalengyere (about 800 km away), making seed the most expensive input in potato production due to its bulkiness hence high transport costs. There is therefore, need to develop "a simple disease-free seed system" to support improved potato productivity in eastern Uganda.

For the resource-poor, small-scale farmers, the use of seed plot techniques is an option to increase availability of seed tubers. The seed-plot technique is an intensive method of producing quality seed by planting at high densities on small bacterial wilt-free plots (Kinyua *et al.*, 2001). Based on findings from earlier studies, wider spacing results in increased tuber weights while closer spacing increases the number of seed-size tubers (Nelson, 1976; Berga Lemaga and Gebrmedhin, 1994). Furthermore, potato grown at high plant density are reported to reduce bacterial wilt incidence through utilisation of small but uninfested portions of land to produce clean seed for ware potato production (Kinyua *et al.*, 2001).The unused land during the season could either be left fallow or planted in rotation to crops that are non-hosts to *Ralstonia solanacearum* hence reducing soil borne inoculum (Berga Lemaga *et al.*, 1999). However, each potato variety has different growth characteristics, and this may vary with location. Therefore there is need to establish optimum spacing for each variety and for each agroecology. Thus, this study investigated the effect of spacing and variety on potato seed tuber production in eastern Uganda.

Materials and methods

The study was conducted in three major potato growing areas in eastern Uganda, namely: Kapchorwa, Wanale-Mbale and Buginyanya ARDC-Sironko. The experiment was conducted during the first (April to August) and second (September to December) seasons of 2002. These seasons are subsequently referred to as 2002A and 2002B seasons, respectively. Two potato cultivars, Victoria and Nakpot3 were each grown at spacings 20 x 20 cm, 40 x 20 cm, 70 x 10 cm and 70 x 30 cm giving plant populations of 125,000 and 142,857 plants per hectare, respectively. Trials were set up using a split plot in a randomised complete block design and replicated three times per site, with the varieties as the main plots and population densities in the sub-plots. Each sub-plot measured 2 m x 4 m, with one-metre alleys between plots. Standard agronomic practices including regular weeding based on weed intensity, earthing-up and basal application of compound fertiliser at a rate of 80 kg ha^{-1} (N.P.K 17:17:17) were carried out. However, for seed plots (20 cm x 20 cm), weeding was minimal and done by hand pulling. Depending on weather conditions, prophylactic sprays were administered to the crop using Dithane M45 (Mancozeb 80% WP) to control late blight. Dithane M45 was obtained from ROHM and HAASITALIA, Sri Via della Filanda, 20060 Gessate (MI), Italy.

Data collection and analysis

Assessment of bacterial wilt disease started at the onset of wilt symptoms. Plants that showed complete or partial wilting were considered wilted, counted and staked to avoid double counting or missing out those that die early during the growth period. The total number of infected plants in the plot was recorded and expressed as a percentage of the total number of plants in the plot. A sample of harvested tubers were further analysed for latent infection by enzyme-linked immunosorbent assay on nitrocellulose membranes, NCM-ELISA (Priou *et al.*, 1999). At harvest, the number of potato tubers per plot were counted and graded into large (>55 mm), seed size (25-55 mm) and under-size (<25 mm) tubers and subsequently expressed as number of tubers m^{-2}. Weights of these grade categories were recorded and subsequently, yield per hectare determined. Data on bacterial wilt incidence, yield and yield components were then subjected to analysis of variance (ANOVA) using Genstat 5 Release 3.2 package and treatment means separated using Standard Error of the Difference (SED) between means

at a probability level of 0.05 (5%). Data for each season and location were analysed separately but where no significant differences were observed between the varieties, seasons and the three locations, data were pooled.

Results

Large tubers (>55 mm)

The ANOVA results indicated that number of over-size tubers produced was not significantly affected by varieties, variety x spacing and all the 3-way interactions. However, there were significant ($P<0.001$) differences between the two seasons, three locations, and spacing, hence, the data for the three locations were not pooled (Table 1).

During 2002A at Buginyanya (Table 1) plant spacing of 70 x 10 cm and 70 x 30 cm produced more tubers than close spacing (20 x 20 cm). However, during 2002B, spacing did not have a significant effect on number of large tubers produced. A similar trend was observed at Kapchorwa. At Wanale, plant spacings of 70 x 10 cm and 70 x 30 cm produced the highest number of large tubers (16 tubers m^{-2}) and the least was produced at 40 x 20 cm spacing in 2002A. In 2002B, 70 x 30 cm spacing had the highest number of large tubers (8 tubers m^{-2}) and the spacing of 20 x 20 cm produced the least number of tubers (5 tubers m^{-2}).

Seed size tubers (25-55 mm)

Spacing x season x location interactions significantly ($P = 0.005$) affected the number of seed size tubers produced (Table 2). At Buginyanya, plant spacing of 20 x 20 cm resulted in highest seed-size tuber production of 61 and 68 tubers m^{-2} while the lowest (19 and 15 tubers m^{-2}) was from the 70 x 30 cm spacing during the first and second seasons of 2002, respectively. At Kapchorwa, the spacing of 20 x 20 cm also produced significantly ($P<0.001$) more seed-size tuber yields (107 and 74 tubers m^{-2}) compared to plants spaced at 70 x 10 cm (65 and 41 tubers m^{-2}), 40 x 20 cm (52 and 36 tubers m^{-2}) and 70 x 30 cm (9 and 11 tubers m^{-2}) during the first and second seasons of 2002, respectively. The trend was similar at Wanale where seed plots spaced at 20 x 20 cm produced most seed tubers (Table 2).

Table 1. Effect of spacing on large tuber production (tubers m^{-2}) of two potato varieties grown at three locations in eastern Uganda during the first (2002A) and second (2002B) seasons.

Spacing	Buginyanya		Kapchorwa		Wanale-Mbale		Overall mean
	2002A[1]	2002B	2002A	2002B	2002A	2002B	
20 x 20 cm	4.5	2.1	13.0	4.1	13.3	4.6	6.9
40 x 20 cm	13.3	2.1	10.4	3.6	10.4	5.9	7.6
70 x 10 cm	18.5	4.8	16.4	3.2	16.4	4.9	10.7
70 x 30 cm	12.6	3.0	16.8	3.2	15.9	8.3	10.0
SED (0.05)	4.1	Ns[2]	1.2	Ns	1.0	6.0	
CV (%)	59.5		14.3		12.9	16.7	

[1]2002A and 2002B refer to first rain (March - June 2002) and second rain (September - December 2002) seasons, respectively.

[2]Ns = not significant at 5%.

Under-size tubers

Under-size tuber category was observed during the second season of 2002 but was negligible in 2002A. In 2002B season at Buginyanya, there were significantly more under-size tubers at higher plant density (20 x 20 cm), comprising 14% of the total tuber production; the wide spacing of 70 x 30 cm produced the least number of under-sized tubers, i.e., 5.5% of the total tuber production (data not presented). The trend was similar at Kapchorwa and Wanale.

Number of total tubers

There was non-significant (P>0.05) variety x spacing and variety x spacing x location interaction effects on total number of tubers (Table 3). However, spacing and spacing x season x location interaction effects were significant (P = 0.005). Thus, results of number of total tubers m^{-2} are presented for only spacing x season x location interaction (Table 4). At Buginyanya, plant spacing of 20 x 20

Table 2. Effect of spacing x location x season interaction on number of seed-size potato (25-55 mm) tubers m^{-2} of two potato varieties grown at three locations in eastern Uganda during the first (2002A) and second (2002B) seasons.

Spacing	Buginyanya		Kapchorwa		Wanale-Mbale		Overall mean
	2002A[1]	2002B	2002A	2002B	2002A	2002B	
20 x 20 cm	60.5	67.7	106.7	74.4	82.1	73.9	77.6
40 x 20 cm	37.4	37.9	51.5	41.2	26.9	38.9	39.0
70 x 10 cm	51.0	41.7	64.5	36.4	22.4	39.9	42.6
70 x 30 cm	18.8	15.4	8.9	11.1	9.7	13.8	13.0
Mean	41.9	40.7	57.9	40.8	35.3	41.8	43.0
SED (S x L x S)							6.83
CV (%)							28.0

[1] 2002A and 2002B refer to first rain (March - June 2002) and second rain (September - December 2002) seasons, respectively.
[2]Pooled data for two potato varieties.

Table 3. Effect of variety x spacing x location interaction on number of under-size (<25 mm) tubers m^{-2} of two potato varieties grown at three locations in eastern Uganda during the second season of 2002.

Variety	Spacing	Buginyanya	Kapchorwa	Wanale	Mean
Victoria	20 x 20 cm	10.0	16.9	6.7	11.2
	40 x 20 cm	4.2	4.1	2.1	3.4
	70 x 10 cm	5.7	4.8	2.9	4.4
	70 x 30 cm	1.0	1.4	1.2	1.1
	Mean	5.2	4.8	3.2	5.0
Nakpot 3	20 x 20 cm	13.0	11.9	20.0	15.3
	40 x 20 cm	5.2	2.2	13.8	7.1
	70 x 10 cm	5.2	2.3	5.8	4.4
	70 x 30 cm	1.0	1.0	4.2	1.9
	Mean	6.2	4.3	10.9	7.2
	SED (V x S x L)[1]				1.9
	CV (%)				35.8

[1] V x S x L = variety x spacing x location.

cm compared to 70 x 30 cm generally resulted in higher total tuber production of 65 and 82 tubers m^{-2}; the lowest total number of tubers (33 and 19 tubers m^{-2}) was at 70 x 30 cm spacing, during the first and second seasons of 2002, respectively. Similarly, at Kapchorwa, seed plot spacing of 20 x 20 cm resulted in significantly ($P<0.05$) higher total tuber yield (109 and 93 tubers m^{-2}) compared to plants spaced at 70 x 10 cm (71 and 43 tubers m^{-2}), 40 x 20 cm (56 and 48 tubers m^{-2}) and lowest at 70 x 30 cm (21 and 15 tubers m^{-2}) during the first and second seasons of 2002, respectively. At Wanale, seed plots spaced at 20 x 20 cm also produced more tubers than the other spacings (95 tubers m^{-2}) during both seasons. Across locations and seasons, potatoes planted at 20 x 20 cm spacing produced the highest number of tubers (90 tubers m^{-2}) while the spacing of 70 x 30 cm resulted in the least number of tubers.

Total tuber yields (t ha^{-1})

There was significant variety x spacing x location x season interaction effects on total tuber yields (Table 5). Thus, further discussion of yield results is based on the significant 4-way interaction. At Buginyanya, close spacing of 20 x 20 cm resulted in significantly ($P = 0.025$) highest tuber yields of 26.0 t ha^{-1} for both Victoria and 26.3 t ha^{-1} for Nakpot 3. Planting Nakpot 3 at 20 x 20 cm resulted in significantly ($P = 0.014$) higher yield (22.9 t ha^{-1}) compared to only 13.2 t ha^{-1} obtained at 70 x 30 cm and 17.1 t ha^{-1} at 70 x 10 cm spacing. A similar trend was observed in 2002B. In Kapchorwa, plant spacing of 20 x 20 cm resulted in significantly ($P = 0.024$) higher tuber yields (57.5 t ha^{-1}) compared to only 22.4 t ha^{-1} obtained at 70 x 30 cm spacing for variety Victoria; 49.6 and 14.5 t ha^{-1} for Nakpot 3, in 2002A, respectively. A similar trend was observed for tuber yields in 2002B with highest yield of 40.4 and 39.8 t ha^{-1} at 20 x 20 cm spacing and lowest (11.0 and 9.1 t ha^{-1}) at 70 x 30 cm spacing, for Victoria and Nakpot 3, respectively (Table 5). At Wanale, a spacing of 20 x 20 cm also resulted in significantly ($P<0.001$) higher tuber yields (44.4 and 62.8 t ha^{-1} for Victoria; 38.5 and 25.9 t ha^{-1} for Nakpot 3) during 2002A and 2002B, respectively, compared to only 16.2 and 19.3 t ha^{-1} for Victoria; 13.7 and 11.2 t ha^{-1} for Nakpot 3 at 70 x 30 cm spacing. Victoria spaced at 40 x 20 cm also yielded 30.0 t ha^{-1} in the first and 35.5 t ha^{-1} in the second seasons, while Nakpot 3 grown at the same spacing produced 16.7 and 19.6 t ha^{-1}; which was significantly higher than for those spaced at 70 x 30 cm.

Bacterial wilt incidence and latent infection of tubers

Generally, irrespective of plant spacing, the highest bacterial wilt incidence was recorded at Wanale with mean incidences of 11.8% for Victoria and 12.6% for Nakpot 3, compared to only 3.9% and 6.7%

Table 4. Effect of spacing x location x season interaction on total tuber number per m^2 of potato grown at three locations in eastern Uganda during the first (2002A) and second (2002B) seasons.

Spacing	Buginyanya		Kapchorwa		Wanale-Mbale		Overall mean
	2002A	2002B	2002A	2002B	2002A	2002B	
20 x 20 cm	65.0	81.8	109.2	92.9	95.4	95.4	90.0
40 x 20 cm	50.2	41.2	55.8	48.1	37.2	53.0	47.7
70 x 10 cm	69.6	52.0	70.7	43.2	38.1	49.1	53.8
70 x 30 cm	32.7	18.8	21.1	15.4	27.2	20.6	22.6
Mean	54.4	48.4	64.2	49.9	49.5	54.6	53.5
SED (S x L x S)[1]							7.11
CV (%)							23.3

[1]S x L x S = Spacing x location x season.
[1]Pooled data for two potato varieties (variety effects were not significant).

for the respective cultivars at Kapchorwa (Table 6). However, differences among various spacings were not significant except for Victoria at Wanale. Bacterial wilt was not observed at Buginyanya. At Wanale, highest wilt incidence occurred in Victoria spaced at 70 x 10 cm (14.6%) followed by 70 x 30 cm spacing (11.3%) for Nakpot 3 and 70 x 10 cm and 40 x 20 cm for Victoria. Likewise, at Kapchorwa, plants spaced at 70 x 30 cm recorded highest bacterial wilt incidence of 12.5% for Nakpot 3 and 6.3% for Victoria. There was low bacterial wilt incidence in 2002A with mean incidences of only 2.8% and 1.4% at Wanale and Kapchorwa, respectively. In this season, crop spacing of 70 x 10 cm registered highest incidence of bacterial wilt (7.5%) at Wanale and 5.0% at Kapchorwa (data not presented).

All potato tubers obtained from different plant spacing at Wanale reacted positively to NCM-ELISA test, except Victoria obtained from a spacing of 40 x 20 cm (Table 6). In Kapchorwa, only tubers from plants spaced at 20 x 20 cm for both varieties reacted positively with ELISA test. Contrastingly, all potato samples from Buginyanya were non-reactive with NCM-ELISA irrespective of plant spacing (data not presented).

Discussion

The number of seed size tubers, total tubers per unit area and fresh tuber yield were highest under close plant spacing (20 x 20 cm, 40 x 20 cm) compared to wider spacings (70 x 10 and 70 x 30 cm) probably due to the higher number of plants planted and harvested per unit area under close spacing. Thus, the lower yields from the wide spaced crop (70 x 30 cm) was probably due to fewer numbers of plants per unit area. In a related studies, Beukema and Van der Zaag (1990) and Berga Lemaga and Caeser (1990) observed that increasing plant density (close spacing) increased yields and attributed this to increased number of stems per unit area. Generally, however, for both varieties, highest yields were obtained at high plant density and variety x spacing interaction effects were not significant indicating that spacing was the overriding factor in determining tuber numbers and hence yield. Although close spacing resulted in significantly highest number of seed-size tubers, total tubers and yield per unit area,

Table 5. Influence of variety, spacing, season and location on tuber yield (t ha^{-1}) of two potato varieties grown at three locations in eastern Uganda during the first (2002A) and second (2002B) seasons.

Variety	Spacing	Buginyanya		Kapchorwa		Wanale-Mbale		Overall mean
		2002A	2002B	2002A	2002B	2002A	2002B	
Victoria	20 x 20 cm	26.0	27.1	57.5	40.4	44.4	62.8	43.1
	40 x 20 cm	19.3	17.6	34.0	18.2	30.0	35.5	25.8
	70 x 10 cm	23.3	25.5	37.5	21.0	18.6	34.1	27.8
	70 x 30 cm	18.9	8.6	22.4	11.0	16.2	19.3	16.1
	Mean	21.9	19.7	37.9	22.7	27.3	37.9	28.2
	SED	3.0	3.2	8.0	6.1	2.0	4.7	
	CV (%)	15.7	19.7	25.8	33.0	9.0	15.2	
Nakpot 3	20 x 20 cm	26.3	31.7	49.6	39.8	38.5	25.9	35.3
	40 x 20 cm	22.9	13.1	27.3	32.5	16.7	19.6	22.0
	70 x 10 cm	17.1	16.4	44.3	27.5	17.0	21.4	25.6
	70 x 30 cm	13.2	6.9	14.5	9.1	13.7	11.2	11.5
	Mean	19.9	17.0	33.9	27.2	21.5	19.5	23.6
	SED	2.8	4.8	9.8	0.9	3.8	2.2	
	CV (%)	17.4	34.2	35.5	4.0	21.6	12.4	

[1] 2002A and 2002B refer to first rain (March - June 2002) and second rain (September - December 2002) seasons, respectively.

the same spacing also produced the highest proportion of under-size tubers, which is undesirable. The implication is that agronomic practices should be altered according to plant density.

Close plant spacing and hence high plant density in excess of that required for ware yields still produced tubers in the large size (>55 mm) category. This suggests that potato tubers respond differently to competition. As such, some tubers may be preferentially positioned in relation to assimilate supply such that they remain largely unaffected by competition while other tubers are highly affected and with increased competition may lead to reduced size. Gray (1973) and Oparka (1987) also reported similar results.

Mean tuber weight generally increased with wider spacing probably due to more competition for assimilates for tuber bulking and other plant growth factors at high plant density. Also, at close spacing, low average tuber weights could have been as a result of the plants' reaction to high level of shading. The study by Ebwongu *et al.* (2001) revealed that potato plants in an attempt to place their leaves in light, partition more assimilates for stem growth and became taller due to phototropism, at the expense of tuber bulking. Similarly, Burton (1989) reported that shading reduced total radiation intercepted and consequently the net assimilates directed to tubers, resulting in light tubers. Hence close spacing is only appropriate for seed and not ware tuber production.

Higher bacterial wilt incidence was recorded on potato from farmers' fields at Wanale-Mbale and at Kapchorwa but no wilt was observed at Buginyanya, yet planting materials were from the same source and were clean. These results imply that soils in Wanale were more infested by *Ralstonia solanacearum* than at Kapchorwa. The NCM-ELISA test confirms these results. Secondly, absence of bacterial wilt at Buginyanya was probablly due to the fact that the crop was established on plots previously under fallow for over three years while at Kapchorwa, farmers had previously practiced crop rotation by planting their plots with maize and wheat. These factors are known to reduce bacterial wilt inoculum in the soil (Berga Lemaga *et al.*, 1999). At Wanale, on the other hand, due to small land holdings, plots were under continuous cultivation with vegetables such as tomatoes and eggplants, which are hosts to *R. solanacearum*.

Table 6. Effect of spacing and variety on bacterial wilt incidence and latent infection of potato tubers during 2002B[1] in eastern Uganda.

Variety	Spacing (cm)	Wanale		Kapchorwa	
		[2]BW%	Latent infection	BW%	Latent infection
Victoria	20 x 20	9.2	++	2.0	+++[3]
	40 x 20	12.2	-	3.3	-
	70 x 10	14.6	+++	4.2	-
	70 x 30	11.3	+++	6.3	-
	Mean	11.8		3.9	
	SED	0.77		Ns	
	CV (%)	6.5		-	
Nakpot 3	20 x 20	12.5	+++	4.6	+++
	40 x 20	8.3	+++	4.3	-
	70 x 10	13.4	+++	5.1	-
	70 x 30	16.3	+++	12.5	-
	Mean	12.6		6.7	
	SED	Ns		Ns	
	CV (%)	26.7		-	

[1]2002B refer to second rain (September - December of 2002) season.

[2]BW = bacterial wilt disease.

[3]-, +, ++, +++ = non-reactive, reactive, very reactive and highly reactive, respectively, with NCM-ELISA.

Generally, however, in fields where *Ralstonia solanacearum* inoculum existed, all plots irrespective of plant spacing, exhibited some potato plants with wilt symptoms. This shows that manipulation of plant spacing alone can not singly control the pathogen. However, the advantage with close spacing (seed plot) especially at 20 x 20 cm is that more number of seed-size tubers is produced per unit area as exemplified by the results of this study. The implication therefore is that even potato farmers with small land holdings (as is the case with hilly and densely populated potato growing areas of eastern Uganda) could set aside small but clean plots for seed tuber production and also practice crop rotation using non-hosts to *R. solanacearum* in the cropping sequence to reduce bacterial wilt inoculum in the soil. Since manipulation of plant spacing alone could not control the pathogen, an Integrated Disease Management (IDM) approach is viewed as the best approach for bacterial wilt control. It also implies that greater care should be placed in selecting plots for seed-potato production. Furthermore, studies on other IDM protocols such as soil solarisation and using plastic sheets which may be possible under small seed plots could be part of a strategy for bacterial wilt management.

Conclusion

The results of the study generally showed lack of significant varietal effects, and in most cases, variety x environment interaction were not significant. Thus, the two varieties can be used in all the study areas for seed and for ware production. What is crucial is to determine whether production is for ware or for seed. For seed production, spacing of 20 x 20 and 40 x 20 cm appear appropriate, while for ware, the spacing of 70 x 10 cm could be used. Seed plot spacing at 20 x 20 cm compared to wide plant spacing (70 x 30) produced significantly highest proportion of seed size tubers and total number of tubers per unit area. Therefore, to enhance potato seed production in eastern Uganda, high plant density should be used. However, there is still a problem of bacterial wilt latent infection. Therefore, farmers should be assisted by researchers, extension workers and other partners in potato production to identify bacterial wilt-free areas in their plots for seed-potato production.

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Production potential for sesame in the forest-savanna transition zone of south-west Nigeria

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Abstract

The performance of fourteen sesame (*Sesamum indicum* L.) cultivars was evaluated at the Teaching and Research farm of University of Agriculture, Abeokuta (7° 15' N, 3° 25' E) in the forest – savanna transition zone of south west Nigeria during the late cropping seasons of 1999 and 2000. Data were collected on phenology, growth characteristics, grain yields and yield components of sesame. The results revealed that the sesame varieties flowered, produced capsules and matured at 49-60, 58-74 and 96-106 days after planting (DAP), respectively. Number of branches and capsules per plant ranged between 2-4 and 13-37, respectively. The sesame entries recorded grain yields that ranged between 374.0 (Domu) and 899.46 kg ha^{-1} (530-3). All the varieties except Yandev 55 (a local variety used as check in the study) (448.94 kg ha^{-1}) and Domu (374.94 kg ha^{-1}) produced grain yields higher than the current Nigerian average yield of 487 kg ha^{-1}. Grain yield was highly associated ($P<0.01$) with number of nodes and capsules per plant. The overall performance of these varieties in this study, is similar to results reported for sesame generally in the southern Guinea savanna region of Nigeria. Consequently, this confirmed the suitability of the forest – savanna transition zone for sesame production. The distribution of rainfall and temperature during the two late cropping seasons and their relevance to the productivity of the sesame varieties is discussed.

Key words: Agronomic performance, productivity, *Sesamum indicum*, yield

Introduction

Sesame or beniseed (*Sesamum indicum* L.) is one of the oldest cultivated plants in the world and it probably originated from Ethiopia. Sesame gained prominence in Nigerian agriculture in the mid sixties when Mokwa experimental station of The Institute of Agricultural Research located in the southern Guinea savanna region was chosen as the Centre for sesame research in Nigeria (Voh, 1998). Consequently, most of the pioneer research activities were carried out at this station (van Rheenen, 1967, 1970, 1973). The crop which is now well established in the African savanna is cultivated mainly for its seeds which contains approximately 50% oil and 25% protein (Oplinger *et al.*, 1990). The oil of sesame is free from undesirable nutritional or flavour components and is very stable because it contains natural antioxidants such as *sesamin* and *sesamolin* which prevent ageing and malfunctioning of liver. The oil is also used in the manufacture of paints, soaps, perfumes, pharmaceuticals and insecticides. Sesame meal (residue after oil extraction) is an excellent high protein (34-50%) feed supplement for livetock (Oplinger *et al.*, 1990). The largest buyer of Nigerian sesame is Japan where sesame seeds are used in baking, candy making, other food industries and for industrial purposes (Coote, 1998). The local people in the sesame growing regions of Nigeria also eat sesame seeds fried, roasted or pounded and mixed with sugar (Voh, 1998).

