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Full Length Research Paper

Adaptation of sweet potato [*Ipomoea batatas*) (L.) Lam] genotypes in various agro-ecological zones of Malawi

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Sweet potato [*Ipomoea batatas*) (L.) Lam] is grown by small holder farmers across a wide range of environments in Malawi. A multi-location trial of eight genotypes for three seasons at six research stations was undertaken using additive main effects and multiplicative interaction (AMMI) model analysis to determine the genotypes' stability and influence of genotype × environment interactions (GEI) on storage root yield. ANOVA showed high significant differences in storage root yield of the genotypes among seasons and locations (p≤0.01). Genotype, environment and genotype × environmental interaction significantly influenced storage root yield variation of the cultivars (p≤0.01). The variance in yield was mainly attributable to environment variability (62.86%) than genotypes variation (14.25%) and G × E interactions (15.06%). Semusa was superior for storage root yield (27.77t/ha) and Lu96/334 was the most inferior (11.19 t/ha). AMMI stability analysis revealed that LU96/303 (24.72 t/ha) was the most stable genotype across sites. Biplot analysis showed that Chitedze and Baka were sites conducive for high yields hence can be used for preliminary yield evaluation to capture maximum genotypes' yield potential, while Lunyangwa was the lowest yields site; therefore useful for assessing the potential of worst performance of genotypes under unfavourable environmental conditions.

Key words: G × E interactions, multi-locational trial, stability, sweet potato, genotypes, root yield.

INTRODUCTION

A better understanding of genotypes and environment interactions (GEI) is critical for any crop varieties improvement program (Singh et al., 2006; Osiru et al., 2009; Andrade et al., 2016) as it helps breeders to identify superior genotypes and their best environments (Yan and Rajcan, 2002; Thiyagu et al., 2013). GEI refers

Table 1. Sweet potato genotypes used for GEI study.

Clone/genotype	Source/origin
LU96/220	Lunyangwa 1996 selections
LU96/274	Lunyangwa 1996 selections
LU96/374	Lunyangwa 1996 selections
LU96/303	Lunyangwa 1996 selections
LU96/334	Lunyangwa 1996 selections
Mugande	CIP, Nairobi
Kenya (SPN/O)	Tanzania bred, came through Kenya
Semusa (Cemsa 74-288)	CIP, Nairobi

to differential sensitivity of genotype performance from one environment to another (Chalwe et al., 2017). While modern plant breeders work to improve various attributes of crops ranging from pest and disease resistance to biofortification, yield improvement has remained the main drive for most breeding programs (Yahaya et al., 2015). Yield is a complex quantitative trait that is determined by an interaction of various factors including external environment such as soil fertility, rainfall, pests and diseases (Dia et al., 2016). Sweet potato [*Ipomoea batatas*) (L.) Lam], like other crops suffers yield losses that are due to abiotic and biotics limitations (Tekalign, 2007; Kivuva et al., 2014; Chalwe et al., 2017) hence an understanding of the nature and magnitude of GEI among sweet potato genotypes is essential in both sweet potato breeding and variety release (Singh et al., 2006; Rukundo et al., 2013). From time in memorial, genotypic yield levels have been the focus of many sweet potato farmers but adaptation to environments and stability (consistency of yield) of the genotypes have always been the underpinning determinants of final yields (Eberhart and Russell, 1966; Bilbro and Ray, 1976; Rea and Vieira, 2002). Thus, a variety is considered more adaptive and stable if it has a high mean yield but a low degree of yield fluctuation in diverse environments (Kang, 2002; Osiru et al., 2009; Khamphas et al., 2015). Sweet potato [*I. batatas*) (L.) Lam] is grown under varying agro-ecological conditions in the tropical and subtropical regions (Thiyagu et al., 2013; Boney et al., 2014) and it is postulated that because of its high genetic diversity and expansive distribution, it exhibit large variability in genotypic expression in multi-environmental trials across regions (Grünerberg et al., 2005). Therefore, this study used the additive main effect and multiplicative interaction (AMMI) model to assess elite sweet potato genotypes in Malawi to determine their stability and influence of genotype × environment interactions (GEI) on storage root yield in order to identify superior cultivars.