According to FAO (2000), world projected production in 2001 was 2.84 million tons on 7.40 million hectares. The estimated total production in Africa was put at 741,956 tons with Sudan, Uganda and

Nigeria accounting for 40, 13 and 9.3%, respectively. Harvested area under sesame in Nigeria increased by 37.2% between 1990 and 2001 (FAO,2000). The average yields of sesame in the world (263 kg ha^{-1}) and Africa (383 kg ha^{-1}) are still very low compared to yields of up to 1.2, 1.1 and 0.7 t ha^{-1} in Egypt, Honduras and Ethiopia, respectively (FAO,2000). The earlier reported low yield of 300 kg ha^{-1} of sesame in Nigeria by Philip (1977) has increased from 400 kg ha^{-1} in 1990 to 457 kg ha^{-1} in 2001(FAO,2000). This increase could easily be attributed to cultivation of improved local cultivars under improved cultural practices.

Sesame is adapted to tropical and temperate conditions. In West Africa it is cultivated in areas where annual rainfall ranges from 500mm in the Sudan savanna to 1,100-1,500mm in the southern Guinea and derived savanna. However, the traditional sesame growing regions in Nigeria fall within latitudes 6° and 10° N (Agboola, 1979) and these regions receive a little below 1,000mm of rainfall. In a three year study conducted on sesame in the forest region where rainfall is usually above 1000mm per annum, an encouraging average yield of 693 kg ha^{-1} was recorded (Ogunremi, 1985). This suggests that other regions bordering the traditional growing areas of sesame such as the forest-savanna transition zone with annual rainfall above 1000mm could also be exploited for sesame cultivation.

Despite the increasing demand for Nigerian sesame seeds in the world market, especially Japan, there are still no large scale commercial growers of this crop. The two improved released varieties (E8 and Pb Til) alongside the adapted local varieties like Yandev 55 and Okenne local are still cultivated within the traditional growing regions. In order to meet the increasing local and international demand of Nigerian sesame seeds which is a highly favoured source of foreign exchange income , this study was aimed at evaluating the performance of some sesame improved and promising cultivars in other potential areas of cultivation such as the forest-savanna transition zone of south west, Nigeria.

Materials and methods

The study area

Figure 1 shows the location of the study area and the vegetation of Ogun State of Nigeria. The State covers an area of about 14,409,260 sq. km with a population of about 2.8 million people (OGADEP, 1999). The vegetation of the state ranges between the derived savanna (forest-savanna transition) in the north to deltaic swamp complex in the south. The study area is located in the forest-savanna transition zone where the original vegetation had been altered by bush burning and continous cultivation. Consequently, grasses (*Panicum maximum, Imperata cylindrica)* and some trees (*Afselia africana, Daniellia oliveri)* and shrubs (*Chromolaena odorata*), which are fire resistant now remain in the area. The major arable crops in the area include cassava *(Manihot esculentum)*, maize *(Zea mays)*, yam *(Docus carota)*, cocoyam *(Docus* sp.), rice *(Oryza sativa)*, vegetables, pepper, okra, *Amaranthus* species, *Celosia* species and legumes (cowpea *(Vigna unguiculata)* and groundnut *(Arachis hypogea)* (Tayo *et al.*, 1992). The experimental site was located at the Teaching and Research farm of University of Agriculture, Abeokuta (7°15'N, 3°25'E). The soil of the site was oxic paleudulf (Adetunji, 1991) and it had a pH of 5.6, 0.87% organic carbon, 0.05% total N, 2.23 mq kg^{-1} available P (Bray's P1) and 1.3 mmol(+) kg^{-1} exchangeable K. Traditionally, rainfall distribution in this region is usually bimodal with peaks usually in July and September and a short dry spell in August often referred to as *August Break*. However, this trend was not observed during the period of experimentation in both years. Figure 2 shows the mean monthly rainfall and temperature for 1999, 2000 and fifteen year mean of 1982 - 1996 during the late cropping season. The experimental fields were previously cropped to maize in both years.

Experimental design and treatments

The experimental design was randomised complete block design with three replicates. Fourteen varieties of sesame were collected from National Cereals Research Institute, Badeggi, Niger State which has the national mandate for research (genetic improvement) and development of sesame production in Nigeria. The varieties were: E8, Yandev 55, Goza, Type 4, 73A-11, 73A-79, 530-6-1,69B-88Z, Domu, 73A-97, C-K2, 73A-94, 530-3 and PB Til. E8 (an improved and released variety) and Yandev 55 (an adapted local variety) were used as control checks in the study. Seeds were planted at a spacing of 60 x 5cm on 7th and 31st July, 1999 and 2000, respectively. The seedlings were thinned

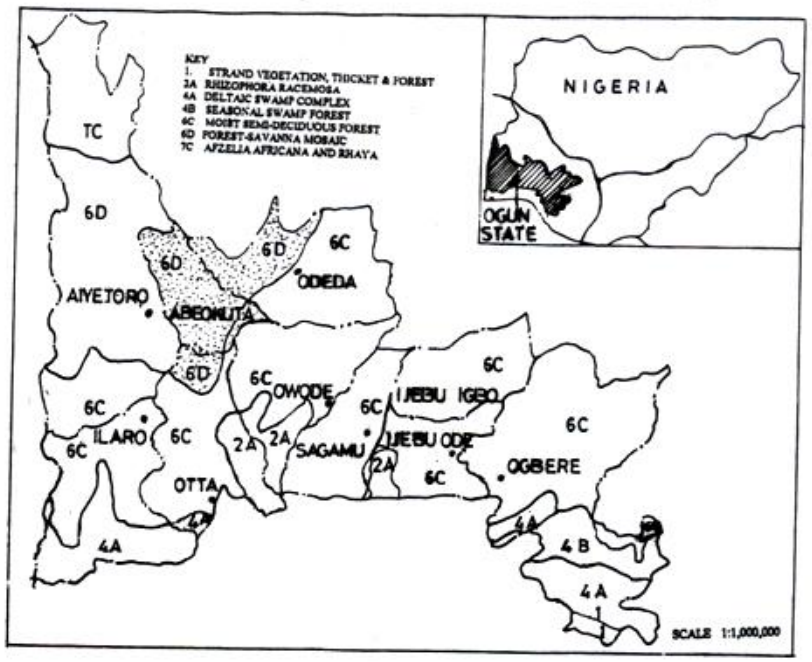

Figure 1. Vegetation map of Ogun State showing the study location. Source: Tayo et al., 1992.

to one plant per stand at three-weeks after planting (WAP). While manual weeding of plots was done at 5 and 9 WAP in both years. Fertiliser was applied at the rate of 60 kg N ha^{-1}, 30 kg $^{-1}$ P_2O_5 and 50 kg K_2O ha^{-1} at 6WAP (Olowe and Busari, 2000).

Observations and staistical analysis

Data were collected on phenology (number of days to flowering, capsule formation and maturity), growth parameters (height at flowering, maturity, height of lowest capsule and number of nodes per plant), yield components (number of branches and capsules per plant, weight of capsules and seeds per plant, average plant weight, harvest index, seed production efficiency, and 1000 seed weight) and grain yields. The data obtained were subjected to combined analysis of variance. Simple correlation analysis was also carried out to determine the level of relationship between eight yield parameters and grain yield. Means of significant treatments were separated using the Duncan's Multiple Range Test (Steel *et al.*, 1997).

Results and discussion

Rainfall and temperature distribution

Figure 2 shows rainfall and temperature distribution for both 1999 and 2000 compared to the fifteen year mean (1983-1994). The total amount of rainfall during the period of experimentation (June – November) in 1999, 2000 and fifteen year mean was 566.8, 917.5 mm and 802.3 mm, respectively. These values were 30 and 14% lower and higher than the fifteen year mean for the study area, respectively. The late cropping season of year 2000 was wetter than that of 1999. Consequently, the period could be described as wet (i.e., irrigation not essential) because the rainfall bars cross the mean temperature curve particularly in July, August and September which happened to be the period of active vegetative and reproductive phases of sesame. However, in 1999 since the rainfall bars did not cross the mean temperature curves throughout the late cropping season except in August, the season

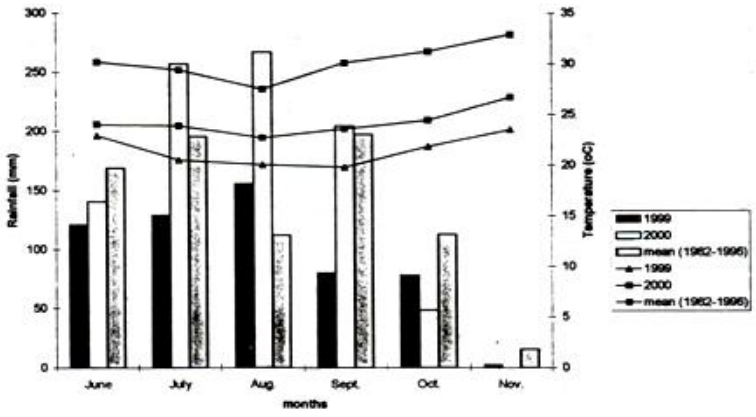

Figure 2. Monthly rainfall and mean monthly temperature for 1999, 2000 and mean for 1982 - 1996 during the late cropping season.

could be said to be dry (i.e., irrigation essential) according to Olasantan (1996). The mean monthly temperature during the late cropping season averaged 21.4°, 24.2° and 30.2°C in 1999, 2000 and for the fifteen year mean, respectively. However, the fifteen year monthly mean temperature was markedly higher than the mean temperature of 1999 and 2000 throughout the late cropping season.

Phenological observations

Significant differences ($P<0.05$) were recorded amongst the fourteen entries for number of days to flowering, capsule formation and maturity (Table 1). Number of days to flowering and capsule formation ranged between 49 – 60 and 58 – 74 DAP among the entries, respectively. Type 4 attained these two stages earlier than all the other entries. The observed range of 49 – 60 DAP to flowering is very similar to the reported ranges of 37 – 58 (Adeyemo and Ojo, 1993) and 42 – 67 DAP (Iwo *et al.*, 1998) from the middle belt zone of Nigeria. Number of days to maturity ranged between 96 DAP (Domu) to 106 DAP (73A-94). Based on these findings, the fourteen entries could be described as early maturing which is a desirable trait for crops growing in regions with limited rainfall particularly in the late season.

Growth parameters

All the varieties exhibited significant ($P<0.05$) height characteristics (Table 2). The two control checks (E8 and Yandev 55) recorded height at flowering and maturity that were comparable to the values recorded by other varieties; although, 530-3 (147.12 cm) was significantly ($P<0.05$) taller than E8 (126.27 cm) at maturity. The range of height of 115.18 – 147.12 cm at maturity is very close to 125.0 – 187.0 and 119.9 – 148.3 cm, reported for sesame in the southern Guinea savanna zone of Nigeria by Adeyemo and Ojo (1993) and Busari and Ajewole (1993), respectively. The height of the lowest capsule ranged between 56.77 (Yandev 55) – 76.75 cm (530-6-1). This trait is more of a varietal characteristic (Weiss, 1984) and it has implication if sesame is to be harvested mechanically because it will determine the height at which the cutter bar must be adjusted to minimize field loss during mechanical harvesting. Variety 530-3 produced the highest number of nodes per plant (104) and 73A-97 the lowest (30). This trait is also a varietal characteristic (Weiss, 1984).

Table 1. Number of days to flowering, capsule formation and maturity of fourteen sesame varieties

Varieties	Number of days to		
	Flowering	Capsule formation	Maturity
E 8	54def	67cd	103bc
Yandev 55	56bcde	69bcd	100de
Goza	57abcd	71ab	104bc
Type 4	49h	58f	100de
73A-11	53efg	71abc	105abc
73A-79	59ab	73ab	103bc
530-6-1	50gh	62ef	99e
69B-88Z	60a	72ab	105abc
Domu	52fgh	66de	96f
73A-97	55cdef	74a	102cd
C-K2	57abcd	71abc	103bc
73A-94	58abc	72ab	106a
530-3	59ab	73a	102cd

Means along columns with a common letter are not significantly different from each other according to Duncan's Multiple Range Test (DMRT).

Grain yield and yield components

Results of grain yield and some yield components of the fourteen sesame varieties are presented in Table 3. They all produced 2 – 4 branches per plant. This confirmed the findings of Iwo *et al.* (1998) that sesame rarely produce more than four branches per plant. The number and weight of capsules per plant and average plant weight produced by E8 and Yandev 55 were similar to the observations recorded from most of the other entries except 73A-97 which had 13 capsules per plant (significant $P<0.05$). However, the number of capsules per plant 13-17 (the lowest) produced by these varieties in the forest-savanna transition location is relatively lower than those (43 - 52) reported for sesame in the southern Guinea savanna environment by Adeyemo and Ogunwolu (1996). Variety C-K 2 recorded the highest weight of seeds per plant (4.27g) while the values for other entries were similar. These values were also slightly lower to the 5.3 – 5.4 gm plant^{-1} reported by Adeyemo and Ogunwolu (1996).

Harvest index (HI) describes the ability of a plant to partition manufactured assimilate into the seeds and is a ratio of weight of seeds to weight of above ground plant part expressed in percentage. Yandev 55 which is a locally adapted variety recorded the lowest value of 6.91% compared with 19.22% by Goza. On the average, all the entries that recorded HI above 10% produced comparable grain yields. Seed production efficiency (SPE) is the ratio of seed weight to capsule weight expressed in percentage and was used to measure the ability of sesame to produce seeds (Ogunremi, 1985). The values of this parameter by the entries ranged between 35.20 to 59.10% and were higher than 29.4% (Yandev 55) and 37.4% (65A-36) reported by Ogunremi (1985).

The fourteen varieties in this study produced grain yields that ranged from 374.0 (Domu) to 899.46 kg ha^{-1} (530-3). When compared to the current world (263 kg ha^{-1}), Africa (383 kg ha^{-1}) and Nigeria (457 kg ha^{-1}) averages, only Domu and Yandev recorded lower grain yields. These values are at par with grain yields reported by Iwo *et al.* (1998) for sesame in the middle belt zone of Nigeria. To date, only E 8, Yandev 55 and PB Til have been released to farmers. Variety E 8 which is an improved variety and a check in this study produced the fifth highest yield of 749.9 kg ha^{-1} which was not significantly different from the four top yielders. Yandev 55 which is a local adapted variety and the second check produced an average yield of 448.94 kg ha^{-1} which is slightly lower than the current Nigerian average of 487 kg ha^{-1}. The fourteen sesame varieties recorded higher yields in 2000 than 1999. The top six

Table 2. Some Height Characteristics of Fourteen sesame varieties.

Varieties	Plant height (cm) at		Height (cm) of lowest capsule	Number of nodes per plant
	flowering	maturity		
E 8	58.46abc	126.27bc	69.13abc	92ab
Yandev 55	61.42ab	132.37abc	76.75a	73abc
Goza	55.63abc	141.75ab	71.22abc	74abc
Type 4	61.15ab	135.03abc	59.05c	91ab
73A-11	62.92ab	132.20abc	76.10a	63abc
73A-79	52.52bc	131.50abc	68.40abc	66abc
530-6-1	56.95abc	134.00abc	56.77c	91ab
69B-88Z	49.63c	134.27abc	74.72ab	93ab
Domu	53.54bc	124.90bc	61.30bc	56bc
73A-97	53.59bc	115.18c	59.20c	30c
C-K2	58.43abc	131.07abc	63.93abc	62abc
73A-94	54.00bc	128.53abc	74.23ab	57bc
530-3	58.37abc	147.12a	76.58a	104a

Means along columns with a common letter are not significantly different from each other according to Duncan's Multiple Range Test (DMRT).

Table 3. Grain yield and some yield components of fourteen sesame varieties.

Varieties	Number of		Average plant weight (g)	Weight (g) of		Harvest index (%)	SPE (%)	1000 seed weight (g)	Grain yield (kg ha^{-1})
	branches per plant	capsules per plant		capsules per plant	seeds per plant				
E 8	3abc	32abc	29.07abc	7.68abc	2.10b	16.83ab	52.05ab	2.97ab	749.90abc
Yandev 55	3abc	25abcd	33.63a	5.47ab	2.16b	6.91c	36.85de	2.45de	448.94cd
Goza	3abc	31abc	27.82abcd	9.38a	2.30b	19.22a	55.82ab	2.75bc	580.37abcd
Type 4	2cd	30abc	30.58abc	8.03ab	1.87b	15.80ab	52.68abc	3.03ab	811.86ab
73A-11	2cd	23abcd	22.78bcd	4.64bc	2.45b	12.68abc	59.10a	3.00ab	564.96bcd
73A-79	2cd	22bcd	22.35bcd	6.18abc	2.40b	15.57ab	49.90abcd	2.95ab	653.51abcd
530-6-1	2cd	27abc	33.15a	7.22abc	2.25b	14.97abc	55.50ab	3.11a	893.07a
69B-88Z	4a	29abc	25.9abcd	8.28ab	2.25b	13.52abc	40.50cde	2.82bc	677.32abcd
Domu	2cd	19cd	22.62bcd	5.12abc	1.67b	12.55abc	47.07abcde	2.60cde	374.31d
73A-97	2cd	13d	19.02d	3.45c	2.38b	10.27bc	49.60abcd	3.23a	555.67bcd
C-K2	3abc	28abc	21.09cd	7.62abc	4.27a	13.58ab	35.20e	2.43e	555.53bcd
73A-94	3abc	23abc	21.92bcd	8.25ab	2.83b	15.28ab	42.32bcde	2.67cd	523.8484
530-3	3abc	37a	26.53abcd	9048a	2.3b	17.73ab	47.02abcde	2.98ab	899.46a

Means along columns with a common letter are not significantly different from each other according to Duncan's Multiple range Test (DMRT).
SPE – Seed Production Efficiency.

yielders (530-3, 530-6-1, PB Til, Type 4, E 8 and 69B-88Z) were the same in both years (Table 4). The total amount of rainfall and mean monthly temperatures were 62% higher and 1.2 - 3.2°C warmer during the late cropping season of 2000 than 1999. Furthermore, the more favourable rainfall distribution in 2000 and the slightly warmer temperature experienced in October and November apparently enhanced the performance of sesame in 2000 over 1999 (Fig. 2).

Relationship between grain yield and eight yield components

The correlation coefficients (Table 5) revealed that grain yield and plant height at maturity, number of nodes per plant, number and weight of capsules per plant and weight of seeds per plant were significantly and positively related suggesting that these traits contributed to the grain yield of sesame. Similarly, weight of seeds, number and weight of capsules per plant, number of nodes per plant and height at maturity were highly positively associated (significant at $P<0.01$) with each other. Harvest index was highly significantly positively associated with weight of seeds ($r=0.85$) and capsules

Table 4 Grain yield of fourteen sesame varieties.

Varieties	1999	2000	Mean
E 8	450.50bcd	1049.30ab	749.90abc
Yandev 55	260.30cd	635.5bc	448.94cd
Goza	156.10d	1004.64abc	580.37abcd
Type 4	569.10abc	1054.59ab	811.86ab
73A – 11	277.41cd	855.87abc	564.96bcd
73A – 79	341.13bcd	989.89abc	653.51abcd
530-6-1	628.57ab	1157.60ab	893.07a
69B-88Z	346.97bcd	1007.67abc	677.32abcd
Domu	283.87cd	464.75c	374.31d
73A-97	153.90d	955.77abc	555.67bcd
C-K2	290.93cd	820.12abc	555.53bcd
73A-94	302.00cd	745.67bc	523.84bcd
530-3	733.49a	1065.41ab	899.46a
PB Til	436.37bcd	1347.17a	891.77a

Means along columns with a common letter are not significantly different from each other according to Duncan's Multiple Range Test (DMRT).

Table 5. Correlation coefficients among nine agronomic characters measured during two years for fourteen sesame varieties.

Traits	Height at flowering	Height at maturity	Number of capsules per plant	Number of nodes per plant	Weight of capsules per plant	Weight of seeds per plant	1000 seed weight	Harvest index
Grain yield	0.32	0.53*	0.67**	0.78**	0.56**	0.57**	0.50	0.45
Height at flowering	-	0.26	0.41	0.34	0.13	0.10	-0.03	-0.21
Height at maturity		-	0.84**	0.75**	0.76**	0.75**	-0.15	0.50
Number of capsules/plant			-	0.90**	0.85**	0.80**	-0.13	0.51
Number of nodes/plant				-	0.73**	0.68**	0.06	0.40
Weight of capsules/plant					-	-	-0.18	0.70**
Weight of seeds/plant						-	0.02	0.85**
1000 seed weight							-	0.27
Harvest index								-

* and ** significant at 5 and 1%, respectively.

(r=0.70) per plant. Ogunremi and Ogunbodede (1986) also reported significant positive association of number and weight of capsules per plant, weight of seeds per plant and plant height at maturity with grain yield. They recommended that number of capsules per plant, weight of seeds per plant and plant height among other traits should be considered in selection for high yield capacity in sesame production. The encouraging average performance of these fourteen sesame varieties in a forest – savanna zone over two years confirms its suitability for the cultivation of sesame.

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Sorghum yield response to kraal manure combined with mineral fertilisers in eastern Uganda

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Abstract

Sorghum [*Sorghum bicolor* (L.) Moench] is a strategic food security crop in the drought prone areas of Uganda. However, farmers' yields are much lower than what is reported in research stations. Low soil fertility is identified as a major contributor to the low grain yields but little has been done to address this problem. The use of kraal manure obtained from cattle that are an integral component of the farming systems in sorghum growing areas is not being exploited probably due to farmers reluctance to take advantage of synergistic relationships between crop and livestock production units. The yield responses of sorghum to kraal manure and mineral fertiliser applications are largely unknown in Uganda and besides, application rates have not been determined. This study was conducted to evaluate sorghum yield response to kraal manure (KM) combined with mineral nitrogen (N) and phosphorus (P) fertilisers in Kumi district, eastern Uganda. On-farm experiments were conducted for three rain seasons; from August 2000 to December 2001. We found that combining 2.5 t KM with 22.5 kg N and 8.5 kg P ha^{-1} gave the highest grain yields (2.1 and 4.0 t ha^{-1}) for the first and second seasons of 2001, respectively. Further studies are, however, needed to establish the feasibility of this input combination for increased and sustained sorghum production.

Key words: Drought prone areas, food security, grain yield, nitrogen, phosphorus, *Sorghum bicolor*

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench.] is a staple cereal in the drought prone areas of Uganda. Such areas are found in the eastern, northeastern and southwestern parts of the country and are the areas where sorghum production is concentrated (Esele, 1988). However, farmers' get on average 700 kg ha^{-1} of the grain, compared to 4000 kg ha^{-1} reported in research stations. Use of low-yielding varieties (Cox *et al.*, 1984), poor soil fertility (Esele, 1988), low soil moisture (Seetharama, 1995), and pests and diseases (Esele, 1995a) are the major contributors to the low farmers' yields.

Earlier, FAO (1981) had projected that higher yields per unit area would account for 60 % of sorghum yield increases by the year 2000. Accordingly, World Bank (1982) sounded calls for increased research in neglected areas of rain fed crops, particularly coarse grains, such as sorghum and millet (*Elusine coracana* L. Gaertn) in drought prone areas. In particular, World Bank (1982) stressed the need to develop location-specific technologies that would increase grain production while maintaining high quality of the soil. Recognizing its strategic importance in the drought prone areas of Uganda, NARO (1991) re-sounded calls by World Bank (1982) by ranking sorghum third after maize (*Zea mays* L.) and finger millet, in order of research priorities within the framework of the country. Significant scores have since, been made from breeding for drought tolerance (House, 1995; Seetharama, 1995; Omanya *et al.*, 1996), grain quality improvement (Asante, 1995) and higher yields (Cox *et al.*, 1984; Esele, 1995b). These concerted efforts saw Esele (1995b) release varieties like sekedo with a yield potential of 3 to 5 t ha^{-1}. However, farmers have not yet realised this yield potential, owing to the inherently low

soil fertility in the areas where sorghum is produced. Since cattle are an integral component of the farming system in Kumi district, we conducted this study to evaluate sorghum grain yield response to KM combined with modest quantities of mineral N and P fertilisers.

Materials and methods

This study was conducted in Kanyum parish, Kanyum sub-county, Kumi district, in eastern Uganda. Kumi lies in the agro ecological zone where the Teso Farming System (TFS) is practiced (Wortmann and Eledu, 1999). The landscape is gently undulating, with occasional inselbergs. Kumi lies on latitude 1° 43' N, longitude 33° 37' E and on elevation ranging from 1030-1127 m above sea level (Yost and Eswaran, 1990).

The climate is mainly tropical, with a bimodal type of rainfall, averaging 900-1200 mm yr^{-1} (Yost and Eswaran, 1990). First rains start from March, peaking in April, and subsiding in June. The second rains start from August, peaking in September, and subsiding in October, or persisting up to November. The intervening periods are dry. Prolonged dry spells may be experienced in the east and northeastern parts of the district, especially areas bordering Karamoja. Annual temperature ranges from 15 to 36 °C, with a mean temperature of 25 °C (Yost and Eswaran, 1990).

The typical farming system comprises of cotton (*Gossypium hirsutum L.)*-finger millet system. Other major crops are sweetpotatoes (*Ipomoea batatus* L. Lam), cassava (*Manihot esculenta* Crantz) and sorghum (Mugisha, 1996). These are often cultivated in a crop rotation sequence with legumes like groundnuts (*Arachis hypogaea* L.) and cowpeas (*Vigna ungiculata* L. Walp.) on light-textured soils, ranging from sandy loam to loam. In addition to cultivation, cattle were an integral component of the TFS before they were rustled away in 1987 (Walaga *et al.*, 2000). However, some farmers have managed to restock kraals.

On-farm experiments were conducted with farmers who had access to KM either from their own cattle or from neighbours for three planting seasons; second rains of August - November 2000, first and second rains of March-June and August-November 2001, respectively. Different plots were used during each planting season. Each season, 20 farmers each as a replicate, were involved in the execution of on-farm trials.

Farmers' fields where soil samples had been analysed (Olupot *et al.*, 2003 unpubl.) were ploughed twice using oxen, as practised by the farmers. The plough depth was about 0-15 cm. Second ploughing was done two weeks after the first ploughing, to turn the decomposing plant materials. This also helped to pulverise the soil to produce a suitable tilth for the relatively small sorghum grain.

The fields were marked into experimental units of dimension 5 m x 5 m. Each of the plots within a block was separated by 0.5 m alleys. Each farmer had 7 treatments arranged in a randomized complete block design. The treatments included: T_0 (P_0+N_0+KM_0), T_1 (2.5 t KM), T_2 (17 kg P + 2.5 t KM), T_3 (45 kg N + 2.5 t KM), T_4 (8.5 kg P + 22.5 kg N + 2.5 t KM), T_5 (8.5 kg P + 2.5 t KM) and T_6 (22.5 kg N + 2.5 t KM) ha^{-1}.

Field dry KM was broadcast and mixed with the soil before planting. Five to ten seeds of the Sekedo variety of sorghum were planted at a recommended spacing of 60 cm x 20 cm (Obuo, 1995). The rationale for the choice of sekedo variety was based on its drought tolerance, resistance to birds and higher yield potential (3-5 t ha^{-1}) (Esele, 1995b).

Phosphorus as single super phosphate (7.9 % P), was spot applied once at planting, 5 cm deep and 5 cm away from the seed holes. Nitrogen as calcium ammonium nitrate (26.6 % N) was split into two doses. The first dose (25 % of the total dose) was spot applied at planting as for P, to supply some starter nitrogen (de Geus, 1967). Thinning to one seedling per hole was done during the first weeding, to give 83,400 plants ha^{-1}. The second dose of nitrogen was top-dressed at anthesis. According to de Geus (1967) and Clegg (1996), these are the critical stages for nutrient demand for grain filling in sorghum.

The crops were sprayed with Fenkil and Ambush whenever necessary, to control particularly shoot

flies and stalk borers, the common pests of sorghum. The purpose was to minimize as much as possible, the influence of external factors on the yield of sorghum. The experiments were closely monitored by both the farmers and the researchers.

Total above ground biomass of sorghum was determined at harvest, by cutting whole plants at ground level for each plot. Field dry biomass was weighed in kg per plot. The sorghum heads were then cut off from the stover and weighed in kg per hectare. The heads were then threshed per plot, and the grain weights determined in kg per hectare. The moisture content in the stover and grain were standardised by oven drying sub-samples in the oven at 70 °C for 48. The threshing percentage (TP) was calculated as the fraction of grain expressed as the percentage of sorghum head weights (HW) in kg ha^{-1} (equation 1).

$$TP = \frac{GY * 100}{HW} \quad \ldots\ldots 1$$

Where: GY = Sorghum grain yield (kg ha^{-1})

The harvest index (HI) under each treatment was estimated from equation 2 below.

$$HI = \frac{GY * 100}{AGB} \quad \ldots\ldots 2$$

Where: AGB = above ground biomass (kg)

The data collected were subjected to ANOVA using the GenStat computer programme version 6.1. The significantly different means were separated using Fischer's LSD (Steel *et al.*, 1997).

Results and discussion

Effect of combining kraal manure with mineral N and P on above ground biomass of sorghum

The second season experiment for August - November 2000 failed because of late planting which coincided with drought, coupled with devastation of the crop by the smut fungus *(Sporisorium sorghi)* (Esele, 1995a). The above ground biomass (AGB) yields of sorghum for the first and second rain seasons of 2001 in response to KM combined with mineral N and P are presented in Figure 1. All the treatment combinations influenced AGB of sorghum variably ($P < 0.05$) in both seasons. Treatment T_3 gave the highest AGB (14.21 t ha^{-1}). This yield was as good ($P > 0.05$) as 13.66 t obtained when 2.5 t KM was combined with 22.5 kg N and 8.5 kg P ha^{-1} (T_4). These yields were close to the 13.33 t ha^{-1}, which Omanya *et al.* (1996) got as a mean of 20 sorghum genotypes at the University of Nairobi Dry Land Research Field Station during the second season of their experiments. The control treatment, T_0 gave the lowest AGB (9.65 t ha^{-1}). The second season AGB was generally higher in all the treatments than during the first season. Again T_3 and T_4 significantly ($P< 0.001$) outperformed the other treatments. Application of nitrogen and phosphorus fertilisers was also instrumental in increasing the AGB yield of maize in the south-east lowveld of Zimbabwe (Nyakatwa *et al.*, 1996).

Nitrogen stimulates the vegetative growth of a plant whereas phosphorus encourages root growth and uptake of nutrients like nitrogen. These two factors contributed to the higher AGB of sorghum. Vegetative and root growth also helped both rice and wheat to attain higher straw yields in the Sudan (Ayoub, 1986; Kolar and Grewal, 1989).

Attainment of higher AGB is desirable for more utilisation of available water and uptake of nutrients. This, according to Seetharama (1995) enables a crop to thrive in drought prone areas through a mechanism known as drought avoidance. High AGB enables the crop to accumulate assimilates that

kg N (T_3) and with 22.5 kg N and 8.5 kg P ha^{-1} (T_4), respectively. The KM and mineral N and P rates used were much lower than the levels used by Clegg (1996) in the USA, Gono (1996) and Zengeni (1996) in Zimbabwe. The manure rate used was also lower than the 8 t ha^{-1} that Ikombo (1984) recommended for maize grain yield of 5 t ha^{-1} in eastern Kenya. The results highlight the crucial importance of both N and P in increasing sorghum grain yields in Kumi district.

Nutrient transfer to the grain is important in terms of the physiology of grain filling, yield and nutritive value of the grain. Mengel and Kirkby (2001) reported that the transportation of nutrients from xylem to the grain appears to be strongly influenced by the availability of water and plant nutrients in the root environment. The role of manure in moisture and nutrient retention could have accounted for the more than doubling of the grain yields obtained under the farmers' practice (T_0) during the first season, where 2.5 t KM alone (T_1) was applied. The poor quality of the manure used (1.1 % N, and 0.6 % P) (Olupot *et al.*, 2003 unpubl.), however, highlights the need to supplement it with modest quantities of mineral N and P fertilizers. This was evidenced by the more superior grain yields ($P < 0.05$) obtained where 2.5 t KM was combined with 22.5 kg N and 8.5 kg P ha^{-1}.

Nitrogen and phosphorus play a key role in increasing the leaf area index (LAI) as well as chlorophyll content in leaves of plants. These factors enabled rice and wheat to trap more photosynthetically active radiation (PAR), which increased the number of ear-bearing shoots, grains per panicle, grain weight and yield (Kolar and Grewal, 1989). Nitrogen is particularly important in determining grain number and quality, and hence yields. Its deficiency results in abortion of the initial florets, production of a small panicle with fewer primary and secondary branches, and fewer visible florets. These factors reduce yields, depending on the on-set of the deficiency. Asher and Cowie (1974) found that N deficiency between floral initiation and anthesis caused between 16 to 30% of the florets to abort whereas N stress following anthesis had little effect on grain yield, but greatly reduced grain N concentration. Phosphorus was, on the other hand, found to promote extensive lateral and fibrous root development (Hajabbasi and Schumacher, 1984). Ayoub (1986) found that phosphorus applied to irrigated rice increased uptake of soil- fertiliser-nitrogen, resulting in higher straw and grain yields. The synergistic relationship between nitrogen and phosphorus was the reason for the superior performance of sorghum where they were jointly applied.

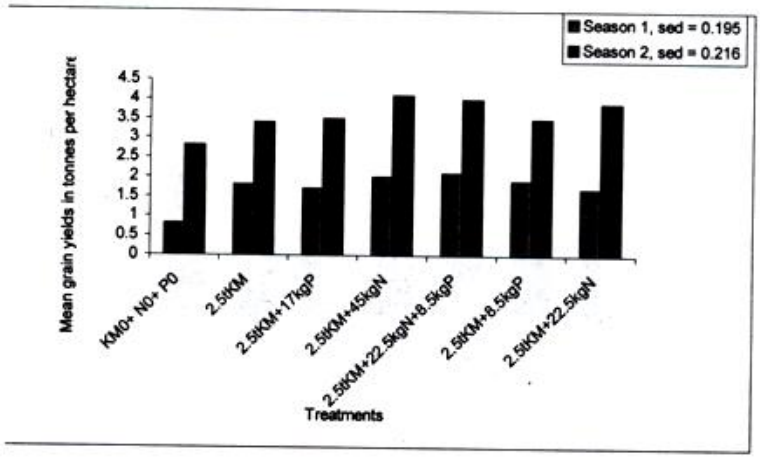

Figure 2. Sorghum grain yield response to N- and P-enriched KM for seasons 1 and 2.

Effect of KM with N and P on the threshing percentage and harvest index of sorghum

The results of threshing percentage (TP) and harvest index (HI) of sorghum are presented in Table 1. The T_0 treatment gave the poorest ($P < 0.05$) TP (51.3 %) during the first season. During the second season, none of the treatments influenced the threshing percentage of sorghum ($P > 0.05$). The results indicate that application of soil inputs is more critical in increasing the proportion of the grain in sorghum heads during the first season than during the second season.

Some farmers planted sorghum in fields where groundnuts had been harvested. Groundnuts is usually grown during the first season, whereas sorghum is mainly a second season crop. The symbiotic association between the *Rhizobia* and legumes like groundnuts was found to fix substantial quantities of N (22.5 kg ha^{-1}) Olupot *et al.* (2003 unpubl.). The symbiotically fixed N may have benefited the sorghum crops particularly in the control treatment.

The harvest index (HI) of sorghum was highly influenced ($P < 0.001$) by the various treatments. Again T_4 gave the highest harvest indices ($P < 0.001$) of 0.153 and 0.229 for the first and second seasons, respectively. Combining KM with small doses of mineral N and P fertilisers increased the proportion of nutrients that ended up in the economic (grain) yield of sorghum.

One of the major factors constraining the use of mineral fertilisers in Uganda (Bekunda and Woomer, 1996) is their high costs. Reduction in costs of sorghum production is important because the crop is grown mainly for food security reasons. Besides, the people who produce this crop in Kumi district are small scale farmers who are already resource constrained, and can not therefore, afford use of higher rates of mineral fertilisers. Interventions that minimise the costs of producing sorghum such as combining KM with small quantities of N and P may receive wide acceptance in Kumi district.

Conclusions and recommendations

Kraal manure has a potential of enhancing productivity and thus bridging the wide gap between farmers' and research station grain yields. Farmers are advised to apply 2.5 t KM ha^{-1}, if they are to benefit from its yield advantages. The poor quality of the manure suggests the need to combine it with mineral N and P fertilisers. For this particular study, combined application of 2.5 t KM with 22.5 kg N and 8.5 kg P ha^{-1} resulted in grain yields comparable to those reported in research stations.

Table 1. Sorghum threshing percentage and harvest index as influenced by the inputs.

Treatment	TP_1	TP_2	HI_1	HI_2
	— — (%) — —			
T_0 (P_0+N_0+KM_0)	51.3a	75.2	0.071a	0.185a
T_1 (P_0+N_0+2.5 t KM ha^{-1}	63.7	76.5	0.137bc	0.235b
T_2 (17 kg P+N_0+2.5 t KM)	65.2	77.3	014bc	0.219a
T_3 (P_0+45 kg N+2.5 t KM)	62.9	76.8	0.137bc	0.227
T_4 (8.5 kg P+22.5 kg N+2.5 t KM)	64.6	77.7	0.153bc	0.229
T_5 (8.5 kg P+N_0+2.5 t KM)	63.2	78.2	0.141bc	0.242
T_6 (P_0+22.5 kg N+2.5 t KM)	59.1	78.4	0.132c	0.243
CV (%)	12.4	6.5	21.7	19.3

Means followed by the same letter (s) or without any letter (s) within each column were not significantly different ($P > 0.05$). 1 & 2 (first & second seasons).

Agronomic practices should aim at ensuring timely maximum production of above ground biomass, since this later translates into higher grain yields. Further studies to establish the feasibility of this input combination and to provide plausible explanations for the more superior performance of sorghum during the second rain season than during the first season are recommended.

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The physicochemical properties of banana starches

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Abstract

The main objective of this study was to establish the physicochemical properties of native banana starches with particular reference to key granular and molecular characteristics. The design was targeted to provide for a comparison of landrace cultivar varieties (AAA-EA) with three hybrid varieties with particular reference to: *Gonja* (AAB), *Musa Kayinja* (ABB) and *Sukali Ndizi* (AB). Starch was extracted from the study samples using a modified sedimentation technique. All banana starches were established to be of exceptionally high purity. The determined characteristics included: amylose content using an amperometric technique, crystallinity and crystal type using X-ray diffraction characterisation, granular size using a laser beam technique, gelatinization endotherms using differential scanning calorimetry (DSC) and pasting viscosity using brabender visco amylograph. The amylose content ranged between 8 – 21%. The X-ray diffraction measurements showed no remarkable difference in the crystallite structure and although the crystallinity was unusually high for B crystals, all the banana starches conformed to the latter crystal type. The starch granule shape ranged between 6 - 60mμ in size. The DSC results showed the range for the maximum endotherm temperature (70.4 -72.8 °C). The results also indicated a relationship between molecular composition and unique cooked characteristics of the AAA-EA varieties while the high endotherm values and broad ranges concurred with the level of crystallinity obtained. The Brabender cycle curves were characterised by a two stage-swelling phenomenon, very high peak viscosities (1780 -1910 VE), low cook stability and extremely high setback (2080 –2940 VE). The physicochemical properties helped to explain the extensive shelf life of banana flours besides, rendering the banana starches potentially useful in a wide range of application in the food industry.

Key words: Amylose viscosity, characteristics, granular, *Musa* spp., X-ray diffraction

Background

Native starch physicochemical properties fall under two broad subtitles, granular and molecular characteristics. The granule size has been proved to be species specific with reference to the level of maturity. Many authors (Franco and Ciacco,1992; Sahai and Jackson,1996) have reported the impact of granule on starch physicochemical properties. The amylose concentration of normal starches of higher plants has also been established to be equally wide among species of the starch origin with the species range for amylose at 11-37% (Deatherange *et al.*, 1955). Consequent to conformation of starch macromolecules, starch has been proved to be partially amorphous and partially crystalline with percentage crystallinity ranging between 15 – 45% (Zobel *et al.*,1988). Starch granules are also broadly grouped according to distinction in diffraction patterns of X-rays as a result of the cell geometry and the amount of water in the packing of the granular cell. Cereals are generally characterised with the "A" pattern while roots, tuber and fruits have the "B" pattern. There is also a hybrid of both patterns known as the "C" pattern (Zobel, 1984; Imberty *et al.*, 1991; Zobel, 1992).

Zobel (1992) projected granular structure to partially account for starch properties including: granular density, resistance to chemical and enzymatic degradation and gelatinisation, gelation and retrogradation characteristics. Starch granule is generally insoluble in cold water but on heating in cold water starch undergoes the pasting process which was described by Meyer and Gibbon (1957) as constituting two opposing forces; i.e., the swelling of the granule, followed by the release of the exude (often amylose).

The latter phenomenon has the effect of increasing paste viscosity while at the same time breaking intra molecular forces within the granule, which affects granular integrity and subsequent paste mechanical properties. Consequently, the mechanical properties of starch gels have been reported to be influenced by the rheological characteristics of the amylose gel matrix, the volume fraction and rigidity of the gelatinised granule as well as interaction between the starch components (Eliason, 1986).

Viscosity studies using the Brabender visco amylograph have enabled the characterisation of starch from different sources or history with reference to six critical points as indicator parameters:

The pasting temperature - which registers the beginning of paste formation and is therefore a good indicator of the starch origin, modifications and additives present.

Peak viscosity - which is a measure of the maximum peak and an indicator of the beginning of the cooking stage.

Viscosity at 95°C - which is a measure of the ease of cooking of the starch.

Viscosity after a specified period of holding at 95°C - which gives a good indicator of the paste stability/ instability during cooking under relatively low shear.

Viscosity at 50°C - give a measure of the setback that occurs on cooling the hot paste.

Viscosity after holding at 50°C - gives a measure of the stability of the cooked paste under simulated use conditions (Zobel,1994).

The physicochemical properties of starches, therefore are good indicators of the potential for the starch application both in food and non-food industries for good consumer acceptability and textural properties. It is therefore, imperative to have appropriate data before undertaking processing/ modification of any starch or starchy raw material.

Data on some granular characteristics of banana starches has been generated by different researchers. Patil and Magar (1974) established physicochemical properties of banana starches from five banana varieties (3 of *M. cavendishii* and 2 of *M. paradiciaca*) which included granular and molecular characterisation but they established no varietal dependency. Kayisu *et al.* (1981) reported on the morphological and physico-chemical properties of banana (dessert variety) starch with an amylose of 16 %. Lii *et al.* (1982) reported chemical composition of an unspecified banana variety whose gelatinisation endotherm transition range was 74 - 81°C. Ling *et al.* (1982) characterised starch from *M. cavendishii* and found an amylose concentration of 19.5%. Eggleston *et al.* (1992) worked on plantain; plantain hybrids and cooking bananas and reported an amylose, content range of 9.11-17%, which concurred with work of earlier researchers. They suggested that properties of hybrid starches were ploidy level dependant and that ABB starches were more restricted in their swelling with high gelatinisation and pasting temperatures. They worked with low concentration and subsequently obtained no peaks but nonetheless recommended that banana starches could be applied in most applications, which require corn starch.

Starch studies to date have, however, not covered the triploid acuminata East African highland varieties (AAA-EA). This study, therefore, was conducted to establish the physicochemical properties of the starch of the cooking AAA-EA banana varieties and compare them to those of four hybrid

varieties with particular emphasis on: *Gonja* (AAB), *Musa Kayinja* (ABB) and *Sukali Ndizi* (AB). Results would help to establish whether all banana varieties could be used as composite starches as well as help line up their corresponding potential industrial application.

Materials and methods

Sample selection

Five banana varieties were selected purposefully on basis of their genotype, local use and perceived cooked textural quality. The textural classification is based on conventional market values, which were also documented by Semwanga (1996). The samples included two AAA-EA cooking varieties (one hard and one soft, *Bukumu* (Bm) and *Nandigobe* (Nb), respectively); one dessert AB (*Sukali Ndizi (SN)*) one AAB roasting plantain (*Gonja* coded *Gj)* and one ABB juice variety (*Musa Kayinja* coded *-MK*).

Starch extraction

The starch extraction procedure followed by Eggleston *et al.* (1992) was modified to suit the requirements of the different varieties as follows:

The bananas were peeled, sliced and thereafter cooking varieties were extracted using 0.045M NaOH and blended for 35 sec. The non-cooking varieties were extracted with 0.065M NaOH and blended for 45 sec. The mashes were then filtered through a muslin cloth to separate the starch, which was washed (3-5 times) and centrifuged before drying.

Starch composition

The proximate composition of starch with respect to moisture content , starch, protein and ash content was determined to help estimate the purity of the starches using standard analytical procedures.

Amylose determination

The amylose determination was based on amperometric adaptation of the dead stop titration technique, which was modified to suit the Titro Processor 670. The principle of the method is that first reported by Larsson *et al.* (1953).

X- ray diffraction characterisation

The x-ray examinations were carried out using a technique of the Fraunhoffer Institute (IAP) Germany. The tests were done using a Siemens' Diffraktometer D5000 in symmetrical transmission using a Ge-Primary monochromator. The resultant diffraction curves were interpreted using Programmes WAXS 7 which is based on Ruland – Vonk's method to establish the percentage chrystallinity (Xc %) and the disorder value (a measure of the lattice disturbance in the crystallite). After a peak separation in the corrected diffraction curves, the mean lateral crystallite size (Dhkl) was determined.

Determination of size

Particle size distribution was determined using a Laser beam system, Mastersizer of the Malvin Instruments Ltd.(U.K) version 2.15. The system details were: Range lens 300RF mm; beam-length 2.40mm; sampler MS17, Obscuration 26.6%.

Gelatinisation endotherms

The changes in the endotherm values were monitored using a Direct Scanning Calorimeter (DSC) 120,220 Seiko Instruments (Japan) calorimeter module which was integrated to a pesonal computer and printer through a Disk station. Starch slurries in a ratio of 1:5 were prepared in Al / Ag pans which were hermetically sealed and measured against water as standard. The temperature regime was between 10° C to 106°C and the heating rate was 1 K min^{-1}. The cooling at the end of each endotherm measurement was through ice. The endotherm values were calculated manually to establish peak temperature (Tp), temperature at the beginning of gelatinization (Tb), temperature at the end (Te) and enthalpy value (ΔH).

Viscosity measurements

The viscosity was measured using Brabender Visco/Amylograph following standard procedures as outlined by Haase *et al.* (1995). The starch concentration for peak formation was, however, 8.6% for the Brabender technique.

Results and discussion

Starch composition

The starch extracted from the different varieties using the procedure outlined above yielded starch which was exceptionally pure (97 -100%) (Table 1). The protein content was within a range of values obtained by Lii *et al.* (1982) but these authors obtained lower values for ash content. Eggleston *et al.* (1992), however, obtained higher values for both protein and ash content.

Granular characteristics

Particle size range lay within the maximum range of 6-60 mμ which has been quoted by different authors for starches from different banana varieties (Shantha and Siddappa, 1970, Patil and Magar, 1974, Lii, *et al.*, 1982, Eggleston *et al.*,1992). In contrast to other starches which have been measured using similar techniques, the median granular size of the study samples lay between that of the big wheat granules (19 mμ) and potato (41 mμ) (Seidemstücker and Fritz, 1997). The percentage crystallinity measurements showed no remarkable difference in the crystallite structure, but, the lateral crystallite size and crystallinity of sample 6-ABB were somewhat smaller. All the banana samples belonged to the B crystal type. The crystallinity was, however, appreciably lower in MK-ABB (Table 2). The crystallinity values were closer to the range of cereal starches which are A-crystal types (Zobel, 1984). The amylose content of the banana starches in this study was generally low. This concurs with Eggleston *et al.*,(1992) earlier report on banana starches. The data can be segregated along genotype lines whereby the hybrid varieties (non-cooking) presented higher levels of amylose.

Table 1. Banana starch composition.

**Sample	M.C %	Starch%	Protein%	Fat %	Ash %
Gj-AAB	10.7	98.6	0.1	Tr.	0.1
Bu-AAA-EA	11.2	98	0.1	Tr.	0.1
Nb-AAA-EA	11.3	99.7	0.2	Tr.	0.1
MK-ABB	10.8	97.4	0.1	Tr.	0.1
SN-AB	11.1	100	0.1	Tr.	0.1

All the data in Table 1 are means of two determinations. Starch , protein, fat and ash are determined on dry basis.