MATERIALS AND METHODS

Table 1 presents eight genotypes of sweet potato used in this study. Materials included selections from open pollinated seeds in a crossing block that was established at Lunyangwa Research Station in 1995. Scarified (using sulphuric acid in 99 parts of water) true seeds were initially planted in a seedling nursery in 1996 where clonal selections were made, hence the coding of LU96. LU96 clones were combined with introductions from the International Potato Centre (CIP) and evaluated together in preliminary, advanced and uniform (multi-location) yield trials. Inferior clones in terms of pests and diseases, root yield, root shape, dry matter content, palatability, fiber content, etc. were dropped while the rest were maintained over the seasons. In the final entry, six genotypes were evaluated against Kenya (low yield check genotype) and Semusa (high yield check genotype) (Table 1) at six sites.

Evaluation sites and seasons

The multi-location trial was conducted during the 2002/2003, 2003/2004 and 2004/2005 seasons at Bvumbwe, Makoka, Chitedze, Chitala, Lunyangwa and Baka government agricultural research stations (Table 2). The testing sites represent sweet potato agro-ecological zones in Malawi. The sites differ by altitudes, soil texture, pH and climatic characteristics (rainfall and temperatures) as presented in Table 2 and Figures 1 and 2.

Trial design and field lay out

The clones were laid out in three replicates using a randomized complete block design (RCBD) at the six locations. The plots were planted and maintained following standard procedures with no fertilizer and herbicide application.

Collection of data

Harvesting was done five months after planting (5MAP) at all sites

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Table 2. Altitude and soil characteristics of the study sites (MoALD, 1995).

Location	Altitude (masl)	Soil texture	Soil pH
Bvumbwe (SR)	1164	Sandy clay loam	4.5-6.0
Makoka (ER)	1026	Sandy clay loam	5.2-5.7
Chitedze (CR)	1097	Sandy clay loam	5.5-6.7
Chitala (CR)	600	Sandy clay	6.1
Lunyangwa (NR)	1342	Clay to sandy clay	4.4-5.6
Baka (NR)	460	Sandy clay to sandy clay loam	6.0

SR = Southern region; ER = eastern region; CR = central region; NR = northern region.
Source, MOALD (1995).

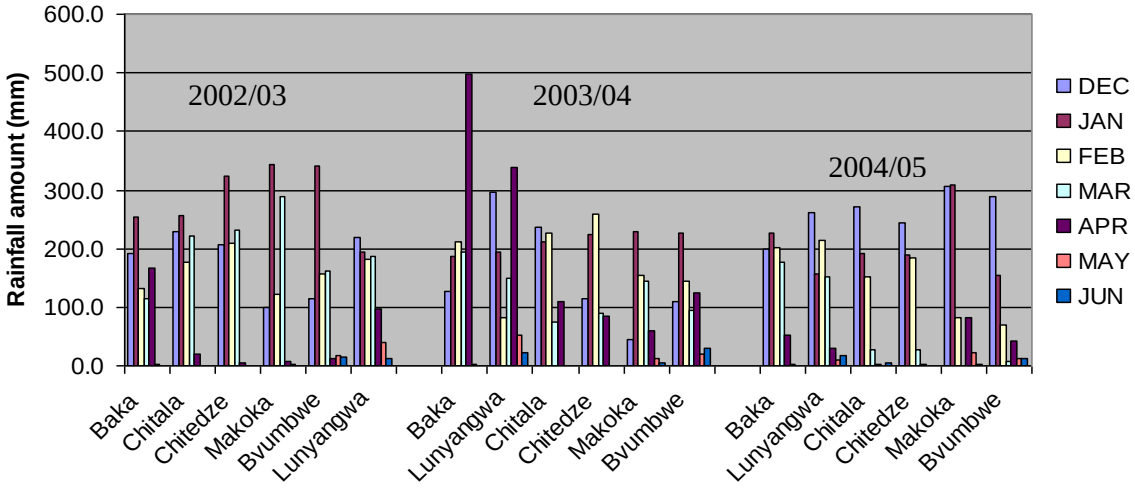

Figure 1. Rainfall amounts (mm) for testing sites over the study seasons (December to June).