The gelatinization endotherms lay within close range of those reported by Patil and Magar (1974) for an unspecified variety and Lii *et al.* (1982) for Cavendish. They were, however, appreciably higher than those obtained for Plantain Bobby, Plantain hybrid and Bluggoe by Eggleston *et al.*(1992). T_{max} had a positive correlation to granule average particle size of r = 0.5 which agreed with the observation of Sahai and Jackson (1996) that larger granules with low crystallinity were more susceptible to heat. On the contrary *Nandigobe* (AAA-EA) which had the lowest mean particle size also had a relatively lower T_{max}

The granular characteristics on the whole showed no clear genotype or functional segregation. The X-ray diffraction data, however, tended towards the higher range (cf. potato 24%) which supports the low amylose/amylopectin ratios. The gelatinization endotherms did not show genotypic dependency but were generally high which concurred with the level of crystallinity obtained.

Pasting viscosity

Table 3 shows the results of the Brabender visco amylograph, which is a low shear. These banana starches like that studied by Kayisu (1980) did not produce peaks at concentration under 6% (s/w) which agree with the highly restricted swelling typical of the banana starches. The above results were obtained at a concentration of 8.6% (s/w) and they showed two types of swelling characteristics. Gj-AAB displayed rapid swelling but with a flat peak (Fig. 1) while the other three samples showed rapid swelling within the first few minutes of the pasting cycle. This was followed by a phase of restricted swelling of varying length commencing around 85°C which ended in sharp peaks (Fig.1). The latter behaviour is indicative of a two - stage swelling mechanism similar to that of milo starch (Leach *et al.*, 1959). However, in the case of banana starch the rapid swelling period precedes the restricted swelling. Leach *et al.* (1959) attributed the behaviour of milo starch to two sets of bonding forces within the starch granule relaxing at different temperature levels.

The high sharp peaks were accompanied by pronounced thinning on cooking for 30 minutes as evidenced by the initial and final viscosity at 92°C (Fig. 1 and Table 3). The thinning effect is indicative

Table 2 Granule particle size and X-ray diffraction measurements of bananas starches.

Sample	Particle (median) _ d mμ		Xc %	K -10^2 (nm)	Dhkl (nm)			Crystal type
	size	Range			D_{100}	D_{120}	D_{300}	
Gj - AAB	24.05	10.06-45.47	2.25	9.4	7.2	6.6	B	B
Bu-AAA-EA	22.25	8.27- 38.3	ND	ND	ND	ND	ND	ND
Nb - AAA-EA	19.67	6.42-35.19	2.39	9.1	8.5	7.1	B	B
MK - ABB	25.8	14-08-38.18	2.24	8.5	6.8	6.8	B	B
SN -AB	24.1	9.42-41.36	2.54	9.2	7.9	7.2	B	B

Xc =Chrystallinity, K = radom parameter, Dhkl = mean lateral Crystallinity values Dhkl .

TABLE 3. Banana starch granular and pasting characteristics.

Sample/ genotype	Amylose %	Gelatinisation					Amylograph Data VE			
		ΔH mJ/mg	T_{max}	T_{begin}	T_{end}	Trange	Peak max	Peakmin hold	Difference Peak-(max-min)	Peak-max after hold 50°C
Gj-AAB	19.19	12.30	70.40	66.35	76.45	10.1	1910	840	1070	2260
Bu-AAA-EA	12.94	13.20	71.25	66.75	78.05	11.3	-	-	-	-
Nb-AAA-EA	8.44	11.10	70.90	65.90	76.58	10.68	1885	910	975	2080
MK-ABB	15.49	11.05	72.5	68.00	77.28	9.28	1780	1080	700	2775
SN-AB	20.59	11.45	72.75	67.50	78.60	11.1	1870	1125	745	2940

of progressive fragmentation and solubilisation of the starch granule and therefore, a measure of cook/shear stability. The flattening of the peak of sample Gj-AAB is most likely a direct consequence of mechanical breakdown of the excessively swollen granule at this point. The percentage remaining viscosity at the end of the 30 min cook period at 92°C was 44% for Gj-AAB, 48% for Nb-AAA-EA, 60 % for SN-AB and 61% for MK-ABB. Consequently 2-AAB and 5-AAA-EA were less cook - stable, whereas samples 8-AB and 6-ABB showed only moderate thinning. At concentrations of 6% or below, however, all the banana starches have been reported to display remarkable stability to cooking with some increases in viscosity on cooking (Kayisu, 1980; Eggleston *et al.* 1992). The swelling and thinning of the paste, therefore, appear to have a high concentration dependency under low shear.

The extent of increase in viscosity at 50°C has been said to reflect the retrogradation tendency of the starch product (Mazurs *et al.*, 1957). The setback was extremely high for all the banana starches with no parallel from common native starches but perhaps only comparable to that of acid modified corn starch (Mazurus *et.al* 1957). The higher restriction to swelling of the banana varieties enabled use of high starch concentrations for pasting studies which appear to enhance the associations of molecules on cooling. The setback was more dependent on the cook/shear stability ($r = 0.9$) than on the amylose content ($r = 0.6$). In other words the effect on starch pastes of increasing starch concentration may be relative to that of using a higher amylose starch which for the same variety is only attainable through breeding.

Conclusion

The banana starches level of purity especially with respect to fat would make them generally favourable for making gum confections (Osman, 1965). The high gelatinisation temperature favours application of simple hybrid dryers without apparent loss of product quality. The low level of amylose in the cooking variety may apparently favour its soft texture as waxy starches generally have poor

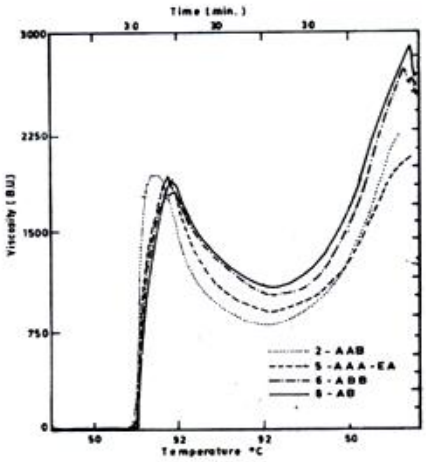

Figure 1. Brabender viscoamylograph for four banana starches.

retrogradation properties. The starches from the soft cooking banana therefore, most likely would have good thaw stability which would make it good for thickening sauces and confectioneries that require cold storage cycles. The banana high restricted swelling which necessitated the higher concentrations (>6%) to producing peak viscosity would favour the use of all the banana starches in the study with exception of Gj-AAB in similar applications that require wheat or corn starch such as replacement of wheat flour in fine bakery products. The restricted swelling also would favour: application of banana starch as a diluent in powders, the extremely long shelf-life of banana flours and chips, incorporation of higher amounts of flour in porridges/soups targeting protein energy malnutrition disorders. The restriction to swelling may also contribute to shelf stability of banana flours and may favour the incorporation of banana starches in the manufacture of biodegradable plastic where normally corn and rice starch have been used.

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Effect of threshing, drying and storage methods on purity and germination of farmer managed rice seed in Uganda

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Abstract

A total of seventy farmer-saved rice seed samples from three districts of Uganda namely Bugiri, Pallisa, and Lira were examined for purity and germination following procedures described by the International Seed Testing Association. The results revealed that postharvest handling practices (threshing, drying and storage) significantly ($P<0.001$) affect the quality of rice seed and their effects are additive as indicated by observed significant differences for the 2 and 3-way interaction effects of storage and threshing, and storage, threshing, and drying, respectively. Threshing by "beating" significantly reduced the quality of the seed while drying on cemented floors and polyethylene sheets reduced the germination percentage but maintained the physical purity of the seed. Rice seed stored in polyethene and polyline bags exhibited the highest proportion for germination and purity tests. Thus, postproduction operations need to be considered as part of an approach to minimise losses in quality of farmer saved rice seeds.

Key words: Farmer-saved seed, postharvest, seed quality

Introduction

In Uganda, much of the rice crop is produced by small-scale farmers. They save and use their own seed for rice production year after year. Under such a production system, there is little control over seed quality (Mew, 1998) yet seed quality is a key to raising a healthy crop (Amana *et al.*, 2003). Consequently, most rice farmers in Uganda raise their crop from inferior seed. Such seed are characterized by low germination percentage, weak seedlings, slow growth rate, susceptibility to stress and poor crop stand. Subsequently, poor yields are obtained, making these families more food insecure and unable to have any surplus for sale.

The production and postharvest handling practices used in rice production vary from farmer to farmer. In Uganda, the role of the different practices and their influence on seed quality is not well known since there has been little emphasis on postharvest quality management. However, with increasing commercialisation of rice production in the country, farmers need to recognise the value of high quality seed and therefore, the need for good postharvest handling practices. The aim of this study therefore was to elucidate the effect of the different postharvest handling practices used by farmers on germination and purity of rice seeds in Uganda.

Materials and methods

Rice seed sample collection

Seed samples (70) were collected from three districts of Uganda namely Lira, Bugiri, and Pallisa. These districts were selected basing on differences in agro-ecologies, production practices, history of

rice production and the fact that they are among the main rice producers in the country. Details about these districts with regard to rice production are given by Biruma *et al.* (2003).

The seed samples were collected from individual households situated at least 10-20 kilometres apart. A pre-tested questionnaire was used during seed sample collection to gather postharvest information. Questions focused on methods of threshing, drying and storage.

Purity analysis

All the 70 samples were analysed for purity following procedures outlined by the International Seed Testing Association (ISTA, 1999). Forty grams of each sample were physically separated into pure seed, other seeds and inert matter. Separation was based on examination of each particle in the sample. The respective weights of the different components were then expressed as percentage by composition.

Germination test

All the 70 samples were tested for germination capacity using paper roll method (ISTA, 1999). Four hundred (400) seeds (working sample) in four replicates each of 100 seeds were taken from each sample and placed on previously moistened germination papers following procedures described by (ISTA, 1999). Two sheets of square blotters (AGF 725-230 x 265 mm) were wetted using distilled water, leaving an adequate margin of about 2 centimetres. One hundred seeds were then placed evenly on the wet blotter and rolled. The rolls were then placed upright inside a plastic bag to avoid drying and incubated at 28 ±2°C, in alternating 12 hours near ultra violet (NUV) light and 12 hours darkness for 14 days. On the 14th day, normal, abnormal, diseased seedlings, hard seed/dead seeds were counted and placed in separate plastic petri-dishes. Seedlings were considered normal when found intact with all the essential structures or with slight defects and secondary infection. Abnormal seedlings were those with any of the essential structures irreparably damaged, deformed and or decayed. Dead seeds are those that at the end of the test period had not produced any part of a seedling (ISTA, 1999). The numbers of normal, abnormal and dead seeds was recorded.

Data analysis

All data obtained from the laboratory (purity and germination) were subjected to analysis of variance (ANOVA) and the differences between treatment means tested using Fishers' protected Least Significance (LSD) test at 5% probability level (Steel *et al.*, 1997). Quantitative data from the questionnaires were analysed using the statistical package for social scientists (SPSS).

Results and discussion

Rice postharvest handling methods

All farmers used the traditional method of sun-drying where the harvested grains was spread under sunshine. However, the drying surfaces or grounds differed among farmers and within the three districts (Table 1). Four types of drying surfaces/materials were encountered and these were; drying on bare ground, ground smeared with cowdung, polyethelene sheets, and cemented floors. Further, three storage methods were employed and included; storage in baskets (15%), polyethelene bags (62.3%), and polyline bags (22.7%). Two methods of threshing, namely, hand threshing and "beating" were commonly used. In the former method, rice panicles are squeezed between hands to dislodge grains from the panicles while in the later, grains are dislodged by beating with sticks. The relative use of the two threshing methods is given in Table 2.

Effect of postharvest practices on rice seed purity

The threshing techniques significantly influenced seed purity. Generally, hand threshed seed had higher purity percentage values compared to beaten seed (Table 3). This is probably because hand threshing results in intact seeds with minimal cases of broken seeds compared to beating. Hand threshing is also a more controlled activity with seed most likely being carefully collected in a clean container while beating to some extent is uncontrolled, with seed scattering widely, and, in the process of gathering such seeds, other materials are inevitably included in the seed which eventually reduces the quality of the seed. Proctor (1994) reported a 4% reduction in purity due to broken seed resulting from threshing by beating. In an earlier study, Chancellor (1965) noted that besides the high percentage of broken seed associated with beating, it also leads to mixing of rice with debris of varied origin notably soil and small pebbles of which winnowing does not completely remove and thus contributes to reduced purity of the seed.

Overall, drying material had a significant effect on seed purity ($P<0.05$). The highest purity was obtained from seeds dried on cemented floors fcllowed in descending order by those dried on polyethene sheets, ground smeared with cowdung and bare ground (Table 4). However, there were no significant differences in seed purity among seeds dried on polyethene and cemented floors. Nevertheless, the seed purity attributed to these two drying methods was significantly different from that attributed to bare and ground smeared with cowdung (Table 4). The present findings suggests that seeds dried on bare ground are more prone to contamination compared to the rest of the drying surfaces used. Kregyer (1972) observed that contamination with dirt of seeds dried on bare ground cannot be avoided and thus recommended plastic sheets to ensure clean dried grains. Therefore the role of the drying material in maintaining the seed quality highly depends on how best the material restricts contamination of the seed with other materials.

Storage methods significantly ($P<0.001$) influenced seed purity. The highest purity (98.2%) was obtained from seeds stored in polyline bags while the lowest (96.5%) was obtained from seeds stored in baskets (Table 5). The drastic reduction in purity of the rice seed with respect to storage methods

Table 1. Percentage of farmers using the different drying surfaces and storage methods in the districts of Pallisa, Lira and Bugiri.

Drying surface	Pallisa	Lira	Bugiri
Bare ground	48.2	0.0	27.3
Ground smeared with cowdung	33.3	9.4	9.0
Cemented floor	3.7	59.4	18.2
Polythene sheets	14.8	31.2	45.5
Storage method			
Polythelene bags	66.7	65.6	54.5
Polyline bags	18.5	31.3	18.2
Baskets	14.8	3.1	27.3

Table 2. Percentage of farmers using the two threshing methods in Lira, Bugiri and Pallisa districts.

Threshing method	Pallisa	Lira	Bugiri
Hand threshing[1]	55.6	9.4	72.7
Beating[2]	44.4	90.6	27.3
Total	100	100	100

[1] Rice grains dislodged from husks by squeezing between hands.
[2] Rice grains dislodged from husks by beating with sticks.

might be attributed to a number of factors either acting independently or in combination. For example, Verma (2002) noted that at higher moisture levels, the heat of respiration can cause much damage to stored seeds especially where the seed is not well protected from weather and this partly accounts for the very low percentage purity observed in seeds stored in baskets. On the other hand baskets are made from locally available materials, which cannot completely keep out moisture, and other materials including dust, stones and vermin, which collectively lower the purity of the seed. Thus, the implications of the present findings is that storage techniques that minimise contamination of the seed and prevent stored seed from reabsorbing moisture should be used in order to maintain the quality of the seed.

Effect of postharvest practices on rice seed germination

Hand threshed seed had significantly higher percentage germination compared to seeds dislodged from panicles through beating (Table 3). The low percentage germination obtained in seeds threshed by beating is attributed to the mechanical stress and damage imposed on the seed during the threshing process. Verma (2000) noted that traditional methods used by rice farmers such as threshing by 'stick beating' significantly affects the viability and vigour of rice seed and seedlings. Proctor (1994)

Table 3. Mean percentage purity and germination of rice as influenced by threshing methods.

Threshing method	Percentage purity	Percentage germination
Hand threshing	98.3	69.4
Beating	96.9	66.3
Mean	97.6	67.8
$LSD_{(0.001)}$	0.1	1.4
CV%	0.4	5.0

Table 4. Mean percentage purity and germination as influenced by drying methods.

Drying method	Percentage purity	Percentage germination
Bare ground	95.0	75.8
Ground smeared with cowdung	97.7	68.4
Polyethene sheets	98.6	64.2
Cemented floors	99.0	63.1
Mean	97.7	67.9
$LSD_{(0.001)}$	0.2	2.0
CV%	0.4	5.0

Table 5. Mean percentage purity and germination as influenced by storage methods.

Storage method	Percentage purity	Percentage germination
Polyethene	98.0	72.4
Polyline	98.2	72.7
Baskets	96.5	58.6
Mean	97.6	67.9
$LSD_{(0.001)}$	0.2	1.7
CV%	0.4	5.0

reported that threshing by "beating," mechanically stresses the grain and this may have a direct influence on the endogenous process of the seed and thus affects its viability. It is therefore suspected that threshing by beating reduces the germinability of the seed by negatively affecting the physiological process within the seed. However, the actual damage inflicted and or physiological processes affected remain unknown and need to be investigated.

The drying surfaces significantly affected seed germination. The highest (75.8) and lowest (63.1) germination percentage was obtained from seeds dried on bare ground and cemented floors, respectively (Table 4). Germination percentage was highest in seeds dried on polyethene sheets, and least on those dried on bare ground. The significant variations among the drying surfaces with respect to germination is likely due to three factors; heat absorption, retention and dissipation of the different drying surfaces used. Where the surface absorbed and retained a lot of heat for long hours, the grain probably suffered more damage and *vice versa*. Procter (1994) noted that excessive heat might subject the seed to hydrolytic stress and consequently reducing the viability of the seed. Kreyger (1972) reported that the viability of the grain is directly linked to the temperatures attained by the grain during drying. In particular, Chancellor (1965) and Soetoyo and Soemardi (1979) observed that temperatures above 50°C reduce the viability of the seed. Similarly, Yadav and Sharma (2000) reported that high temperatures accelerate the rate of hydrolytic enzyme activity causing more rapid detororiation and viability loss. From the foregoing, results of the present study suggests that polythene sheets and cemented floors have the capacity to absorb and retain a lot of heat compared to bare ground and ground smeared with cowdung. This explains the lower percentage germination obtained in seeds dried on polyethene sheets and cemented floor.

Storage methods significantly ($P<0.001$) affected seed germination. The highest germination (72.7%) was obtained from seeds stored in polyline bags but this was not significantly different from germination percentage of seeds stored in polyethelene bags (71.9%). However, the two storage methods resulted in significantly higher germination percentage as opposed to seed stored in baskets (58.6%) (Table 5). Studies conducted elsewhere reported similar findings. For example, Verma (2000) observed reduced viability and vigour in seeds stored in open environment than those stored in sealed polyline bags. This was attributed to fluctuations in temperature and moisture content of the stored grains (Roberts, 1972). Similarly, Copeland and McDonald (1985) reported that moisture proof containers such as polyethene bags prevent re-absorption of moisture from the atmosphere and thus, maintains the viability of the seed. In the present study, the low germination percentage observed in seeds stored in baskets was attributed to the inability of baskets to act as desiccation materials and also guard against entry of other materials including possible pathogens and insect pests (Christensen, 1972; Roberts, 1972; Copeland and McDonald, 1985; Agrawal, 1988; Biruma *et al.*, 2003). Several studies indicate that increases in seed moisture content during storage encourages insect pest and fungal activity which eventually leads to reduced viability and vigour of the seedlings. Therefore, the variations in germination with respect to different storage materials used were partly attributed to the ability of the different materials to control grain moisture fluctuations.

Conclusions

Results of this study have provided evidence that postharvest handling practices influence the quality of rice seed. The study demonstrated that storage method is an important aspect influencing seed quality as indicated by the third order significant interaction effects. Though threshing and drying affects the quality of grains, they are practices that take relatively short period and if done properly may not affect seed quality as much as storage. Despite the fact that these processes are generally recognised by farmers, the question is how best they can be applied and integrated in the postharvest production process so as to maintain the quality of the seed. From this study, seeds with both high percentage purity and germination can be obtained when the seed is hand threshed, dried on ground smeared with cowdung and stored in polyline bags. This however, may not be practical for large scale farming.

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

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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
Cpiletotrichum 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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Effect of time of harvesting, storage and fungicide seed dressing on soybean seed health and germinability

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Abstract

A key constraint to soybean *(Glycine max)* production worldwide is the rapid loss of viability after harvest. This study examined effects of time of harvesting, moisture content, storage period and fungicide treatment on seed fungal flora infection and germinability of two local soybean cultivars (Nam 1 and Nam 2). At maturity (R8 stage), a portion of the soybean crop was randomly selected from each of six fields and divided into three sections that were harvested at 90, 97 and 104 days after planting (DAP). After harvesting, seeds were dried to two moisture levels; 9.5% and 12%. For each moisture content, the seeds were divided into 2 sub-samples, one treated with Vitavax 200 FF fungicide at a rate of 3 ml kg^{-1}, and the other was left untreated (control). Each sub-sample was then packed in polyethene bags and tightly sealed and stored at room temperature (25 ± 2°C) and subsequently assayed every 30 days for three months using International Seed Testing Association procedures to determine levels of fungal flora infection and seed germinability. Results indicated that time of harvesting and fungicide treatment significantly influenced incidence of fungal flora and seed germinability, regardless of the season of growth and variety. The highest percentage germinability was recorded from the earliest harvested seeds (R8) compared to the late harvested seeds (7 - 14 days after R8). Seed dressing with Vitavax 200 FF significantly reduced levels of seed-borne fungi and improved germinability compared to the untreated seeds, although this varied with seed moisture content in storage. However, storage period had no significant effect on soybean seed germinability and incidence of most fungal micro-flora. Varietal and seasonal effects were also not significant for most fungal microflora species, except *Cercospora kikuchii* and *Penicillium* spp. The fungal microflora identified were, *Aspergillus flavus, A. niger, Alternaria* spp., *Cercospora* spp., *Cercospora kikuchii, Fusarium* spp., *F. equiseti, F. oxysporium, F. moniliforme, F. semitectum, Phoma* spp., *Phomospsis* spp., *Penicillium* spp., *Cladosporium, Curvularia* spp. and *Colletotrichum truncatum*. These results imply that soybean should be harvested at R8 stage, and seeds dressed with a fungicide so as to reduce fungal microflora incidence and increase germinability.

Key words: Fungal micro-flora infection, *Glycine max*, Uganda

Introduction

Globally, one of the most recognised problems in soybean production is the rapid loss of viability in seeds. In the United States of America, for example, farmers and seed dealers experience a lot of difficulties in maintaining viability in storage (Justice and Brass, 1978). Similarly, in Ghana, uneven seedling establishment in the field has been attributed mainly to loss of viability in storage (Nangju, 1977). In Uganda this problem has long been recognised as well (Mukasa, 1970; Leakey, 1971). Unfortunately, although several interventions were put in place to minimise the problem (USP, 1973), soybean viability continues to be low due to rapid loss of viability in storage (Kabeere, 1977; Tukamuhabwa, 1992). Furthermore, studies done elsewhere indicate that seed borne fungal inocula contribute significantly to loss of viability (Onesirosam,1986; Mycock and Berjark, 1995). Fortunately,

studies done in Ghana have shown that time of harvesting could be manipulated to lengthen viability of soybean seeds and hence, storage duration. For example, Nangju (1977) observed that soybean seeds harvested 100 days after planting registered higher germination and longer storability than those harvested at a later date. It is suspected that delayed harvesting and long storage duration promote build-up of seed-borne inocula, thus contributing to rapid loss of seed viability.

Thus, the objectives of this study were to:1) assess the effect of time of harvest on soybean seed health and germinability, 2) identify the fungal flora associated with soybean seed in storage and their effect on seed germinability, and 3) assess effect of seed moisture content during storage on soybean seed viability.

Materials and methods

Six randomly selected contract growers planted two soybean seed crops, in September 2001 and March 2002. Two local cultivars of soybean (Nam 1 and Nam 2) were used in the study, each variety being planted by three farmers. The three farmers were considered as a block with each farmer as a replicate. The seeds were planted at a spacing 60 cm between rows and 5cm within rows, placing 1 seed per hill. All required agronomic practices were done as recommended by Tukamuhabwa (2000). At maturity (R8 stage), when 95% of the pods had turned dark-gray and brown, a portion of the soybean crop was randomly selected from each of the six fields and divided into three sections that were harvested at 90 (R8), 97 and 104 days after planting (DAP). After harvesting, seeds were dried to two moisture levels, 12% and 9.5%. For each moisture content, the seeds were divided into two sub-samples, one treated with the fungicide (Vitavax 200 FF) at a rate of 3ml kg^{-1}, and the other left untreated (control). Each sub-sample was then packed in tightly sealed polyethene bags and stored at room temperature (25 ± 2°C) and subsequently assayed every 30 days for three months using standard procedures outlined by the International Seed Testing Association (ISTA, 1996) to determine levels of fungal flora infection and seed germinability.

Seed health essay

A sample of 200 seeds was drawn from each sub- sample following procedures recommended by the International Seed Testing Association (ISTA, 1996). Ten seeds were plated on three layers of moist blotters, evenly placed in each Pyrex petri- plate of 9cm diameter, previously oven sterilized. For each test, the petri-plates were arranged in a completely randomised design with three replicates. The plated seeds were incubated at 21 ± 2 ° C under alternating 12 hours of near Ultra- Violet (NUV) light and darkness to encourage sporulation of the fungal flora (ISTA, 1996). After incubation, each individual seed was examined for the presence of fungal flora infection under a stereo microscope (x 50 – 60 magnification). The fungal flora identity was ascertained on the basis of their habitual characters (Marthur *et al.*, 1992). The number of seeds infected was also recorded and these values were used to calculate the incidence (%) of each fungal species infection. The seed health assays were done once every 30 days on each sample for a period of 3 months.

Seed germination assay

Each sample was thoroughly mixed and a representative sample of 200 seeds taken and divided into four replicates each of 50 seeds. Each seed was surface sterilised by soaking in 1% sodium hypochlorite solution (Reckitt, Benkiser East Africa Limited, Nairobi Kenya) for two minutes, rinsed three times in sterile distilled water and dried between two sterile blotters. The samples were tested using "between paper rolled method" as described by ISTA (1996). The surface sterilized seeds were evenly placed on four layers of moist germination paper (newsprint) measuring 16.54"x11.69" and two layers of the

same type of paper were laid on top to cover the seeds. The bags were tied with a rubber band to prevent drying of the germination papers. The rolls were placed upright in a water-proof bag then put in a wire rack in a completely randomised design (CRD) with 3 replicates. The seeds were then incubated in the germination room and maintained at 23 ± 2 °C for 7 days. After incubation, the seedlings were examined and categorised as normal (seeds with well developed roots, hypocotyls and cotyledons) or abnormal (roots, hypocotyls or cotyledons when absent or rotten or malformed). Percent seed germination was calculated as the number of normal seeds that germinated over the total number of seeds plated/plate.

Moisture content assay

The moisture content of the seed samples was determined using the oven method. After threshing and cleaning each sample was transported to the laboratory in an air- tight package to minimize the change in moisture content. With a precision divider, a representative sample was portioned for moisture content testing. The seeds were ground, 4.5 gm put in a moisture content testing tin of known weight then oven dried at 103 ± 2 °C for 16 hours. Subsequently the samples were allowed to cool in a desiccator, reweighed and the moisture content (Wet basis) calculated as described by ISTA (1996).

Effect of storage duration on soybean seed germinability and fungal flora infection

The harvested seeds were stored at room temperature at 22-25 °C for 90 days. Periodically (once every 30 days) the seed samples were subjected to moisture content, seed health and germination tests to determine fungal flora types and population, and seed viability. Seed health and germination tests were done following procedures described earlier.

Effect of seed treatment on fungal flora infection and soybean seed germinability

For each moisture content group, the seeds were divided into two sub-samples of which one was treated with Vitavax 200FF fungicide at a rate of 3 ml kg^{-1} of seed (USP, 2001). The other half was not treated and this served as a control. Each of these sub-samples was then packed in polythene bags used by USP and stored at room temperature (25 ± 2 °C) for up to three months. All the sub-samples were assayed for seed health and germination every 30 days to determine levels of fungal flora infection and seed germinability. The laboratory assays were done following the procedure described by ISTA (1996), and as outlined earlier. Data collected for the different trials was subjected to either one or two way analysis of varience (ANOVA) using Genstat Computer program (Genstat, 1995) and standard error of the difference (SED) values obtained were used to assess the difference between two treatment means.

Results and discussion

Effect of harvesting time and seed moisture content on incidence of fungal microflora on soybean seed

Delayed harvesting significantly ($P<0.05$) increased the incidence of the majority of fungal microflora on soybean seeds during both seasons (Table 1). On the contrary, the varietal, season x harvest time interaction and harvest time x variety interaction effects were only significant ($P<0.05$) for a few fungal microflora. Also delayed harvesting significantly ($P = 0.001$) reduced soybean germinability in both seasons. However, variety and its interactions with harvesting time and season were not significant ($P>0.05$). Irrespective of the harvest period, seeds stored at 12% moisture content recorded significantly higher incidences of fungal microflora than those stored at 9.5%.

The incidence of most fungal microflora on soybean seeds, especially the storage fungi were significantly influenced by high seed moisture (Table 2). However, there was no consistent trend for the effect of moisture content on seed germination (Table 3).

Table 1. Summary of main effect of time of harvesting on incidence (%) of fungal microflora on soybean seeds harvested in 2001 and 2002.

Fungal species	Time of harvesting (days after planting)													
	2001			2002			Across seasons			Across harvest times		Across seasons and harvest times	Statistics (combined seasons)	
	90	97	104	90	97	104	90	97	104	2001	2002		CV (%)	SED
Aspergillus flavus	1.9	2.1	4.7	1.4	2.6	4.5	1.7	1.7	4.6	2.9	2.4	2.6	149.2	0.3
Aspergillus niger	2.0	2.0	5.7	1.5	3.0	5.1	1.5	1.7	5.1	3.2	2.8	3.0	150.6	0.5
Penicillium spp.	1.4	1.7	9.6	1.1	3.2	4.1	1.3	1.4	6.9	4.3	2.1	3.2	160.8	0.6
Rhizopus spp.	1.1	1.3	2.5	1.2	1.9	4.2	1.2	1.2	3.4	1.7	2.2	1.9	172.6	0.4
Cladosporium	2.3	1.7	5.5	1.9	2.8	4.2	2.1	1.4	4.8	3.2	2.4	2.8	144.7	0.5
Fusarium oxyspor	0.7	0.8	3.6	0.7	1.5	2.4	0.7	0.7	3.0	1.7	1.2	1.5	198.9	0.1
Fusarium equiseti	0.0	0.1	0.3	0.1	0.1	0.3	0.1	0.1	0.3	0.1	0.2	0.1	391.0	0.1
Fusarium semicectum	0.1	0.2	0.3	0.1	0.2	0.3	0.2	0.1	0.3	0.2	0.2	0.2	171.1	0.1
Fusarium moniliforme	0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	267.2	0.1
Other *Fusarium* spp.	0.6	0.8	0.7	0.5	0.7	0.8	0.5	0.7	0.7	0.7	0.6	0.7	171.1	0.1
Phomopsis sojae	0.6	0.6	0.5	0.5	0.5	0.6	0.6	0.5	0.5	0.6	0.5	0.5	154.7	0.1
Phoma spp.	0.4	0.6	0.5	0.3	0.4	0.3	0.4	0.5	0.4	0.5	0.4	0.4	171.5	0.1
Cercospora spp.	0.0	0.4	0.4	0.1	0.3	0.3	0.1	0.4	0.4	0.2	0.3	0.3	209.9	0.1
Collectotrichum truncata	0.0	0.2	0.5	0.1	0.2	0.4	0.1	0.2	0.4	0.2	0.2.	0.2	244.6	0.1
Cercospora kikuchii	0.4	0.2	0.3	0.4	0.3	0.2	0.4	0.2	0.3	0.3	0.3	0.3	209.9	0.1
Alternaria spp	0.4	0.2	0.4	0.4	0.3	0.4	0.4	0.2	0.4	0.3	0.3	0.3	188.8	0.1
Curvularia spp.	0.3	0.3	0.2	0.3	0.3	0.2	0.3	0.2	0.2	0.3	0.3	0.3	208.9	0.1

DAP = Days after planting; CV= Coefficients of variations, SED = Standard error of difference between means.

Table 2. Summary of effect of seed moisture content on the incidence of fungal microflora on soybean seed stored during 2001 and 2002 seasons

Fungal species	Moisture content (%) : 9.5 and 12 %							
	2001		2002		Across moisture levels		Statistics (combined seasons)	
	9.5	12	9.5	12	9.5	12	CV (%)	SED
Aspergillus flavus	1.7	4.0	1.8	3.1	1.7	3.5	152.8	0.4***
Aspergillus niger	2.2	4.3	1.7	3.8	1.9	4.1	156.7	0.5***
Alternaria spp.	0.2	0.4	0.3	0.4	0.3	0.4	189.6	0.1*
Cercospora kikuchii	0.3	0.4	0.3	0.3	0.3	0.3	210.0	0.1n.s
Cladosporium spp.	2.3	4.0	1.6	3.2	2.0	3.6	151.4	0.4***
Colletotrichum truncatum	0.2	0.3	0.2	0.3	0.2	0.3	253.1	0.1*
Curvularia spp.	0.2	0.3	0.3	0.2	0.3	0.3	208.3	0.1n.s
Fusarium equiseti	0.2	0.1	0.2	0.1	0.2	0.1	394.4	0.1n.s
Fusarium moniliforme	0.2	0.1	0.2	0.1	0.2	0.2	268.4	0.1n.s
Fusarium oxysporium	1.4	2.0	0.8	1.6	1.1	1.8	212.4	0.3*
Fusarium semitectum	0.1	0.2	0.2	0.2	0.1	0.2	266.1	0.1n.s
Other *Fusarium* spp.	0.6	0.8	0.5	0.7	0.6	0.7	171.0	0.1n.s
Penicillium spp.	3.6	5.0	1.3	2.9	2.4	4.0	182.9	0.6**
Phoma spp.	0.5	0.5	0.4	0.3	0.4	0.4	175.0	0.1n.s
Phomosis sojae	0.5	0.5	0.5	0.5	0.5	0.6	153.6	0.1n.s
Rhizopus spp.	1.2	2.1	1.4	2.9	1.3	1.2	179.5	0.3***

*,**,*** = means significant at 5%, 1% and 0.1% level, respectively; n.s = not significant at 5% level; CV = Coefficient of variation; SED = standard error of difference between means.

Effect of seed treatment with fungicide on incidence of seed- borne fungi

Fungicide treatment significantly ($P \leq 0.05$) reduced incidences of all the fungal flora species more especially in 2001 season (Table 4). Seed dressing significantly influenced germinability in 2002, but not in 2001 (Fig. 1).

Effect of storage duration on incidence of microflora on soybean seeds and subsequent seed germinability

Storage duration significantly ($P \leq 0.05$) influenced the incidence of only two fungal species namely *Fusarium semitectum* ($P = 0.023$) and *Rhizopus* spp. ($P = 0.019$). The incidence of *Fusarium semitectum* was higher on the seeds stored for 30 and 90 days, than on seeds stored for 60 days . On the contrary, the incidence of *Rhizopus* spp. was significantly higher on the seeds stored for 90 days (2.6%) compared to those stored for a shorter duration i.e.,30 or 60 days after harvest.

Pooled data for both seasons revealed that germinability of soybean seeds declined with storage duration averaging 86.3, 85.1, and 83.0% for seeds stored for 30, 60, and 90 days after harvest, respectively.

Table 3. The effect of seed moisture content on the germinability of soybean seeds grown during 2001 and 2002.

Seed moisture content (%)	Seasons		
	2001	2002	Mean
9.5%	86.81	91.02	88.90***
12%	73.56	78.93	76.26***
Mean	80.22***	85.00***	82.61ns
$LSD_{0.05}$	2.00		
CV (%)	10.9		

1*** = significant at 0.1%.

Table 4. Summary of the effect of seed treatment with fungicide (Vitavax 200FF) on the incidence of fungal microflora on soybean see d grown during the 2001 and 2002 seasons.

Fungal species	2001		2002		Across seasons		Across vitavax treatment		Across seasons and fungicide treatments	Statistics for combined seasons	
	+ Vit	-Vit	+ Vit	-Vit	2001	2002	+ Vit	-Vit		CV (%)	SED
Aspergillus flavus	0.0	5.8	0.2	4.6	2.9	2.4	0.1	5.2	2.7	122.1	0.3
Aspergillus niger	0.0	6.5	0.2	5.3	3.2	2.8	0.1	5.9	3.0	126.9	0.4
Alternaria sp.	0.0	0.6	0.0	0.6	0.3	0.3	0.0	0.6	0.3	163.3	0.1
Cercospora sp.	0.0	0.6	0.0	0.4	0.3	0.2	0.0	0.5	0.3	219.3	0.1
Cercospora kikuchii	0.0	0.7	0.0	0.6	0.3	0.3	0.0	0.6	0.3	182.9	0.1
Cladosporium sp.	0.0	6.3	0.2	4.6	3.2	2.4	0.1	5.4	2.8	118.3	0.3
Colletotrichum truncatum	0.0	0.5	0.0	0.4	0.2	0.2	0.0	0.4	0.2	230.9	0.1
Curvularia sp.	0.0	0.5	0.0	0.5	0.3	0.3	0.0	0.5	0.3	182.9	0.0
Fusarium equiseti	0.0	0.3	0.0	0.3	0.1	0.2	0.0	0.3	0.1	110.2	0.1
Fusarium moniliforme	0.0	0.3	0.0	0.3	0.2	0.2	0.0	0.3	0.2	250.3	0.0
Fusarium oxysporum	0.0	0.4	0.1	2.4	0.7	1.2	0.1	2.9	1.5	188.1	0.3
Fusarium semitectum	0.0	0.4	0.0	0.3	0.2	0.2	0.0	0.4	0.2	247.1	0.0
Other *Fusarium* sp.	0.0	1.4	0.0	1.5	0.7	0.6	0.0	1.3	0.7	1.39.7	0.1
Penicillium sp.	0.0	8.5	0.2	4.0	4.3	2.1	0.1	6.3	3.2	151.5	0.5
Phoma sp.	0.0	1.0	0.0	0.7	0.5	0.4	0.0	0.8	0.4	142.7	0.1
Phomosis sojae	0.0	1.1	0.0	1.0	0.6	0.5	0.0	1.1	0.5	117.2	0.1
Rhizopus sp.	0.0	3.3	0.2	4.2	1.7	2.2	0.1	0.7	1.2	140.1	0.2

Discussion

In this study, time of harvesting and fungicide treatment significantly influenced levels of fungal flora infestation and soybean seed germinability. The highest germinability was recorded on the earlier harvested seeds (R8) compared to the late harvested seeds (7-14 days after R8). Similarly, seed dressing with Vitavax 200 FF significantly reduced levels of seed-borne inoculum and improved germinability compared to the untreated seeds, although this depended on the level of seed moisture content in storage. Thus, the results support the hypothesis of the study that time of harvesting influences levels of seed infection by fungi and that seed dressing significantly reduces levels of the fungal infection. The low germinability associated with the delay in harvesting has been attributed to weather as well as fungal infection and accumulation of carbon dioxide (Mondrogon and Potts, 1974; Pascal and Ellis, 1978).

Unlike results of other studies on groundnuts *(Arachis hypogea)* (Rao *et al.*, 1996), sorghum *(Sorghum bicolor)* (Gupta *et al.*, 1996) and onions *(Allium cepa)* (Singh *et al.*, 1996), the germinability of soybean seeds in this study was not significantly influenced by storage period. This may have been due to the short storage duration (90 days) as well as the fact that the seeds were promptly well dried soon after harvest. Similarly, the incidence of most fungal microflora on soybean seeds was not significantly affected by storage duration, varietal and seasonal differences. This is in agreement with Bankole (1993) who also observed that in melons, one of the varieties *(Citrullus lanatus)* maintained higher germination percentage for 4 months while the other *(Citrullus vulgaris)* only for 2 months. In the present study, variety did not affect the incidence of most fungal microflora indicating that the conditions which affect soybean seed viability are similar regardless of variety being handled. However, it must be noted that these recommendations refer to Nam 1 and Nam 2 soybean varieties.

The use of fungicides in seed dressing is a widely recognized control strategy against many seed-borne fungi. According to Gay (1970), fungicide treatment of seeds is a necessary requirement for the

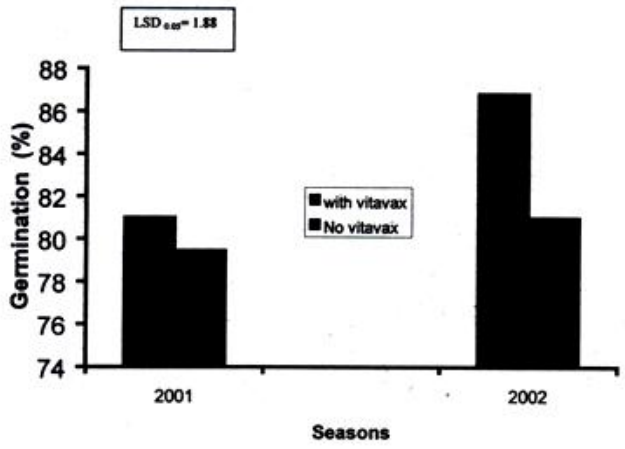

Figure 1. Effect of seed dressing with fungicide on soybean seed germinability. Only the main effect was significant.

protection of seedlings against seed- borne fungi before adult plant resistance develops. Although in this study fungicide treatment improved germinability and reduced the occurrence and the levels of fungal flora infestation, the effects were dependent on the seed moisture content.

As observed in many previous studies (e.g., Murrthy and Raveesha (1996) on soybean, Sachan and Agarwal (1994) on rice and Prokinova and Buresova (1996) on pea and barley), a number of fungal and bacterial pathogens associated with seeds of many crop species are responsible for the low germinability as well as poor quality seeds. The detrimental effects of seed-borne pathogens in soybean seeds are accentuated by long storage period under especially high moisture levels. The practical implication of our results therefore is that in order to produce soybean seeds of high quality. or viability capable of long term storage, the seeds must be harvested at R8 growth stage, dried to 9.5% moisture content and dressed with appropriate fungicide before storage.

Conclusions

The major findings of this study are that: harvesting time influences the germinability of soybean seeds irrespective of the cultivar grown. Seed harvested at physiological maturity appear to store longer and exhibit higher germinability than those harvested after R8 stage. The R8 stage would of course depend on the maturity period of the variety, in which case harvesting date would be adjusted accordingly. Since high seed moisture content in storage resulted in increased fungal infection and reduced seed viability of soybean seeds, the seeds should be dried to the required moisture content soon after harvest. The following fungi were found associated with soybean seeds; *Aspergillus flavus, Aspergillus niger, Alternaria* spp., *Cercospora* spp., *Cercospora kikuchii, Fusarium* spp., *Fusarium equiseti, Fusarium oxysporum, Fusarium moniliforme, Phoma sojae, Phomopsis* spp., *Penicillium* spp., *Cladosporium* spp., *Curvularia* spp., *Fusarium semitectum* and *Colletotrichum truncatum.* Their incidence can be reduced significantly by harvesting soybean at harvest maturity (R8), proper seed drying and seed dressing with appropriate fungicides.

Arising from the above results it appears that in order to produce seeds of high quality capable of long storage, the seeds must be harvested at R8, dried to 9.5% and dressed with an appropriate fungicide.

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Traumatic indigestion in dairy cattle in rural Kisoro district

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Abstract

Cattle (*Bos* sp.) are not selective feeders and often ingest foreign objects that cause traumatic injury in their forestomachs that result in indigestion, loss of production and death. However, the prevalence of this problem especially in rural areas is largely unknown. Traumatic indigestion, is frequently encountered in industrialised countries and is a major disease in intensively kept dairy cattle. There was need to investigate the prevalence of traumatic indigestion in rural areas. A study relying on clinical examination and use of a metal detector was conducted on herds under semi-zerograzing, zerograzing and open grazing systems in Kisoro district. Results showed that 24% of the dairy cows sampled had ingested metals with some degree of indigestion. Prevalence of metal ingestion was 61% in semi-zerograzed, 57% in zerograzed and 12% in open grazed cows. Fifteen cows received rumen magnets orally administered by a balling gun. Ten cows were operated upon with removal of various metals and other foreign objects, most spectacular being a two by two meter cotton cloth. Agricultural extension workers, farmers and veterinarians need to emphasize the importance of preventive procedures such as sorting out cattle feeds carefully, provision of mineral salts and administration of rumen magnets to minimise occurrence of traumatic indigestion and subsquently optimum production.

Key words: *Bos* sp., *reticuloperitonitis*, rumenotomy, zerograzing

Introduction

One of the tenets of modernising agriculture in developing countries is to encourage change in norms and culture to fit with changes in the global world (GoU, 2000). Kisoro district, in southwest Uganda bordering Congo to the West and Rwanda to the South, has been a region of peasant hoe cultivators keeping goats and few zebu cattle. In 1992, Muhabura Diocese (Church of Uganda) introduced dairy cattle farming. The dairy drive got further boost from European Development Fund and the Presidential donations of in-calf-heifers largely to women groups. Zerograzing has been the main system of management due to land shortage. In this system animals are confined in stalls or kraals and are hand fed with chopped herbage, fodder and household feed remains.

Cattle are careless feeders and do not discriminate against non-feed materials (Reaves and Henderson, 1963). Therefore, they often ingest foreign objects like nails, metallic wire, plastics and clothing that cause traumatic injury to their forestomachs leading to indigestion, gastritis, peritonitis or even death (Bosshart, 1926; Begg, 1950; Radostitis *et al.*, 1994) depending on how sharp they are and on how deep they perforate the internal organs. Traumatic indigestion, a complex syndrome, is an important disease in developed industrialised countries where it is responsible for great loss of production and high mortality (Frazer *et al.*, 1991; Radostitis *et al.*, 1994). Various descriptive terms such as traumatic gastritis (Bosshart, 1926), traumatic reticulitis (Hansen, 1953) hardware disease (Wrinkler, 1982) or traumatic reticuloperitonitis (Frazer *et al.*, 1991) have been used synonymously with traumatic indigestion.

Preliminary work on hardware disease in Uganda has been carried out around Kampala and in central areas of Uganda, and established that dairy cows were more prone compared to free range local cattle

(Mwanani, 1998; Bizimenyera *et al.*, 2000a, b). Since dairy farming and especially zero grazing is recent in rural Kisoro district, cases of traumatic indigestion were likely to go un noticed and would most likely result in cattle death. Some of these animals are given to farmers on condition that they give out the first calves to other women. There was need to investigate the prevalence of traumatic indigestion in dairy cattle in rural Kisoro that had recently been introduced to dairy farming to make sure that corrective measures are taken as fast as possible.

Materials and methods

A clinical study employing stratified random sampling of herds kept on zero grazing, semi-zero grazing and open grazing in each of the three sub-counties of Kisoro district was undertaken. Using cluster sampling, taking a herd as a unit, all animals within a herd were clinically examined. Eighty four (84) herds were chosen as sampling number (n) using the following formula formula:

$n = 4PQ/L^2$ (Martin *et al.*, 1987).

where:

P = suspected prevalence of the condition = 30%
L = allowable error (diagnosis difficult) = 10%
Q = L-P

Of the 84 herds selected, 30 were on zero grazing, 30 on semi-zero grazing and 24 on open grazing. Semi-zero grazing was where cows were partially confined in stalls and partially let out on paddocks.

The study relied on clinical examination and use of a metal detector (Vet-Tec ®, Alfred & Cox). Clinically, diagnosis of traumatic indigestion was based on depressed appetite, low production, loss of condition, diarrhoea and abdominal pain (Bizimenyera *et al.*, 2000 a). The metal detector screened cows for presence of metals and was applied around the xiphoid region. Clinically sick cases associated with severe loss of production or presence of metals were subjected to rumenotomy operations. The operations were carried out on the farm using a technique described by Turner and McIlwraith (1989) as modified by Bizimenyera *et al.* (in press). In this modification 15-20 ml of local anesthesia was used to block all the T13, L1 and L2 paravertebral nerves in a standing and feeding animal. Some other cows, depending on cooperation of the owner received a rumen magnet (BOVIVET ®, Kruuse) each, orally administered by a balling gun.

Results

Of the 300 cows examined, 72 (24%) were found to have ingested metals and other foreign materials with some varying degree of indigestion (Fig. 1). Metal ingestion was 61% in semi-zero grazed animals, 57% in zero grazed and 12% in open grazed animals (Fig. 2). Foreign materials recovered from operated animals included stones, plastics, nails, pieces of metallic wire, textile items, pieces of fishnet and a two by two meter cloth, the type worn by women in Kisoro (Fig. 3).

Rumen magnets were administered to fifteen cows. One of the cows doubled production from 8 liter to 16 liter of milk per day within five days of receiving the magnet. All the ten operated animals recovered well and registered improved production and condition. The cow that had ingested cloth was 8 months pregnant at the time of operation.