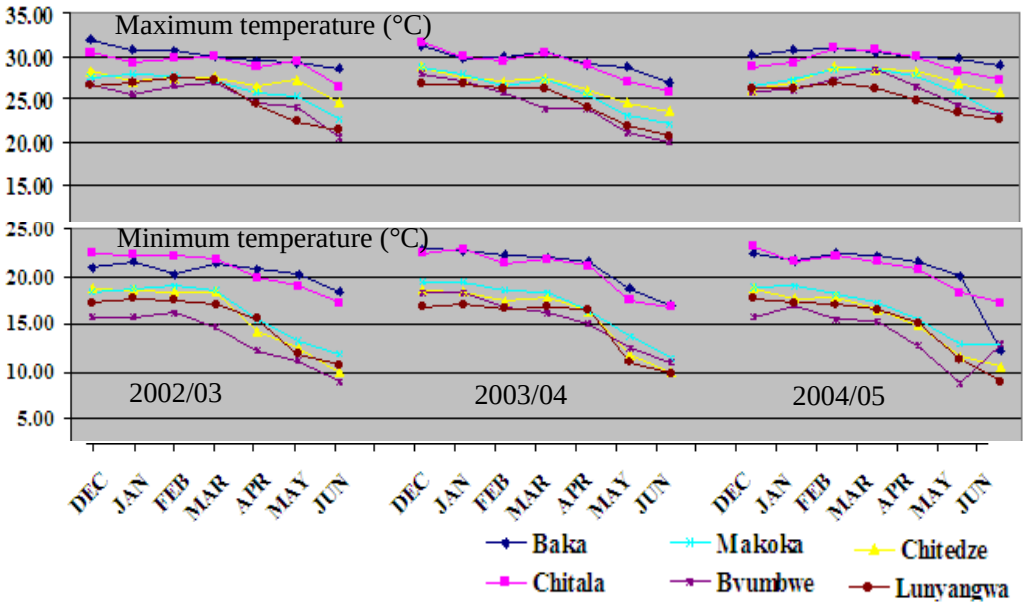

Figure 2. Maximum and minimum temperatures for six study sites and three seasons.

Table 3. Models of ANOVA used for analysis of interaction variance.

Source	DF	MS
Seasons (S)	(S-1)	
Locations (L)	(L-1)	
S × L	(S-1)(L-1)	
Reps (L and S)	LS(R-1)	
Genotypes (G)	(G-1)	$\sigma^2e+r\sigma^2_{gls}+rl\sigma^2_{gs}+rs\sigma^2_{gl}+ rs\sigma^2_{g}$
G × L	(G-1)(L-1)	$\sigma^2e+r\sigma^2_{gls}+rs\sigma^2_{gl}$
G × S	(G-1)(S-1)	$\sigma^2e+r\sigma^2_{gls}+rl\sigma^2_{gs}$
G × L x S	(G-1)(L-1)(S-1)	$\sigma^2e+r\sigma^2_{gls}$
Error	LS(G-1)(R-1)	σ^2e

Table 4. Combined ANOVA for storage root yield (t/ha) over three seasons.

Source	Degrees of freedom	Sum of Squares	Mean squares	Contribution to total sum of squares (%)
Location (L)	5	17476.84	3495.37***	27.60
Seasons (S)	2	8523.73	4261.86***	13.46
L × S	10	13803.89	1380.39***	21.80
Genotype (G)	7	9020.83	1288.69***	14.25
G × L	35	5232.82	149.51***	8.26
G × S	14	1100.64	78.62***	1.74
G × L × S	70	3204.92	45.79***	5.06
REP (L × S)	36	964.55	26.79*	1.52
Error	252	3994.91	15.85	6.31
Total	431		63323.13	
		r^2=0.94	%CV=19.19	

*=p≤0.05; ***p=≤0.01; r^2=% repeatability.

and data on final stand count, representing final surviving plants in a net plot was recorded. Using a weighing scale, storage root yield (kg) per plot was determined with only marketable roots considered for analysis.