Discussion

The prevalence of metal ingestion of 24% in rural Kisoro was lower than 56% reported in a previous study in central (urban) areas of Uganda (Bizimenyera *et al.*, 2000 a). Developed countries report

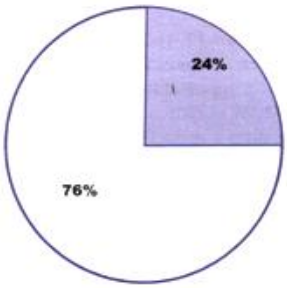

Figure 1. Overall prevalence of hardware disease.

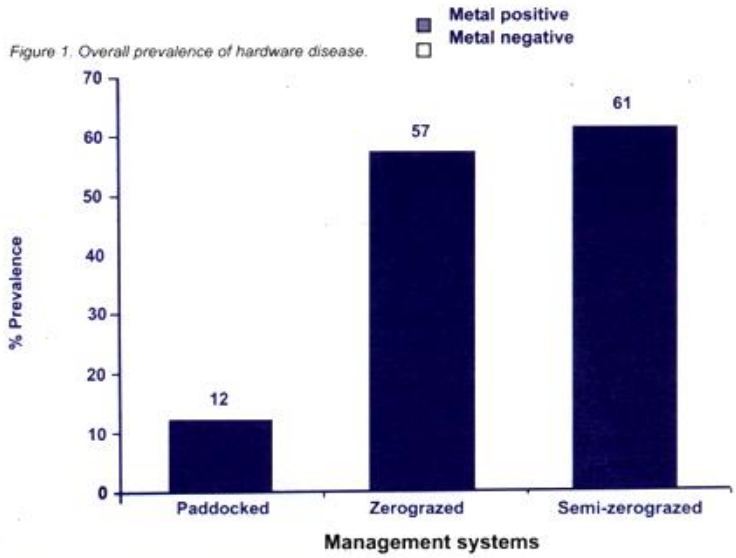

Figure 2. Prevalence of hardware disease per management system.

Figure 3. 2x2 meter cloth removed from 8 month pregnant cow by rumenotomy operation.

prevalence of 70 - 90% (Radostitis *et al.*, 1994). This indicated that traumatic indigestion or hardware disease is a widespread disease even in remote rural areas.

Zero grazing management system appeared to be a risk factor in traumatic indigestion. Mwanani (1998) reported prevalence of 82.5% in zero-grazed herds around Kampala and Bizimenyera *et al.* (2000 b) reported that hardware disease was a big problem among zero-grazed dairy cows. It would appear that in the process of gathering feed materials from household food remains, markets and restaurants, some foreign materials get carried along and accidentally are put into feed troughs. Felony or malice by neighbours has been cited also as a source of foreign objects.

Clinical diagnosis of traumatic indigestion or hardware disease has been a difficult subject since there are no pathognomonic signs (Hofmeyr, 1957; Pinsent, 1962; Bizimenyera *et al.*, 200a). Most affected animals depict depressed or low appetite, loss of production and loss of condition (as a result of low feed intake), diarrhea and abdominal pain. Where perforating metals or sharp objects are involved, there is in addition grinding of teeth (sign of pain), rough hair coat, recurrent fevers (from peritonitis) and neutrophilia. Radiological diagnosis has been described to be useful (Durchame *et al.*, 1983). Metal detectors do not tell the size or shape of the object or whether it has perforated internal organs or not. Moreover, occasionally there are metals lying harmless in the reticulum without any obvious abnormality in the animal.

Control of traumatic indigestion is as difficult as its diagnosis; both metallic and non-metallic objects cause similar signs of disease. Many methods of control have been devised with limited success. Sorting out of cattle feeds manually to remove foreign objects is the first approach. But the method is cumbersome and prone to human error and fatigue especially where there is a large number of animals. Provision of mineral salts tends to limit the depraved appetite that is licking or ingesting foreign bodies (Poulsen, 1976). Rumen magnets have proved useful in preventing traumatic injury by ferrous metals (Carrol, 1956; Lundvall, 1957) and have been administered to 90% of breeding age heifers in developed countries. However, magnets are less effective when the offending metal is non-ferrous, longer than 3 inches (the size of rumen magnet), crooked or where there are many of them at the same time. Rumenotomy operations, carefully carried out have been reported successful (Michael and McKinley, 1954; Williams, 1955; Bizimenyera *et al.*, 2000a). Bizimenyera *et al.* (in press) have reported a cost – effective operation carried out at farm premises costing $70.0 as compared to developed countries where similar operation costs $400.0. Furthermore, although some operations have failed to access foreign objects, operations, generally provide definitive diagnosis. Therefore, an appropriate combination of some of the methods of control outlined may prove more useful in practice.

In conclusion, traumatic indigestion should not be looked on as a disease of cattle in developed industrialised countries only; it is found in all intensive management systems like zero grazing even in rural areas. Agricultural extension workers, farmers and veterinarians need to emphasize the importance of preventive procedures such as sorting out cattle feeds carefully, provision of mineral salts and administration of rumen magnets for optimum production. Carefully performed rumenotomy operations are safe and can save valuable animals.

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Chemical changes during spontaneous and lactic acid bacteria starter culture fermentation of *bushera*

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Abstract

Bushera is a fermented sorghum beverage which is widely consumed in Uganda. It is not clear whether the process of fermentation affects some nutrient attributes. Thus a study was conducted to monitor changes in dry matter, total soluble solids (TSS), protein content, and composition of sorghum *(Sorghum bicolor)* storage proteins (kafirins) during spontaneous and lactic acid bacteria starter culture fermentation of *bushera* for up to 96 h at 30°C. Results show that dry matter and TSS decreased by about 41% and 58%, respectively, during spontaneous fermentation of *bushera*. Starter cultures had very little effect on the dry matter and TSS of *bushera*. Fermentation had no marked effect on protein content of *bushera*. The SDS-PAGE banding pattern showed the presence of various proteins of different molecular weights, in addition to α_1-, α_2-, β-, and γ-kafirins. The predominant kafirin protein was α-kafirin. SDS-PAGE did not show any protein degradation during fermentation.

Key words: Kaffirins, protein content, SDS-PAGE, soluble solids

Introduction

Sorghum *(Sorghum bicolor)* is a staple food for millions of people who live in the semi-arid tropical regions of Africa, Asia and Latin America (Chandrashekar and Kirleis, 1988; Rom *et al.*, 1992; Watterson *et al.*, 1993; Oria *et al.*, 1995a; Charlotte *et al.*, 1998). For consumers of sorghum-based diets, the grain represents a high percentage of protein and energy intake (Oria *et al.*, 1995a). Sorghum proteins are grouped as albumins (water-soluble protein), globulins (salt-soluble proteins), prolamins (alcohol-soluble protein) and glutelins (alkali-soluble proteins). The prolamin fraction of sorghum, kafirins is further divided into α-, β-, and γ-kafirins based on differences in solubility, molecular weight and structure (Shull *et al.*, 1991: Watterson *et al.*, 1993; El-Nour *et al.*, 1998).

A major problem associated with sorghum as a food is the poor nutritional quality of its proteins (Sastry *et al.*, 1986). The factors contributing to low-quality of protein are low solubility in aqueous media, insolubilisation of proteins by tannins present in the grain pericarp and testa and deficiencies in essential amino acids especially lysine. Additionally, sorghum proteins are unique among the plant food proteins in that they become markedly less digestible after cooking (Oria *et al.*, 1995b; Charlotte *et al.*, 1998). Studies using human subjects have shown that protein from tannin free sorghum porridge and Indian bread is poorly digested in comparison to other cereal proteins (MacLean *et al.*, 1981; Oria *et al.*, 1995b).

Some processing methods such as fermentation and extrusion have been shown to increase digestibility (Chavan and Kadam, 1989). A study on sorghum germination revealed that proteins are degraded during the process (Mazhar and Chandrashekar, 1993). However, such studies have not been extended to the effects of fermentation on the sorghum protein in traditional fermented sorghum

products such as *bushera*. *Bushera* is one of the traditional fermented sorghum beverage widely consumed in Uganda. This study was aimed at investigating the effect of spontaneous and lactic acid bacteria (LAB) starter culture fermentation on sorghum protein content and composition, total soluble solids (TSS), dry matter (DM) and sugars of *bushera*, a traditional sorghum based spontaneously fermented beverage.

Materials and methods

Sorghum flour (*Sorghum bicolor* (L) Moench) made from germinated sorghum grains was purchased from local markets in Kabale district in western region of Uganda. The flour was stored at -40°C, to prevent insect infestation, until airfreighted to the Department of Food Science, Agricultural University of Norway, and then stored at 3-4°C until *bushera* was produced.

Preparation of starter cultures

Lactic acid bacteria starter cultures were isolated frora traditionally fermented *bushera*, characterised and identified using biochemical test and API 50 CH strips and API CHL medium according to manufacturer's instructions (API system, Bio-Merieux, France). Detailed procedure for isolation and characterisation of LAB are described by Muyanja *et al.* (2002). Five pure LAB starter strains were selected and used for fermentation of *bushera* under controlled conditions. The strains used were *Lactobacillus* (Lb) *fermentum* MINF99, *Weissella* (W) *confusa* MINF8, *Lb plantarum* MINF277, *Lb brevis* MINF226 and *Lb paracasei* subsp *paracasei* MINF98. Each strain was grown in 250 ml of MRS broth, incubated for 18 hours at 30°C and centrifuged at 6000 rpm (5440 x g) for 10 minutes at 4°C (Sorvall 5RB, du pont Instruments, Delaware, USA). The cell pellets were resuspended in 25 ml of Ringers solution containing 10% glycerol and stored at -80°C until required for use.

Preparation of bushera

The *bushera* samples were prepared in 320 ml screw capped glass jars by mixing the prepared sorghum flour (30 g) with 250 ml of distilled water, and then steamed at 98°C for 30 minutes. The steamed samples were cooled to 30°C before inoculation. The *bushera* samples to be fermented spontaneously and by LAB starter cultures were treated in a similar manner except that sorghum malt (75 g) was used to initiate spontaneous fermentation. Samples for LAB starter culture fermentation were inoculated at about 7 log cfu ml^{-1}. The mixtures were incubated at 30°C and samples taken after 0, 4, 8, 12, 24, 48, 72 and 96 h. Each sampling interval was allocated a separate fermentation jar. All samples were analysed for total soluble solids and dry matter. Samples for crude protein determination and gel electrophoresis were freeze-dried (Heto Drywinner, 85, Model DW 6-85, Copenhagen, Denmark). The experiment was repeated using two independent times.

Dry matter determination

Dry matter was determined according to AOAC method (AOAC, 1995). Samples (5 g) of spontaneously and starter culture fermented *bushera* were weighed (Mettler AE, Delta Range, Switerzland) in pre-weighed aluminium dishes and dried overnight in a hot air oven at 100°C. Thereafter, samples were cooled in a desiccator for 1 h. The loss in weight was used to calculate the dry matter content. Dry matter was determined at zero time and after fermentation for 96 h. Determinations were carried out in duplicate.

Determination of total soluble solids (TSS)

Total soluble solids (°Brix) of fermenting or fermented *bushera* were determined at 20°C using an Abbe refractometer (Model IT, Atago, Japan) according to the method of Joslyn (1970).

Determination of sugars

Maltose, glucose and fructose were determined during spontaneous fermentation by high performance liquid chromatography according to Narvhus *et al.* (1998). The sugar detection was done by a Refractive Index detector (Series 2000, Perkin Elmer, Norwalk, USA). Standard sugar solutions (Sigma, St Louis, MO, USA) were used for calibration.

Determination of crude protein

Crude protein of freeze-dried fermented *bushera* was determined by the micro-Kjeldahl method (AOAC, 1995). The sorghum protein conversion factor of 5.65 was used as reported by Mossé (1990). Samples from each fermentation interval were analysed in duplicate.

Protein extraction

Proteins were extracted from freeze dried fermented *bushera* samples according to the method described by Wallace *et al.* (1990) as modified by Oria *et al.* (1995a). Freeze dried fermented *bushera* (200 mg) was weighed into a 15 ml plastic screw cap test tube and extracted with 6 ml 0.0125 M sodium borate (Merck, Dermstadt, Germany) buffer, pH 10, containing 1% (w/v) Sodium dodecyl sulphate (SDS) (Koch-Light Laboratories, Colnbrok-Bucks, England) and 2% (v/v) 2-mercaptoethanol (2-ME) for 16 h on an orbital shaker at 25°C and 280 revolutions/minute (Gallenkamp, UK). The suspension was centrifuged at 9000 rpm (8160 x g) for 10 minutes at 4°C (Beckman J2-MC, Beckman Instruments, California, USA). The supernatants were frozen at -80°C overnight and then freeze dried (Heto Drywinner, 85, Model DW 6-85, Copenhagen, Denmark).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out using a horizontal Pharmacia Phast (Pharmacia, Sweden) electrophoresis system. The running gels used were Phast Gel Homogenous (Pharmacia) with 20% polyacrylamide. Freeze-dried protein extracts (0.05 g) were diluted in 5 ml sample buffer 10% (w/v) Tris-HCl, pH 8.8, containing 1% (w/v) SDS, 2% (v/v) 2-ME and 0.05% (w/v) Bromophenol blue. The samples were boiled for 3 min and immediately cooled with ice. One microlitre of protein solution was loaded into each well. Proteins were separated and stained according to the protocol as described in Phastsystem Owners Instruction manual (1987). The proteins were fixed using a solution of 25% (v/v) glutaraldehyde, 15% (v/v) iso-propanol, 30% (v/v) ethanol and 0.03% (w/v) sodium acetate at 30°C. The gels were then washed in 10% ethanol (v/v) and 5% (v/v) acetic acid and then stained using 0.4% (w/v) silver nitrate. Gels were then developed using a solution containing 25% (w/v) sodium carbonate, 16% (w/v) sodium thiosulphate and 37% (w/v) Tris-HCl, pH 8.8). Gels were preserved in a solution with 10% (v/v) acetic acid and 10% (v/v) glycerol.

Gels were scanned to determine molecular weights using a computerised densitometer (Colour Image Scanner Model JX-330, Sharp Twain/Win Version 22x soft ware, Sharp, Corporation, Japan) and Labscan, version, 201 (Pharmacia, Sweden) The scanned gel images were analysed for band quantification using Image Master 1D Elite, version 201 (Pharmacia) soft-ware.

Molecular weights were determined from a standard curve obtained by plotting log molecular weight against relative mobility. A low molecular weight protein reference standard (LMW, 14-97 kDa)

containing phosphorylase b (97 kDa), bovine serum albumin (66 kDA), ovalbumin (45 kDA) carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa) from Pharmacia Biotech (Sweden) was used.

Results

Dry matter

The changes in dry matter during fermentation are shown in Table 1. The dry matter of *bushera* fermented by the starter cultures slightly decreased from an average of 10.3% at zero time to between 9.9 and 10.1% after 96 h of fermentation depending on the starter. In contrast, the dry matter of spontaneously fermented *bushera* significantly decreased from 13.5 to about 7.94% during the fermentation period. Initially, spontaneously fermented *bushera* had higher dry matter than starter fermented *bushera* due to the sorghum flour added to initiate the spontaneous fermentation.

Total soluble solids (TSS)

The TSS of *bushera* with or without added LAB starter cultures remained unchanged about 7.2 °Brix during the first 24 h of fermentation (Table 2). A slight decrease in TSS (6.4 - 6.8 °Brix) was observed in *bushera* inoculated with starters after 24 h of fermentation. Spontaneously fermented *bushera* showed a significant decrease in TSS after 24 h. The TSS of spontaneously fermented *bushera* was reduced from 7.2 to 3.0 °Brix after 96 h of fermentation.

Table 1. Changes in dry matter (%) in bushera during fermentation.

Time (h)	Starter cultures					
	Spontaneous fermentation	*Lb. *paracasei* MINF98	Lb. *plantarum* MINF227	Lb. *brevis* MINF8	*W. confusa* MINF8	Lb. *fermentum* MINF99
0	13.5±0.2	10.3±0.07	10.1±0.0	10.3±0.01	10.4±0.00	10.1±0.03
96	7.94±0.01	9.94±0.02	10±0.1	10.1±0.02	9.98±0.01	9.97±0.00

Values are means of two experiments. *: *Lactobacillus* (Lb.) *paracasei* subsp. *paracasei*.

Table 2. Changes in total solube solids (°Brix) in bushera during fermentation.

Time (h)	Starter cultures					
	Spontaneous fermentation	*Lb. *paracasei* MINF98	Lb. *plantarum* MINF227	Lb. *brevis* MINF226	*W. confusa* MINF8	Lb. *fermentum* MINF99
0	7.2±0.0	7.2±0.0	7.2±0.1	7.1±.0.1	7.2±0.0	7.2±0.0
4	7.1±0.1	7.0±0.0	7.0±0.0	7.0±.0.0	7.0±0.0	7.0±0.0
8	7.0±0.0	7.0±0.1	7.0±0.0	7.0±.0.0	7.0±0.0	7.0±0.0
12	7.0±0.0	7.0±0.1	7.0±0.0	7.1±.0.1	7.0±0.0	7.0±0.0
24	7.0±0.0	7.0±0.1	7.0±0.0	7.0±.0.0	7.1±0.1	7.0±0.0
48	5.0±0.0	6.5±0.0	6.9±0.1	6.5±.0.0	6.7±0.1	6.5±0.1
72	3.5±0.0	6.5±0.1	6.8±0.0	6.8±.0.4	6.7±0.1	6.4±0.0
96	3.0±0.0	6.5±0.1	6.8±0.0	6.7±.0.2	6.9±0.1	6.4±0.0

Results given as averages of duplicate determination ±S.D.

Protein content

The protein content expressed as percent of dry matter (DM), of spontaneously fermented *bushera* and of *bushera* with added starters is shown in Table 3. The protein %DM of *bushera* with LAB starters was between 9.4 and 9.6 % at zero time and varied between 9.0 and 10.2 % after 96 h of fermentation. The protein %DM of spontaneously fermented *bushera* showed about the same development as observed for *bushera* with added starters up to 48 h. After 48 h, however, an increase up to 16.5% protein in DM was observed (Table 3).

Changes in sugar content

The changes in sugar content during spontaneous fermentation are shown in Figure 1. Maltose content increased during the first 48 h from 12181 to 50233 mg kg^{-1}, and then decreased rapidly during the following 48 h to 2826 mg kg^{-1}. The glucose levels of spontaneously fermented *bushera* increased markedly from 12 to 48 h (from 6136 to 29349 mg kg^{-1}), but then decreased to undetectable level after 96 h. Fructose levels decreased from 1700 to 500 mg kg^{-1} during the fermentation period.

Table 3. Changes protein content (%) in bushera during fermentation.

Time (h)	Starter cultures					
	Spontaneous fermentation	*Lb. *paracasei* MINF98	Lb. *plantarum* MINF227	Lb. *brevis* MINF226	*W. confusa* MINF8	Lb. *fermentum* MINF99
0	10.0±0.4	9.4±0.1	9.5±0.2	9.4±.0.1	9.60±0.1	9.6±0.1
4	10.5±0.1	9.5±0.1	9.4±0.2	9.6±.0.5	10.8±0.1	10±0.1
8	10.8±0.1	9.5±0.2	9.5±0.1	9.5±.0.0	10.3±0.4	9.9±0.1
12	9.6±0.2	9.6±0.3	9.7±0.1	9.8±.0.0	10.4±0.6	9.6±0.3
24	9.9±0.1	9.8±0.1	9.5±0.2	9.5±.0.1	10.1±0.2	9.8±0.1
48	9.4±0.1	9.5±0.1	9.9±0.2	9.7±.0.1	10.1±0.0	9.5±0.1
72	12.6±0.0	9.2±0.1	10±0.2	9.3±.0.1	9.8±0.0	9.2±0.1
96	16.5±0.1	9.3±0.1	10±0.2	9.2±.0.1	10.2±0.0	9.0±0.1

Results given as averages of duplicate determination ±S.D.

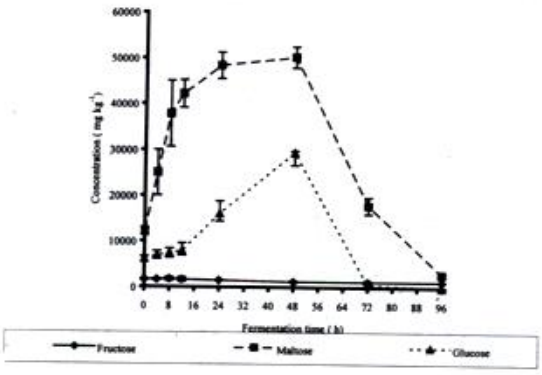

Figure 1. Changes in sugar content in bushera made from germinated sorghum flour during spontaneous fermentation. Results given as averages and standard deviation indicated by bars.

Relationship between protein content and dry matter loss

Figure 2 shows the relation between protein content and dry matter loss before and after spontaneous fermentation and fermentation with starter cultures. The loss of dry matter in form of maltose reduction was found to be high in spontaneously fermented *bushera* and negligible in *bushera* fermented with starters. For *bushera* fermented using starters, the protein content (0.97g 100 g^{-1} *bushera*) expressed on wet weight basis remained unchanged after 96 h fermentation. Protein content in spontaneously fermented *bushera* before fermentation, expressed on the wet weight basis was 13.5 g 100 g^{-1} of *bushera*. The protein content remained constant throughout the fermentation period when expressed on both wet and dry weight basis. The results indicated that the loss of dry matter does not have any effect on the actual protein content in the fermenting mixtures.

SDS-PAGE

The SDS-PAGE electrophoretograms of the *bushera* samples showed no change in band patterns during the fermentation period (Fig. 3a and b). Seven bands were detected with molecular weight 93, 63, 42, 28, 25, 23, 19, and 15 kDa. Bands of lower molecular weight between 13 and 11 kDa were also detected. Kafirins have been classified into α (M_r 25 and 23 kDa), β (20, 18 and 16 kDa), and γ-kafirins (28 kDa) on the basis of solubility, molecular weights and structure (Shull *et al.*, 1991).

Discussion

The protein content of *bushera* products did not change during fermentation when expressed on a wet weight basis (Fig. 2). However, if expressed as a percent of dry matter an increase was observed for the protein concentration (Table 3). The protein content on dry weight basis, in spontaneously fermented *bushera* after 96 h, was higher than at start of fermentation due to starch degradation to maltose and glucose followed by utilisation. This increase is apparent but the absolute amount of protein in *bushera* was unchanged and identical to 13.5g protein (Fig. 2). The apparent increase in protein became obvious when the population of yeast was at its highest (7 log cfu ml^{-1}) during spontaneous fermentation (Muyanja, 2001). This indicates that the major factor influencing the dry matter loss was the presence of yeasts since these were not present in *bushera* produced with starters.

Using the starters, the decrease in dry matter was minimal and the actual protein content remained the same. Fields *et al.* (1981) observed that dry matter losses increased from about 6% to 16.5% for maize solids: water ratio of 1:1 and 1:8, respectively after 4-day fermentation of maize meal. Wang

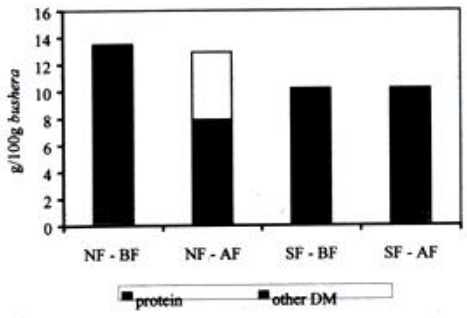

Figure 2. Changes in dry matter components during bushera fermentation. NF: Natural/spontaneous fermentation, SF: starter fermentation, BF: before fermentation and AF: after fermentation.

and Fields (1978) reported about 50% and 14% dry matter loss within 3 days during maize (1:10, solids: water) fermentation when *Saccharomyces cerevisiae* and *Candida tropicalis*, respectively were used as starters. These findings indicated that loss in dry matter is influenced by the solids:water ratio, the nature of microorganism involved and the duration of fermentation.

Spontaneously fermented *bushera* contained active amylases from the germinated flour, and this was responsible for the increase in fermentable sugars observed during the first half of fermentation. These sugars were then fermented by yeasts in the spontaneously fermented *bushera*, probably an alcoholic fermentation, producing carbon dioxide, water and ethanol. Some volatile components would be lost from the fermenting *bushera* and most probably during the drying of the samples for dry matter analysis. In *bushera* produced by starter cultures, not only was the availability of fermentable sugars much lower (Muyanja, 2001) but also the acidic fermentation became self-limiting due to the low pH. This probably accounts for the greater loss of dry matter in spontaneously fermented *bushera* as opposed to *bushera* fermented by starter cultures. The loss of dry matter can be deducted from Figure 1, where there was an increase in the fermentable sugars, which later decreased. It can be seen that the decrease in maltose and glucose (Fig. 1) corresponds approximately to the loss of DM in spontaneously fermented *bushera*.

Numerous authors have reported increase in protein content during fermentation Yousif and El-Tinay (2000) reported increase in protein during the first period of maize dough fermentation. Shayo *et al.* (2000) indicated that the protein content of *orubisil/amarwa* increased from 2.0 to 2.7% during 120 h fermentation. Azoulay (1978) reported 15 - 30% increase in protein as a result of maize fermentation with *Candida tropicalis*. Ikemefuna and Atti (1994) reported protein increase in pearl millet *(Eleuseine coracana)* with the fermentation period. El-Tinay *et al.* (1979) indicated that there was a slight increase in protein content as a result of *kisra* fermentation. Other authors have suggested that the increase in protein content is due to synthesis of proteins by microorganisms (Abdelmoneim

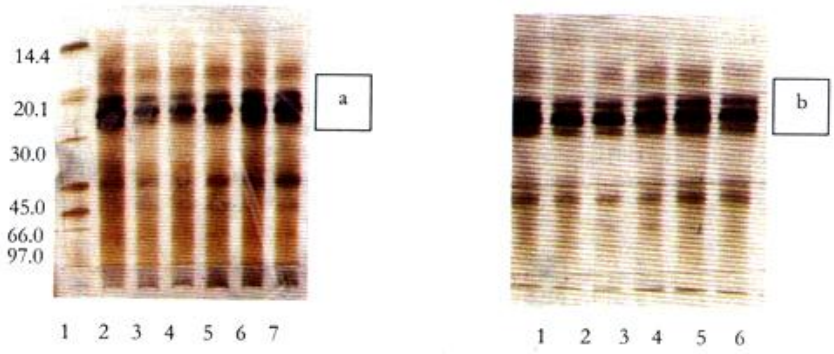

Figure 3(a). SDS-PAGE separation of sorghum kafirins. Lane 1, standard proteins (Mwt 14.4-97.0 kDA), lane 2 and 3, spontaneous fermentaiton after 4 and 8 h respectively, Lane 4 and 5 W.confusa MINF8 (S+) after 4 and 8 h respectively,lane 6 and 7, L. plantarum MINF227 (S-) after 4 and 8 h respectively.

Figure 3(b). Lane 1, spontaneous fermentation after 48 h. Lane 2, W. confusa MINF8 (S+) after 48 h, lane 3, L. brevis MINF226 after 48 h, lane 4 L. plantarum MINF227 (S-) after 48 h, lane 5 L. paracasei subsp. paracasei MINF98 after 48 h, lane 6, L. fementum MINF99 after 48 h.

N.B. All other electrophonetogram at different intervals of fermentation had simialr banding patterns. The above were chosen for their density; S+ = starch degrading strain, S- = non-starch degrading strain.

and El-Tinay, 1994). Rose (1961) as quoted by Abdelmoneim and El-Tinay (1994) in their study of microbial foods reported that microbial cell matter contains appreciable amounts of protein thus accounting for the protein content increase observed. During fermentation, some proteins will be hydrolysed as reported by Ikemefuna and Atti (1994). This may improve the digestibility of the proteins, but will not change the total amino acid content or the amount of nitrogen (as measured by Kjeldahl analysis). This means that synthesis of new proteins from amino acids resulting from proteolytic activity does not increase the total protein content unless an external nitrogen source is added to fermentation mixture.

Other researchers have indicated that fermentation has a slight or no effect on protein content of cereal-based fermented foods. Usha *et al.* (1996) reported that protein content of the millet was unaltered during fermentation. Banigo and Muller (1972) indicated that there was no protein increase during *ogi* fermentation. Hounhouigan *et al.* (1993) also showed that fermentation had only a slight effect on the crude protein content of *mawe* (maize sour dough). Kazanas and Fields (1981) found no significant difference in crude protein of unfermented and fermented sorghum meals.

Tiisekwa *et al.* (2000) reported that TSS is an important parameter, which can be used to monitor the rates of fermentation and alcohol production. This is in agreement with the results for spontaneously fermented *bushera*. However, our study suggested that TSS cannot be relied on as a parameter for monitoring controlled fermentation rates when single starters of lactobacilli are used since only small amounts of fermentable sugars are produced and reduced in relation to the amounts of lactic acid. Yousif and El Tinay (2000) reported an increase in TSS during fermentation of maize dough. Padhye and Salunkhe (1979) also reported an increase of TSS in *idli* prepared from rice and black gram. The results obtained in our study are contradictory to these findings. The contradiction may be attributed to the nature of the product and the raw material used.

The SDS-PAGE showed no differences in the banding patterns for proteins between or within the spontaneous or starter culture fermented *bushera* during the fermentation. The results suggest that there was little degradation if any, of proteins affected by the strains and during spontaneous fermentation. Similar results were reported by Mugula (2001) during *togwa* fermentation. Akinrele (1970) also reported that the predominant microorganisms isolated during the fermentation period of *ogi* showed very little degradation of maize protein. During germination, β-kafirin and γ-kafirin have been shown to be extensively degrade due to their peripheral location (Mazhar and Chandrashekar, 1993). Protein bodies are progressively hydrolysed from their outside surface (Mazhar and Chandrashekar, 1993). It seems that most of the changes in protein bands occurs during the germination of the grains. It may also be suggested that microorganisms use for their growth the easily available nutrients rather than the complex compounds, which have to be hydrolysed to simpler forms.

Conclusion

The study has shown that fermentation of *bushera* using starter cultures has little or no effect on the dry matter, total soluble solids and protein content. The reported increased protein content as observed during spontaneous fermentation of *bushera* is only apparent not absolute and is due to loss of carbohydrate dry matter as a result of their utilisation. It seems yeasts play a major role in this loss of dry matter. This study has shown that little or no protein degradation occurs during spontaneous fermentation of *bushera* and fermentation with starter cultures. The study has shown that the use of starter cultures retains the dry matter content. This is of great importance as far as energy density of the fermented foods are concerned.

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Morphological and agronomic characterisation of climbing bean genotypes

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Abstract

Common bean (*Phaseolus vulgaris*) is an important legume crop grown in Uganda providing a cheap source of protein and income to the resource poor farmers. Basing on growth habit two types of beans occur namely; bush and climbing beans. In Uganda bush beans are generally the most predominant and yet climbing beans have several advantages over bush beans. To exploit such advantages, there is need to avail climbing bean seed to the farming communities. In response, the National Bean Program has bred several varieties of climbing beans but their agronomic traits have not been evaluated. Since release of any new varieties requires characterization and field evaluation for morphology and agronomical traits in order to provide data on genotypes which could be utilized to identify superior characters that will contribute to higher yields as compared to the local varieties. The objective of this study therefore was to characterise and determine the with in genotypes and between genotype phenotypic variation of the 56 climbing bean genotype introductions, and group them in defined groups based on there similarities. Data was collected for the vegetative, inflorescence, fruit, and seed characters of each of the genotypes. Ninety one percent of the total materials in study displayed a narrow genetic diversity, and this reveals the need to collect germplasm to widen the genetic base of climbing bean genotypes. Genotypes, MAC 50, MAC 12-2, DB200-15 and BRC 19 displayed a wider genetic diversity. Genotypes MAC 12-2, LAS 400A and DB 200-15 were identified as large seeded which makes them acceptable for most consumers in Uganda. Promising genotypes with yields greater than the local checks used in the study were MAC 19-1, MAC 35 and MAC 70-2 and are recommended for advanced testing trials.

Key words: Advanced trials, germplasm, introductions, *Phaseaolus vulgaris*, Uganda

Introduction

In Uganda common beans (*Phaseolus vulgaris* L.) is the most important legume crop grown. It is a cheap source of protein and hence an important component in the diet of many Ugandans. Beans provide the bulk of protein to most families who may not afford animal protein (Ugen and Tukamuhabwa, 2000). Besides dietary importance, the bean crop is increasingly becoming an important source of income with a high demand both on the local and international markets. It was identified by Uganda government as a potential non-traditional export crop (Opio *et al.*, 2001). Basing on growth habit, two types of beans are grown, namely bush and climbing bean types.

Climbing beans have several advantages over bush beans including the following; higher yield potential of about 2-3 times higher than bush beans (Niringiye *et al.*, 1994), tolerance to major bean diseases, prolonged harvests, short cooking time and suitability for cultivation in urban areas where arable land is limited. In order to enable farmers exploit these advantages, the Uganda National beans programe has initiated projects to popularise cultivation of climbing beans, and has so far bred and released four varieties (Opio *et al.*, 2001). Further research efforts geared towards development of

other varieties with a view to provide a wide germplasm base are being undertaken. However, before release of any varieties, there is need for assessment and documentation of observed varietal attributes such as agronomic and morphological characters. The objective of this study was to evaluate the morphological and agronomic attributes of candidate climbing bean genotypes and identify varieties that have superior characteristics such as high yielding potential as compared to the other commercial varieties.

Materials and methods

Study area

The study was carried out at Kachwekono Agricultural Research Development Center (ARDC) Kabale district. Kabale receives bimodal rainfall of > 1200 mm yr^{-1} with a distinct dry season during June – July and average temperature of $\leq 20^{0}$ C (Wortmann and Eledu, 1999).

Planting material

A total of 56 climbing bean genotypes were used in the study. These included five varieties as local checks. The materials were obtained from the International Centre for Tropical Agriculture (CIAT), Rwanda. The genotypes were planted at spacing of 50 cm between rows and 20 cm within rows in a plot size of 1 row x 3 meters. The experiment was arranged in a complete randomized block design with each genotype, treatment, replicated twice.

Data collection

Data taken on various agronomic traits. Three randomly selected plants were used for data collection per plot and data were measured and recorded following established procedures outlined by the International Board for Plant Genetic Resources (IBPGR, 1982). Data were analysed using SPSS computer program version 10.0 (Sokal and Rohlf, 1997; Steel *et al.*, 1997).

The bean germplasm was placed into groups using cluster analysis in which genotypes with relatively uniform traits were assigned to a same score (number) and different if the score was significantly different. Additionally, data were subjected to a hierarchical cluster analysis using the rescaled distance cluster method. The genotypes were scored according to closeness and relative differences based on their genetic constitution, which was reflected in the different characters following procedures described by Andreas and Quellette (2000). Where there was relative uniformity, the same score or number was given and if significantly different the score was different. Qualitative and quantitative analyses were analysed separately (Chahal and Gosal, 2002). Results of qualitative analysis were presented as percentages of genotypes that exhibited a particular trait, while for the quantitative trait results presented are traits specific to a genotype.

Results

Classification of 56 climbing bean genotypes

Fifty-six genotypes were classified basing on the attributes evaluated and were grouped into 5 different clusters. The following genotypes exhibited similar morphological and agronomic attributes and were observed to belong to the same cluster: MAC28, MAC16, MAC33, MAC29, MAC70-1, MAC35, MAC70-3, MAC61-2, MAC13, MAC28-1, MAC61-1, MAC12, MAC20, MAC70-2, MAC76-2, MAC34, MAC36-1, MAC19-1, MAC36-2, MAC19-2, MAC26, MAC63, MAC64, MAC17, MAC60, MAC56, MAC12-1, MAC55, MAC76-1, MAC46, RWV1140, RWV524B, RWV1103, RWV1128-

1, RWV1128-2, RWV524A, RWV1139-1, RWV1129, RWV1105, RWV1128-2, RWV1139-2, G2331, LAS400A, LAS405, Melzwelre, RWR1134, NABE7C, NABE8C, NABE9C, NABE10C and SUG31. This implies that they have relatively close or uniform characteristics. The other genotypes MAC50, MAC12-2, DB200-15 and BRC19 were observed as different from each other with regard to morphological and agronomic attributes and each were thus grouped into a separate cluster.

Hierarchical character cluster analysis of 56 genotypes

Figure 1 illustrates the genetic distances among the 56 climbing bean genotypes based on relative closeness or difference from one genotype to another. Four clusters were reflected in the dendrogram. Highest significant difference was found for genotype MAC 12-2 which was grouped separately, this was followed by genotypes DB200-15, MAC50 and BRC19 grouped into one cluster each. Climbing bean genotypes MAC13, MAC36-2, MAC28-1, MAC29, MAC34, MAC12, MAC56, RWV524B, RWV524A, MAC20, MAC70-2, MAC76-1, MAC76-2, NABE9C, RWV1103, SUG31, RWV1140, MAC61-2, MAC63, LAS405, MAC55, MAC46, MAC61-1, RWV1132, MAC35, RWR1134, Melzwelre, MAC36-1, MAC70-3, MAC64, NABE8C, LAS400A, MAC12-1, MAC70-1, RWV1139-2, MAC28, RWV1128-1, MAC19-2 and MAC33 were all scaled in one cluster, reflecting relatively low significant differences among them. The following genotypes, RWR1129, MAC60, NABE10C, MAC16, MAC17, MAC26, RWV1105, RWV1128-2, G2331, RWV1139-1, NABE7C, MAC19-1, and RWV1138 were according to the scale grouped in one cluster with relatively slight differences within the group (Fig. 1).

Qualitative traits of the 56 climbing bean genotypes

Hypoctyl pigmentation

The study demonstrated a predominance of green hypoctyl pigmentation of the genotypes by 96.4%, followed by genotypes with occurrence of a mixture of light green and pinkish pigmentation and others with a purplish pigmentation expressed by 1.8 % of the genotypes.

Leaf shapes and persistence

Two different leaf shapes (triangular and round) and three levels of leaf rentation (when 90% of pods in a plot are dry), were observed among the 56 genotypes (Table 1). The majority of the genotypes (98.2%) had a triangular leaf shape. Types of leaf persistence observed included; all leaves dropped, intermediate and all leaves persisted. The intermediate kind leaf persistence was the most predominant type with 43 out of 56 genotypes in study (76.8%). Twelve genotypes had all leaves persisted (Table 1).

Growth habit

Climbing bean genotypes studied were grouped according to two different classes of growth habit; type IV (intermediate climbing type) and type III b (climbing ability though not well developed). The majority of the genotypes 95% possessed the indeterminate growth habit, type IV.

Colour of flower wings, standard and pods

Data on different types of flower colour (standard and wing) and pod colouration, which were observed among the genotypes are presented in Table 2. The highest frequency of genotypes (34) demonstrated a light green colour of standard. This was followed by two different colours of standard (in freshly

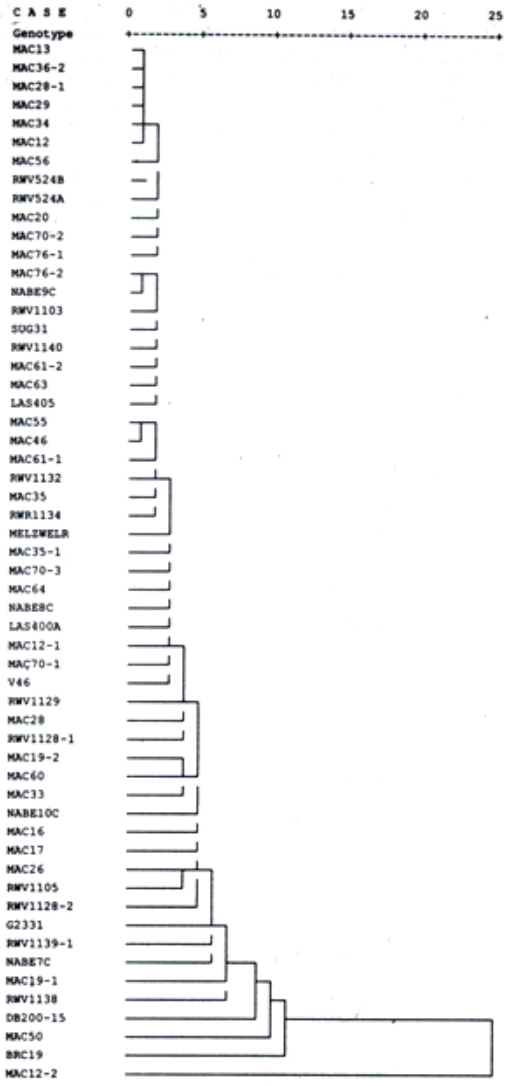

Figure 1. Hierarchical character cluster of 56 genotypes.

opened flower), purple (7 genotypes) and purplish colour expressed by 5 out of 56 genotypes. Six other colours of standard were observed; green, dark green, pink, yellowish, white and pinkish. A total of 42 genotypes had white flower colour of wing. Four other colours of flower wings observed were; purple, dark green and pinkish. Purple and purplish colour of wing among the different genotypes did not have a significant difference of occurrence (6 out 56 genotypes). Of the 56 genotypes 49 possessed light green colour of fully expanded immature pod, and 1 genotype with green pod colour. In addition, three genotypes were variable (Table 2) and exhibited a mixture of either; light green with purple red stripes (7.1%), green with pink (1.8%) or yellow with red stripes (1.8%). Genotypes with pods at

Table 1. The frequency of leaf traits of 56 climbing bean genotypes tested.

Leaf shape	Frequency	Leaf Persistence	Frequency
Triangular	55	All leaves dropped	1
Round	1	Intermediate	43
Others	0	All leaves persisted	12

Table 2. Frequency distribution of flower wing, standard and pods coloration.

Trait	Description	Frequency
Color of standard	Dark green	1
	Purple	7
	Purplish	5
	White	3
	Pinkish	3
	Green	1
	Light green	34
	Pink	1
	Yellowish	1
Color of wing	Dark green	1
	Purple	6
	Purplish	6
	White	42
	Pinkish	1
Color ofimmature pod	Yellow with red strips	1
	Light green with purple strips	4
	Green with pink strips	1
	Green	4
	Light green	49
Pod colour at physiological maturity		
	Yellow	16
	Yellow with red stripes	31
	Yellow with purple stripes	8
	Red	1
Dry pod color	Yellow	1
	Cream	24
	Cream with purple stripes	29
	Yellow with red stripes	1
	Maroon	1

physiological maturity observed having yellow with red strips were the most predominant (55.4%). This was followed by yellow colour at physiological maturity with comparatively a large number of genotypes (28.6%). Unlike yellow with purple stripes that was observed with 8 genotypes, there was only a small number (1 out of 56), which showed red pods at physiological maturity. The cream colour with purple stripes of the dry pod was the most predominant (29 out of 56 genotypes), followed by cream colour possessed by 24 genotypes. Yellow with red stripes, maroon and yellow colour were exhibited by only one genotype each.

Pod characteristics

The proportion of pod cross-section, curveture, and shape of apex, wall fiber, beak orientation and position among the genotypes tested are presented in Table 3. Two types of pod cross sections were observed; the very flat type which was the most frequently encountered (80.4%) and the pear shaped pod cross-section (19.6%). Out of the 56 genotypes 36 (64.3%) were observed with straight pods, 17 genotypes exhibited pods that were slightly curved and 3 genotypes had pods that were curved. Three shapes of the pod apex were observed; slightly curved pod apex and the curved shape were the most predominant shapes possessed by 29 and 25 genotypes, respectively. The straight pod apex shape was represented by two (2) genotypes. Pod wall fiber was expressed at three levels which included strongly contradicting, leathery pods and strong twisting dry pods (Table 4). Two pod beak positions were expressed, marginal and non-marginal and were exhibited by 46 genotypes and 10 genotypes, respectively. The genotypes were divided into two classes of pod beak orientation; up- wards (beak curving to the dorsal side) were 48 (85.7%) and 8 genotypes with straight beak orientation. Three classes of position of pods were observed. Twenty-two genotypes, had pods positioned at the base; other 22 were observed at the center and 12 genotypes (21.4%) had pods evenly distributed, from the base to the top of the plant.

Table 3. Frequency of qualitative traits on pods of climbing bean genotypes.

Trait	Description	Frequency
Shape of apex	Straight	2
	Slightly curved	25
	Curved	29
Pod curveture	Straight	36
	Slightly curved	17
	Curved	3
Pod position	Base	22
	Center	22
	Evenly distributed	12
Pod wall fiber	strongly contracting	5
	Leatherly podded	50
	Strong twisting dry pods	1
Pod beak orientation	Up- wards	48
	Straight	8
Pod beak position	Marginal	46
	Non-marginal	10
Pod cross section	Very flat	45
	Pear shaped	11

Seed characteristics

The frequency and proportion of shape and brilliance of bean genotypes is given in Table 4. Cuboid seed shape was the largest class represented by 23 genotypes. This was followed by an oval shape 35.7% and kidney shape 16.1%. Other shapes observed were truncate-fastigate (3.6%) and round seed shape. Three classes of seed brilliance were recognised; medium brilliance was shown by 39 genotypes, shiny 9 genotypes while the least was observed with matt kind of seed brilliance. There was no apparent seed veining for most of the genotypes studied (98%).

Seed colours of brown, maroon, cream and purple were common in all the seed colouration parameters (Table 5). Eight darker colours were observed on the seed coats of the 56 genotypes. The most predominant was maroon 41.1%, followed by cream, purple and pink. The other seed colours displayed 1.8% of the genotypes studied for each colour; whitish, bluish, brown and pinkish. Twelve genotypes (21.4%) were observed lacking a darker colour in the colour pattern on the seed coat. Cream was the most predominant lighter colour observed on the seed coat 51.8%, followed by maroon colour (21.4%). Others colours observed were, purple, yellow, brown whitish, pink and red. Two genotypes (3.6%) did not display lighter colour in the colour pattern of the seed coat. The majority of the genotypes (51.8%) possessed a maroon colour of ring around the helium. This was followed by brown (16.1%) and black (10.7%). The other colours observed were purple, cream and yellow. Of the 56 genotypes 1.8% had no primary colour on the seed coat. A comparatively large number of the genotype (46.4%) showed a maroon colour followed by cream colour (25), yellow and purple each represented by four genotypes. Other colours observed were; whitish and pink (3.6% each), brown, grey and red (1.8%). The largest number of genotypes (42.9%) was observed with cream, as a secondary colour on the seed coat. Only 14.3% were recorded with maroon and 10.7% with a purple colour. Other colours include; brown and bluish. Among the different colours on the seed coat and or colour pattern of the genotypes in study, 16 genotypes did not display a secondary colour on the seed coat.

Six seed coat patterns were recorded among the 56 genotypes, the most predominant were rhomboid spotted (42.9 %) and the speckled with 14.3% genotypes. These were followed by stripped, constant mottled and marginal colour pattern, respectively. Some genotypes (26.8%) were observed with a plain seed coat (i.e., no pattern observed) (Table 5).

Table 4. Frequency distribution of seed characteristics of 56 climbing bean.

Trait	Description	Frequency
Seed brilliance	Matt	8
	Medium	39
	Shiny	9
Seed shape	Round	1
	Oval	21
	Cuboid	23
	Kidney	9
	Truncate	2
	Fastigate	0
Seed coat pattern	Constant mottled	3
	Stripped	5
	Rhomboid spotted	24
	Speckled	8
	Marginal color pattern	1
	Absent	15

Analysis of quantitative traits

Table 6, shows means of quantitative traits of plant, pod and dry seed of 56 climbing bean genotypes, for all the characters evaluated. The longest hypoctyl length was observed with genotypes MAC 28-1 and SUG 31, which was 4.4 cm long, followed by lines MAC 76-2 and MAC 29 which had hypoctyl length of 4.3 and 4.1 cm, respectively. The shortest hypoctyl length of 2 cm was recorded on genotype NABE 7C which was used as the local check.

Results of days to flowering showed that the earliest genotype to flower was MAC55, which flowered within 51 days. This was followed by MAC 61-2, MAC 61-1 and BRC 19, which flowered within 52 (for both MAC 61-2 and MAC61-1) and 53 days, respectively. The five genotypes that followed flowered within 55 days (Table 6).

Period to maturity for early maturing genotypes ranged from 97 – 99 days. These included genotypes RWV 1140; RWV1139-1, RWV 1128-2, BRC19 and RWR 1134.4. The late maturing genotype MAC 36-1, took 117 days to mature. In general most genotypes (47 in number) reached physiological maturity at 100 - 109 days. Among the local checks only NABE 10C was among the early maturing genotypes.