Data analyses

Analysis of variance

The effects of the genotype, location and season as well as their first and second order interactions were determined using analysis of variance (ANOVA) in Agrobase (1999) Agronomic Software 71. The effects of genotypes were assumed to be fixed, while those of seasons and location effects were considered to be random. Table 3 shows the models of the ANOVA used in the study. The ANOVA for estimating variance components is based on the model proposed by Allard (1960), and further developed by Comstock and Moll (1963) for the determination of interaction variance components. In the model, S, L, G and R are the number of seasons, locations, genotypes and replications, respectively. The σ^2_e and σ^2_g are components of variance of error and genotypes, respectively. Combinations of the subscript identify the components for the interactions.

AMMI stability analysis of root yield

Similarities among test environments based on environmental main and GEI effects were evaluated using additive main effect and multiplicative interaction (AMMI; Zobel et al., 1988) analyses (Agrobase, 1999). The method uses a combination of ANOVA and principal components analysis (PCA). While ANOVA partitioned the variance into three components: genotype, environment and G× E deviations from the grand mean, the PCA partitioned the G × E deviations into different interaction principal component axes (IPCA). These were tested for statistical significance using ANOVA.

RESULTS AND DISCUSSION

Genotypic variability for root yield

The analysis of variance (ANOVA) indicated high significant differences in storage root yield (t/ha) among genotypes at p≤0.01 within and among locations and seasons (Table 4). The variability in yield by different genotypes indicated their differing responses to diverse environments and seasons (Mulema et al., 2008). The

Table 5. Mean storage root yield (t/ha) across all locations, seasons and genotypes

Genotype	2002/03	2003/04	2004/05	Mean
Semusa	30.94	34.39	17.98	27.77
Lu96/303	26.17	28.33	19.65	24.72
Lu96/220	26.20	24.33	16.85	22.46
Lu96/374	22.50	26.11	13.80	20.80
Mugande	21.83	23.72	15.01	20.19
Kenya	21.11	24.28	13.10	19.50
Lu96/274	22.72	24.56	10.90	19.39
Lu96/334	10.56	13.56	9.47	11.19
Mean	22.75	24.91	14.60	20.75±2.36
LSD (genotypes)	2.34	2.77	1.79	
LSD (Location × Season)				1.89
Sig. (Location × Season)				* * *

***p=≤0.01; Sig. = significant; L = location; S = season; LSD = least significance difference.

difference in performance among the genotypes in a given environment is in part due to genetic variability which accounted for 14.25% of the total sum of squares (Table 4). The mean storage root yields of the genotypes (Table 5) ranged from 11.19 (LU96/334) to 28.33 t/ha (Semusa). The coefficient of variance (CV) was 19.19% which was reasonable and reflective of the study results reliability under field and rain fed conditions. The r^2 value = 0.94 represented a high (94%) repeatability (reproducing similar yields) of the trial. The relative yields of the eight genotypes (Table 5) showed that Semusa, the highest yielding reference was the most superior (27.77 t/ha) in two of the three seasons and six locations. The rest of the genotypes had different ranks in different seasons.

The selection criterion for early maturing and high yielding cultivars in Malawi is based on mean root yields of 20 t/ha at 5 months after planting (Chipungu et al.,1999). Such a selection criterion however, is a factor in stability in yield as illustrated by the yields of cultivars in 2005 which were below 20 t/ha. Breeders have always considered both yield levels and stability of performance, in order to present farmers with genotypes that are suitable (Farshadfar, 2008; Fikere et al., 2009). Considering the least significant difference (LSD), the root yield means were over 20 t/ha and above the lower yielding check, Kenya, except for Lu96/274 and LU96/334 (Table 5). Therefore, five of the six tested genotypes are good candidates for release in the national program assuming their performance is equally good in other traits such as tolerance/resistance to pests and diseases, palatability (dry matter content, colour, taste, texture, fiber content, etc.), root sizes and numbers.

Presence and magnitude of GEI on root yield

ANOVA (Table 4) showed significant effects of genotypes, locations, seasons and their interactions (P≤0.01) on root yield. Similar results were shown by Mwololo et al. (2009) in their study on 17 genotypes, three sites and two seasons in Kenya. Khamphas et al. (2015) also found that location, genotypes and G × L interaction were significant for all characters of purple waxy maize genotypes. This underscores the importance of stability studies before recommendation of any crop genotypes for various locations.

In the present study, location which is a predictable environment had a variability of 27.60% (Table 4) of the total variation of yield and could be attributed to differences in soil type and pH and altitude (Table 2). The variability among seasons which is the unpredictable environment (13.46% contribution to total SS) may be attributed to seasonal rainfall and temperatures variations (Figures 1 and 2). When GEI is due to variation in predictable environmental factors, sweet potato breeders can either develop specific varieties for different environments (locations, soil types, management systems, etc.), or broadly adapted varieties that perform well under variable conditions (Farshadfar, 2008; Fikere et al., 2009). However, when GEI stems from variations in unpredictable environmental factors, such as year to year variation in rainfall distribution, as is the case in this study, stable varieties that can perform reasonably well under a range of conditions are needed. Such breeding strategies assist the farmers in risk avoidance. Fikere et al. (2008) indicated that farmers favor genotypes that yield constantly better across seasons and environments. In general, the environment accounted for 62.86% (27.60 + 13.46 + 21.80%) of the total variation in storage root yield. This finding is indicative of a high influence of the environment on root yield, further supporting the need for evaluation of candidate genotypes over multiple sites and seasons for accurate inference (Sial et al., 2001).

Table 6 shows that the best yield was from Semusa in

Table 6. Genotypes yield (t/ha) and performance rank (R) over three seasons and six sites.

Genotype	BK	R	MK	R	CH	R	BV	R	CZ	R	LU	R	OM	R
2002/2003 season														
Semusa	42.67	1	21.67	3	28.67	1	16.00	6	63.33	1	13.33	5	30.94	1
Mugande	32.33	3	13.00	7	14.67	7	16.33	5	39.67	5	15.00	2	26.20	2
LU96/220	38.21	2	21.00	4	24.67	2	19.00	4	42.33	4	12.00	6	26.17	3
Kenya	21.67	7	20.33	6	19.33	5	21.67	1	33.00	7	10.67	7	22.72	4
LU96/303	30.00	4	24.67	1	20.33	4	20.00	3	45.00	2	17.00	1	22.50	5
LU96/274	22.67	5	20.33	5	15.67	6	20.67	2	43.00	3	14.00	3	21.83	6
LU96/374	22.33	6	23.00	2	24.33	3	13.33	7	38.33	6	13.67	4	21.11	7
LU96/334	14.67	8	11.00	8	8.33	8	7.67	8	11.67	8	10.00	8	10.56	8
Mean	28.07		19.38		19.50		16.83		39.54		13.21		22.75	
2003/2004 season														
Semusa	39.33	1	18.33	4	25.67	1	34.67	1	62.33	1	25.67	1	34.39	1
Mugande	19.33	7	17.33	6	24.67	2	20.00	7	43.33	7	24.67	2	24.56	4
LU96/220	25.67	3	17.00	7	21.67	4	21.67	6	46.00	5	21.67	4	24.33	5
Kenya	20.33	5	18.33	5	17.67	7	27.33	3	46.33	4	17.67	7	23.72	7
LU96/303	32.00	2	22.00	1	24.33	3	23.00	4	44.67	6	24.33	3	28.33	2
LU96/274	19.67	6	20.00	3	19.67	5	22.33	5	48.00	3	19.67	5	24.28	6
LU96/374	22.00	4	20.33	2	18.00	6	33.33	2	49.67	2	18.00	6	26.11	3
LU96/334	10.67	8	11.33	8	16.67	8	13.33	8	19.67	8	16.67	8	13.56	8
Mean	23.63		18.08		21.04		24.46		45.00		21.04		24.91	
2004/2005 season														
Semusa	35.7	2	27.00	4	12.04	1	19.00	1	16.98	1	7.20	2	17.98	2
Mugande	19.44	4	24.67	7	8.38	3	17.67	3	12.69	3	7.20	1	13.80	4
Lu96/220	33.95	3	25.00	6	6.58	4	12.67	7	16.72	2	6.17	4	16.85	3
Kenya	14.61	7	26.67	5	5.25	6	16.67	4	10.91	5	4.53	8	10.90	7
LU96/303	36.01	1	28.33	1	9.77	2	17.67	2	10.65	6	5.45	6	19.65	1
Lu96/274	7.51	8	27.00	3	2.57	7	15.67	5	7.20	8	5.45	5	9.47	8
Lu96/374	16.46	5	28.33	2	5.97	5	14.67	6	12.34	4	5.04	7	15.01	5
Lu96/334	14.61	6	15.00	8	1.95	8	10.33	8	8.23	7	6.69	3	13.10	6
Mean	22.29		25.25		6.56		15.54		11.97		5.97		14.60	