Table 5. Frequency distribution of seed coat color and color pattern in the 56. genotypes of beans.

Trait	Description	Frequency
Seed coat primary color	Brown	1
	Marron	26
	Cream	14
	Purple	4
	Whitish	2
	Pink	2
	Red	1
	Absent	1
	Yellow	4
	Grey	1
Seed coat lighter colour	Brown	1
	Marron	12
	Cream	29
	Purple	5
	Whitish	1
	Pink	1
	Red	1
	Absent	2
	Yellow	4
Secondary seed coat colour	Brown	1
	Marron	8
	Cream	24
	Purple	6
	Blueish	1
	Absent	16
Color of ring around the hilium	Brown	9
	Marron	29
	Cream	4
	Purple	5
	Yellow	3
	Black	6
Seed coat darker color	Brown	1
	Marron	23
	Cream	9
	Purple	5
	Whitish	1
	Pink	3
	Absent	12
	Blueish	1

Table 6 Shows means for qualitative traits of the 56 climbing bean genotypes.

Genotype	*HL	DF	DOF	DM	PL	LPP	PW	PPP	SPP	NSPP	SL	SH	SW100	PSW	SYLD
MAC28-2	4.0	59	78	113	17.0	6	10.3	21	6	82	2.0	2.4	40.0	32.0	99
MAC16	4.0	62	74	104	15.0	5	5.0	14	4	31	2.0	3.0	45.0	28.0	40
MAC 33	3.0	67	79	108	14.0	5	4.1	4	5	14	2.0	3.0	52.0	4.0	19
MAC29	3.4	65	79	106	17.0	6	4.2	11	6	51	2.0	2.4	46.0	21.0	75
MAC70-1	4.0	63	82	101	14.0	6	4.4	6	6	31	2.0	3.0	55.1	16.3	51
MAC35	3.3	65	79	103	24.0	7	4.3	14	7	62	2.0	3.0	51.5	34.0	104
MAC70-3	3.2	64	79	103	18.0	6	4.0	12	6	39	2.0	2.2	44.4	18.4	58
MAC61-2	2.4	52	77	103	14.0	7	4.0	14	6	75	1.4	2.1	32.3	26.0	75
MAC13	2.4	66	80	104	15.0	7	4.2	12	6	61	2.0	3.0	51.0	33.0	96
MAC28-1	4.4	66	79	106	16.0	6	4.2	13	6	53	2.0	3.0	45.4	25.4	79
MAC61-1	3.1	52	74	102	16.0	5	4.1	18	5	51	2.0	3.0	56.0	31.2	95
MAC12	3.1	59	75	107	14.0	6	4.0	19	5	69	2.0	2.4	46.1	27.0	88
MAC20	3.0	64	75	105	14.1	6	4.0	12	6	44	2.0	3.0	41.0	20.4	55
MAC70-2	3.4	64	74	101	18.0	7	4.0	14	6	59	2.0	2.4	52.4	37.0	101
MAC76-2	4.3	64	79	111	14.0	8	4.0	15	8	69	1.4	2.4	39.1	27.0	83
MAC34	4.0	59	74	100	15.0	6	4.0	14	5	58	2.0	3.0	46.2	26.3	81
MAC36-1	3.6	65	80	114	15.0	7	4.2	21	7	63	2.0	2.3	53.0	23.0	73
MAC19-1	4.0	63	76	105	15.0	6	4.4	21	5	91	2.0	3.0	51.2	48.3	147
MAC36-2	4.0	62	79	109	14.2	6	4.5	11	6	52	2.0	3.0	51.0	29.0	89
MAC19-2	4.0	59	77	106	15.0	6	5.0	10	5	25	2.0	2.4	34.0	11.0	35
MAC26	4.0	60	80	106	30.2	7	4.1	15	7	63	2.1	3.0	51.4	31.0	95
MAC63	3.0	63	78	108	16.1	6	4.3	13	6	49	2.0	2.5	49.4	23.4	73
MAC64	3.0	59	80	108	17.0	5	4.0	17	5	55	2.0	3.0	57.1	28.0	92
MAC17	4.0	65	79	105	12.5	7	4.2	21	7	233	2.0	3.0	47.1	49.0	98
MAC60	3.1	59	77	103	12.3	6	4.2	11	5	41	1.4	2.5	27.0	12.0	39
MAC56	3.3	64	78	103	14.4	6	4.4	11	6	50	2.0	3.0	53.0	25.4	63
MAC12-1	4.0	66	79	107	15.3	6	4.2	11	6	31	2.0	2.5	49.3	22.0	62
MAC50	4.0	64	77	104	14.0	5	4.0	9	6	28	2.0	3.0	52.0	66.0	100
MAC55	4.0	51	71	105	18.0	6	4.0	10	6	46	2.0	2.5	66.0	30.1	93
MAC76-1	3.0	62	78	104	13.0	5	4.0	11	5	52	2.0	3.0	49.0	17.3	55
MAC12-2	4.0	62	74	105	15.0	6	4.3	18	6	51	1.4	2.1	63.0	34.2	77
MAC46	4.0	62	79	106	17.0	6	4.5	11	6	46	2.0	3.0	63.0	30.0	93
RWV1138	3.0	59	75	100	15.1	6	3.4	17	5	58	3.3	2.3	41.0	23.1	72
RWV1140	3.2	55	74	97	13.2	5	3.5	19	5	62	1.5	2.2	35.0	22.0	67
RWV524B	3.1	56	75	102	14.1	6	4.3	13	6	39	2.0	3.0	47.0	24.2	76

Table 6. Contd.

Genotype	*HL	DF	DOF	DM	PL	LPP	PW	PPP	SPP	NSPP	SL	SH	SW100	PSW	SYLD
RWV1103	3.2	60	75	101	15.0	6	4.0	17	4	56	2.0	2.3	45.0	24.3	76
RWV1128-1	3.1	71	73	101	14.5	6	4.5	13	5	44	2.0	3.0	50.0	21.1	69
RWV524A	3.1	60	79	100	15.5	6	4.1	13	5	49	2.0	3.0	48.0	23.0	72
RWV1139-1	3.1	60	74	98	14.0	6	4.4	16	5	61	2.0	3.0	41.0	28.4	88
RWV1132	2.3	55	74	100	17.0	6	4.0	14	5	49	3.4	2.3	53.4	31.0	95
RWV1129	4.1	57	74	100	14.1	5	4.0	14	5	41	2.0	3.0	44.3	21.0	64
RWV1105	3.1	57	75	103	17.0	6	4.0	19	4	62	2.0	2.4	45.0	30.0	92
RWV1128-2	4.0	55	74	99	13.0	6	11.0	11	5	37	2.0	2.3	45.3	15.0	34
RWV11 39-2	3.0	74	77	101	16.0	7	4.1	17	6	56	2.0	2.4	44.1	29.4	77
G2331	3.0	58	74	103	12.0	7	3.4	18	6	67	1.4	2.3	27.0	17.4	55
LAS400A	3.0	64	79	107	16.0	6	4.3	12	7	36	2.0	3.0	57.0	25.0	74
LAS405	3.5	63	78	106	15.0	6	3.8	12	6	55	2.0	3.0	54.2	28.0	87
DB200-15	3.0	58	71	103	15.0	6	4.0	12	6	55	2.0	2.3	56.0	36.0	51
DRC19	3.1	53	77	99	14.3	5	4.0	17	5	54	2.0	3.0	48.1	58.3	51
MELZWLRE	3.0	55	75	101	16.0	5	4.2	17	5	54	2.0	3.0	45.0	23.0	79
RWR1134	2.5	56	72	99	11.2	7	3.3	16	6	72	1.2	2.1	28.0	22.1	69
NABE7C	2.0	64	74	103	16.0	9	3.1	15	9	75	1.3	2.0	32.0	17.4	55
NABE8C	3.0	55	80	103	15.4	6	4.0	16	5	43	2.0	3.0	40.0	21.0	56
NABE9C	3.0	58	78	104	16.0	6	4.0	17	5	70	2.0	2.3	41.0	26.0	80
NABE10C	3.0	58	74	100	12.3	7	3.4	18	7	88	1.1	3.0	23.2	21.3	63
SUG31	4.4	63	79	105	12.3	6	6.0	12	6	49	1. 5	3.0	45.0	23.1	72
LSD (0.05)	1.0	9	7	5	7.3	1	3.4	9	2	71	0.9	0.4	10.2	23.1	49
Grand mean	3.2	60	76	104	15.2	6	4.3	14	6	56	1.7	2.5	46.2	26.6	74
Standard error	0.5	5	3	2	3.6	1	1.7	5	1	35	0.5	0.2	5.1	11.5	24
CV%	14.9	8	4	2	24.0	13	40.1	32	1	63	27.8	7.4	11.0	43.3	33

*HL = Hypoctyl length, DF =days to flowering, DOF = duration of flowering, DM =days to maturity, PL =pod length, LPP =locules per pod, PW= pod width, PPP = pods per plant, SPP =seeds per pod, NSPP = number of seeds per plant, SL =seed length, SH =seed height, 100 SW =seed weight for 100 seeds, PSW =plant seed weight and SYLD =Seed yield.

Genotypes observed with the highest pod load per plant were MAC 28-2, MAC 36-1, MAC 19-1 and MAC17 with an average number of 21 pods per plant. The lowest pod load per plant was observed with MAC 33 with an average pod load of 3 pods. This was followed with genotypes recorded with eight pods per plant. The highest average number of seeds was observed with genotype MAC17 which had 233 seeds. Genotypes MAC 19-1 and NABE 10C followed with 91 seeds and 88 seeds per plant respectively. The lowest average number of seeds per plant was observed with genotypes MAC 33, MAC 19-2, and MAC 50 (14, 25, and 28 seeds per plant, respectively).

The highest average plant seed weight (66) per plant among the 56 genotypes was exhibited by MAC 50. It was followed by genotype BRC 19 weighing 58.3 seed weight per plant. The least average plant seed weight was observed with genotype MAC 33 (4). There was significant difference among genotypes in terms of seed yield (Table 7). The seed yield was highest for genotype MAC 19-1 (0.147 kg ha^{-1}). Other high yielding genotypes identified were MAC 35 (0.104 kg ha^{-1}), MAC 70-2 (0.101 kg ha^{-1}) and MAC 50 (0.100 kg ha^{-1}). The least seed yield was observed with genotype MAC 33, RWV 1128-2 and MAC 19-2 (0.019 kg ha^{-1}, 0.343 kg ha^{-1} and 0.035 kg ha^{-1}, respectively).

The longest pod length was observed with genotype MAC 26 (30.2 cm) followed by MAC 35 (24 cm) and NABE 9C (20 cm). The lowest pod length 11.2 cm was observed with genotype RWV 1134 followed by G2331. The highest pod width was 11 cm observed in genotype RWV 1128-2, while the genotypes that followed, MAC 28-2, SUG 31 and genotypes MAC 16 and MAC 19-2 exhibited average pod width of 10.3cm, 6 cm and 5 cm, respectively. The least pod width was observed with genotype NABE 7C measuring 3.1 cm. Genotype NABE 7C was identified as having the highest number of locules per pod (9 locules), followed by, MAC 76-2 having 8 locules per pod. The least number of locules observed per pod was 5. Most of the genotypes (60.7%) were observed with six locules per pod. The highest number of seeds per pod was observed with genotype NABE 7C and three genotypes MAC 16, RWV 1103 and RWV 1105 were not significantly different with regard to an average number of seeds per pod. Twenty-two genotypes were observed with an average score of 5 seeds per pod (Table 6). Local varieties NABE 7C and NABE 10C are among the genotypes with a high number of seeds per pod.

Genotype NABE 10C was observed having the lowest seed length of 1.1 cm followed by genotypes RWV 1134 and NABE 7C with a seed length of 1.2 cm and 1.3 cm, respectively. The highest seed length was observed with genotype RWV 1132 (3.4 cm), while genotypes (RWV 1138 and MAC 26) that followed were observed with an average seed length of 3.3 cm and 2.1 cm. Forty-three genotypes were noted to have similar seed length (seed length of 2 cm). Among these were the local checks NABE 8C and NABE 9C. Thirty genotypes were observed with a highest average seed height of 3 cm. Amongst the genotypes that have 3 cm average seed height, are local checks, NABE 10C, NABE 8C and SUG 31. These were followed by MAC 19-1 with an average seed height of 2.7 cm. Local check NABE 7C was observed having the lowest average seed height of 2 cm.

Only one genotype had a weight of 100 seeds less than 25 gm (small seeded), ten genotypes had 100 seed weight of 25 gm to 40 gm (medium seed size) and the largest number of the genotypes (80%) weighed 41 and above. The weights were highest for genotype MAC 55, weighing 66 gm for 100 seed weight. The least average weight for 100 seeds was observed with genotype NABE 10C weighing 23.2 gm. Only one genotype had a weight of 100 seeds less than 25 gm (small seeded), ten genotypes had 100 seed weight of 25 gm to 40 gm (medium seed size).

Correlation between quantitative traits of climbing bean genotypes

The correlations among traits of climbing beans studied are presented in Table 7. Days to maturity were negatively associated with days to flowering and seed yield. Other negative correlations observed were between duration of flowering and days to maturity, pods per plant and number of seeds per pod. 100 seed- weight was positively correlated with number of locules per pod and seed yield was constantly correlated with all the traits in study.

Table 7. Correlation coefficients among qualitative traits measured from 56 climbing bean genotypes.

Traits	*DF	DM	DOF	HL	LPP	NSPP	PL	PPP	PSW	PW	SH	SL	SSP	SW	
1. Conastant	1.000														
2. DF	-0.184	1.000													
3. DM	-0.547	-0.040	1.000												
4. DOF	-0.197	-0.116	-0.492	1.000											
5. HL	0.035	0.021	-0.152	-0.063	1.000										
6. LPP	-0.127	-0.182	-0.071	0.027	-0.043	1.000									
7. NSPP	0.065	-0.019	0.120	-0.137	-0.100	-0.090	1.000								
8. PL	0.024	-0.052	0.113	-0.163	-0.016	-0.105	0.093	1.000							
9. PPP	-0.021	0.028	-0.253	0.105	0.162	-0.065	-0.325	-0.130	1.000						
10. PSW	0.007	0.051	0.060	0.089	-0.033	-0.018	-0.303	-0.074	-0.373	1.000					
11. PW	0.085	0.028	-0.134	0.032	-0.205	-0.014	-0.018	-0.047	-0.035	0.027	1.000				
12. SH	-0.218	-0.042	0.126	-0.333	-0.080	0.126	0.007	0.064	0.022	-0.175	-0.048	1.000			
13. SL	-0.172	0.126	0.009	0.016	0.086	-0.106	-0.006	-0.098	-0.077	-0.013	0.027	-0.030	1.000		
14. SSP	0.130	0.031	-0.163	0.002	0.194	-0.662	-0.138	-0.084	0.222	-0.045	0.002	-0.110	0.229	1.000	
15. SW 100	0.13	-0.147	-0.268	0.061	-0.077	0.345*	0.092	-0.250	0.282	-0.380	0.0461	-0.109	-0.222	-0.135	1.000
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

*HL = Hypoctyl length, DF =days to flowering, DOF = duration of flowering, DM =days to maturity, PL =pod length, LPP =locules per pod, PW= pod width, PPP = pods per plant, SPP =seeds per pod, NSPP = number of seeds per plant, SL =seed length, SH =seed height, 100 SW =seed weight for 100 seeds, PSW =plant seed weight and SYLD =Seed yield.

* Significant at $p < 0.01$.

Discussion

The results of this study reveal a narrow diversity among the population of the genotypes studied. Genotypes, which were grouped in one cluster, were derived from the same parents or related ones, reflecting narrow genetic background. Ninety one percent of the total materials tested therefore, belong to the same group.

It is also deduced that due to breeders' preference of using mostly intraracial populations in the past (Welsh *et al.*, 1995), has resulted in reduced genetic variation. This implies that a wider genetic base of genotypes is highly recommended for the bean-breeding programme (Escribano *et al.*, 1994). Advanced testing could consider genotypes MAC50, MAC 12-2, DB 200-15, BRC19 and MAC 12-2 (Fig. 1) which exhibited significant polymorphic status.

It is most likely that 91% of the climbing bean genotypes studied were hybridized from intraracial population (Welsh *et al.*, 1995). This accounts for the limited variation observed among the genotypes. Thus, growing interest in hybridizing and or combining interracial species to produce heterogenous populations is necessary in order to enlarge the genetic base for more durable and increased levels of resistance to both biotic and abiotic stress factors affecting bean production.

The results of this study suggest that variation of climbing bean genotypes tested is narrow to be used effectively in the breeding programmes. This calls for collection of more germplasm to widen the genetic base of climbing bean germplasm at Namulonge.

Based on results from the study, two types of growth habit were observed. The distinctions among the two growth habits may be explained as a result of differences in internal correlation among the plant parts. It may also be reasoned that the substantial variation in growth habit especially for type IIIb, could be due to environmental effects (soil fertility, plant density, availability of support). Flower colours (standard and wing) produced varied greatly, due to the genetic constitution of the genotype. It was observed that, flower colour (of standard and wing), leaf shape and seed shape could be used to classify beans into Andean versus middle American gene pools and subsequently into races (Allen *et al.*, 1996). Most of the genotypes possessed green colour (58.9%) and dark green colour (39.3%) of leaf chlorophyll. This could be attributed to the ability of the plant to sustain (manufacturing food adequately) the different phases of plant development, which tend to overlap from flowering to maturity. It could be deduced that lack of colour of anthocyanin within leaves, observed in all the genotypes originates from intraracial populations, confirms a narrow genetic constitution for the bean germplasm used.

Climbing bean varieties are reported to having been characterized by having phased flowering and pod formation trait (Debeouck *et al.*, 1986). Due to variation in genetic constitution, some genotypes pod set is concentrated at the base of the plant while in others is at the middle or upper part of the plant. In other genotypes pods are evenly distributed along the plant. It could be deduced that genotypes with evenly distributed pods have high pod load. It was reported that, the presence or absence of fibers in the pod wall and suture is used to identify the French (snap or green) bean from dry bean (Zeven *et al.*, 1999). Pod wall fiber is associated with pod texture. Pod texture would be expected to demonstrate genotypes that will either shatter excessively (with strong twisting dry pods), leatherly podded (dry pod will not spontaneously open) or strongly contracting. Information in this study also explain the fact that bean growers use pod texture to describe the bean varieties using traits such as pod wall fiber, plant growth habit, seed colour pattern and use (Zeven *et al.*, 1999).

The genotypes used in the study were improved climbing bean varieties (indeterminate growth habit), and almost mature uniformly despite phased flowering and pod formation. This explains why the majority of the genotypes (76.8%) were with intermediate of leaf persistence while only 21.4% of the genotypes had all leaves persist up to drying time.

Debeouck *et al.* (1986) reported that Pods can be uniform in colour or streaked and that differences can exist between the immature pod stages and between the mature and completely dry pods. In general

this information agrees with results obtained in the study. Pod colouration could also be attributed to particular genotypes. Colour variation may be related to the different genotypes used in the study.

Seeds are reported to have either one colour or they may have a predominant primary colour along with a secondary colour (Schoonhoven *et al.*, 1987). This information is in agreement with the results of this study. Colours may also be arranged in different patterns, such as mottled and striped. The variations observed among genotypes about seed colour, colour pattern, brilliance and seed shape could form a basis characterising of bean seed for growers and consumers. The majority of the genotypes possessed seeds with medium brilliance (69.6%) and very few were shiny. It should be noted that this character attracted the special attention of the breeders and or farmers. From the different seed colours and pattern observed, it could be concluded that maroon and cream were the most predominant colours. Debeouck *et al.*, 1986 reported that, seeds vary widely in colour, shape brilliance and also colour combinations. Thus, such variability is important for the classification of the genetic diversity existing among the bean varieties used in the study.

Pod shape and pod cross-section, together with other quantitative traits such as seed size, number of seeds per pod and number of pods per plant, determine the reproductive adaptation of the different genotypes, which is more defined by the pod load (Schoonhoven *et al.*, 1987). Nonetheless, characterising bean genotypes using qualitative traits was possible to identify the percentage of genotypes exhibited by each trait.

Considerable variation among the 56 genotypes was recorded for each of the 15 quantitative traits analysed. Analysis of variance showed that significant differences existed among the genotypes for each trait evaluated. Ranges of variation displayed a wide diversity among the genotypes for all the characters studied. In general, these results are in agreement with those presented by Escribano *et al.* (1994). These results prove that the genotypes used in the study have a wide genetic base in terms of quantitative traits. This variation could be used in a breeding programme to improve yield, considering that the yield of the current cultivated bean populations are usually rather low.

Increase in number of days to maturity resulted in more pods per plant, observed genotypes were MAC 28-2 and MAC 36, appearing as both late maturing and having a high pod load. Genotype MAC 28-2 was also observed among genotypes with high seed yield. It is clear that, different genotypes have either the same or varying maturity period, hence influencing the duration of the stages of development in the bean plant. Fernando *et al.* (1985) reported that maturity period causes important differences in the development of genotypes of same growth had it. In general it could be concluded that there is a close genetic proximity in maturity trait among the released climbing beans, NABE 7C and NABE 12C (released currently), which lies between 95 – 115 days. The remaining genotypes in the study were observed to fall in the same ranges.

The results of analysis of pods length and width reveal that pods increase in size depending on the genotypes. The variation in pod size deferred greatly despite the fact that the genotypes were of the same growth habit, this could be due to genotypic constitution. This was confirmed in the report by Fernando *et al.* (1985) that 15 – 20 days after flowering, valves increase in weight markedly after pods have reached their maximum size and weight. Studies by Escribano *et al.* (1994) reported that large pod and seed size of some population, such as PHA-0124, PHA-0196, PHA-0201, PHA-0255 and PHA0256, make them acceptable to consumer and industry. Results of this study, indicated genotypes MAC 12-2, LAS 400A and DB 200-15 to produce large seeds.

Agronomical traits such as days to maturity, pods per plant, seeds per pod and yield were variable. This implies that they are affected by environmental influences. Welsh *et al.* (1991); reported that variation of agronomical traits for morphological, proteins and isozyme markers were larger in interracial populations than in intraracial populations. Seed weight was not positively associated with many quantitave parameters in the study. This could be explained in relation to the inherent characters of the different genotypes. Locules that had premature dead seeds could have attributed to the negative correlation with number of seeds per plant and positive correlation with 100 seed weight. This should explain for the negative correlation between 100 seed weight and number of seeds per pod. Seed weight

in terms of grams of 100 seeds randomly chosen expresses the seed size (Schoonhoven *et al.*, 1987) and the majority of the lines (43) were expressed as large seeded (> 40 gm per 100 seed weight). Report by Gepts and Bliss 1985, grouped the small seed size (100 – seed weight < 40 gm), and the large seed size (100 – seed weight > 40gm), into two different major forms; the Middle American and Andean South America, respectively. Information from the study reveals the forms, which could be of interest in using them in the crossing programmes to enlarge the genetic base. The variability in seed size is the deduced as extremely important for classification of the genetic diversity existing among the large number of bean varieties within the 56 genotypes used in the study.

Thirty-three genotypes have a yield in range between 71 – 147 g plot^{-1}, some of these genotypes could play part in breeding program with a goal to improve yield but also to maintain good seed quality at the same time. The mean yield of 31 genotypes were higher than that of the local checks. This demonstrated the greater usefulness of new introductions for increasing yield potential in common bean (Welsh *et al.*, 1995). Yield variations among the different genotypes could also be subjected to environmental influences. It could also be deduced that genotypes with low yields originate from intraracial populations than interracial. Welsh *et al.* (1991) revealed that, more high yielding lines were from interracial populations than the intraracial populations. The performance of 9% of the genotypes in study was promising and it was observed that they scored desirably (average means) in a number of different traits, though not consistently. They are thought to be originating from interracial populations basing on the explanation above. In general yields of the promising genotypes were below the recommended yield for climbing beans (2500 - 4000 kg ha^{-1}). It was observed by Escribano *et al.* (1994), that dry bean productivity is poor in general, so it is important to find the genotypes with high yields. Further studies revealed that some of the promising genotypes PHA-0029, PHA-0028, PHA-0315 and PHA0419 (with yields > 70 gm per plant) could be parents in breeding programmes with the goal to improve dry bean yield and also addressing the seed quality. These are in agreement with the results of this study, in that genotypes with promising yields MAC 19-1, MAC 35, MAC 70-2 and MAC 50 could be used in the breeding programmme.

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