BK=Baka; MK=Makoka; CH=Chitala; BV=Bvumbwe; CZ=Chitedze; LU=Lunyangwa; OM = overall mean.

2002/2003 and 2003/2004 seasons (30.94 and 34.39 t/ha, respectively) while LU96/303 had the highest yield (19.65 t/ha) in the 2004/2005 season. The most productive season was 2003/2004 (24.91 t/ha), while 2004/2005 (14.60 t/ha) was the worst season (Table 6). The seasonal difference was attributed to uneven distribution of rainfall amounts in 2004/2005 season (Figure 1). The season had recurrent dry spells between January and March. The seasons 2002/2003 and 2003/2004 were similar in terms of rainfall (Figure 1) amounts and distribution. However, all the three seasons were similar in terms of minimum and maximum temperatures (Figure 2). LU96/303 on average was second in yield after Semusa (Table 5) but highest in the drought season (19.65 t/ha) (2004/2005) attaining the Malawian selection criterion (20 t/ha) (Chipungu et al., 1999). This implies that LU96/303 is an ideal cultivar because it combines both drought tolerance and high yield (Makunde et al., 2017). Drought is known to affect root yield negatively (Andrade et al., 2016)

The best site in terms of yield performance was Chitedze in 2002/2003 (39.54 t/ha) and 2003/2004 (45.00 t/ha) while in 2004/2005, Makoka was first (25.25 t/ha). Cultivar Semusa ranked first in five sites in 2003/2004, four sites in 2004/2005 and three sites in 2002/2003. LU96/303 was rated first in each season at Makoka, while LU96/334 was classified as last in each location in 2002/2003 and 2003/2004 seasons and in three sites in 2004/2005 season. The rest of the clones varied from positions 2 to 8 across sites over the seasons (Table 6),

Table 7. AMMI analysis of variance and %GEI explained for root yield.

Source	df	SS	MS	Explained (%)	Cumulative (%)
Environment (E)	17	39804.55	2341.44***	62.86	62.86
Genotype (G)	7	9020.78	1288.68***	14.25	77.11
Rep (G × E)	36	964.56	26.79***	1.52	78.63
G × E	119	9538.29	80.15***	15.06	93.69
Error	252	3994.91	15.85***	6.31	100.00
Total	431	63323.09			
IPCA 1	23	4919.65	4919.65***	51.58	51.58
IPCA 2	21	2545.50	2545.50***	26.69	78.27
IPCA 3	19	866.67	866.67***	9.09	87.35
IPCA 4	17	588.76	588.76***	6.17	93.52
IPCA 5	15	414.98	414.90*	4.35	97.87
IPCA 6	13	130.42	130.42ns	1.37	99.24
IPCA 2	11	72.31	72.31	0.76	100

*and * * *p=≤ 0.05, and 0.01; ns = not significant; SS=sum of square; MS= mean square.

yet good cultivars must show high performance for yield and other essential agronomic traits over a wide range of environments (Becker and Leon, 1988).

Root yield additive main effect and multiplicative interaction (AMMI) for the genotypes across environments

The AMMI analysis of variance of root yield (kg/ha) of eight genotypes in 18 environments showed that genotype main effects, environmental main effects and their interactions were all highly significant for root yield (p≤0.01). This finding concurs with those of Chalwe et al. (2017) whose study on sweet potato genotypes in Zambia showed significant AMMI analysis variance of genotype, environment and their interactions on root yield and weevil damage. Gedif and Yigzaw (2014) and Daba et al. (2015) observed similar significant environment, genotype and genotype × environment interactions influence on yield of potato (*S. tuberosum* L.) and sesame, respectively, in Ethiopia (p≤0.01).

A total of 62.86% of the total sum of square (SS) was attributable to environmental effects, 14.25% to genotypic effects, and 15.06% to GEI effects (Table 7). This means that G × E interaction effects did not impact more on the resultant root yield than the variation in environments, suggesting that the environments (test sites) were highly diverse, hence causing most of the variation in root yield. Mitrović et al. (2012) also reported large portion (77.83%) of the total variation in maize yield as ascribed to the environment, while genotype and genotype × environment accounted for 30% of the total variation. Contrary results which showed greater G × E effects than environmental or genotypic effects were obtained by Andrade et al. (2016) in their study on 58 sweet potato genotypes evaluated over three seasons at one research station. These findings could be due to use of a single site for testing genotypes. In the present study, the total percentage attributed to environment and G × E interaction accounted for 77.92% of the disparity in storage root yield. Comparable results were found by Mwololo et al. (2009) in sweet potato (70%) and Ntawuruhunga et al. (2001) in cassava (50 to 90%).

The magnitude of the GEI (15.06%) sum of squares was above that of genotypes (14.25%), indicating that there were differences in genotypic response across environments (Table 7). Results of the AMMI analysis (Table 7) also showed that the first principal component axis (PCA 1) captured 51.58% of the interaction sum of squares. Similarly, the second principal component axis (PCA 2) explained a further 26.69% of the GEI sum of squares. The mean squares for the PCA 1 and PCA 2 were significant at P≤0.01 and cumulatively contributed to 78.27% of the total GEI. A F-test at P≤0.01 suggested that the two principal component axes of interaction were significant for the model with 44 degrees of freedom. Zobel et al. *(*1988) and Kaya et al. (2002) showed in their prediction assessment that AMMI with only two IPCA axes was the best model. Further interaction principal component axes capture mostly noise and therefore did not help to predict the interactions (Daba et al., 2015). Thus, the interaction of the eight genotypes with 18 environments in this study was best predicted by the first two principal components and the rest were less informative. Thiyagu et al. (2013) reported in their stability study that sweet potato accumulated contribution of IPCA1 and IPCA2 of 85.26%. Comparably, in studies on linseed yield by Adugna and Labuschagne (2002), the two IPCAs accounted for 69.5% of the total interaction

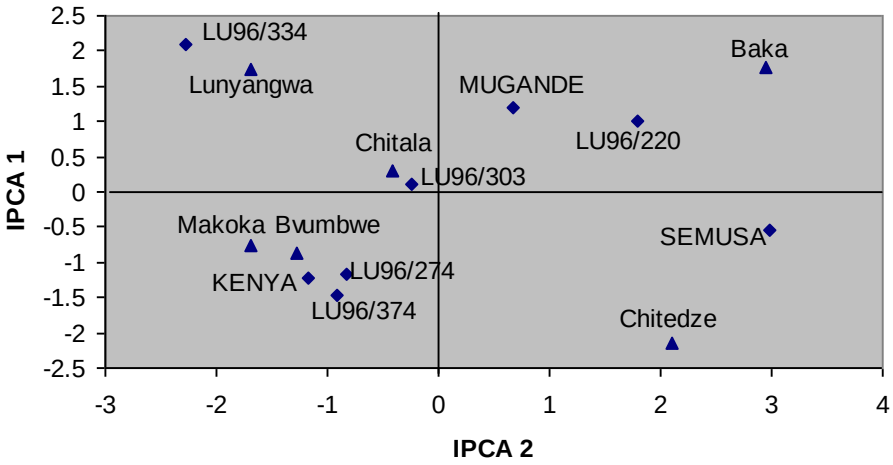

Figure 3. Biplot of eight genotypes and six locations for IPCA scores 1 and 2.

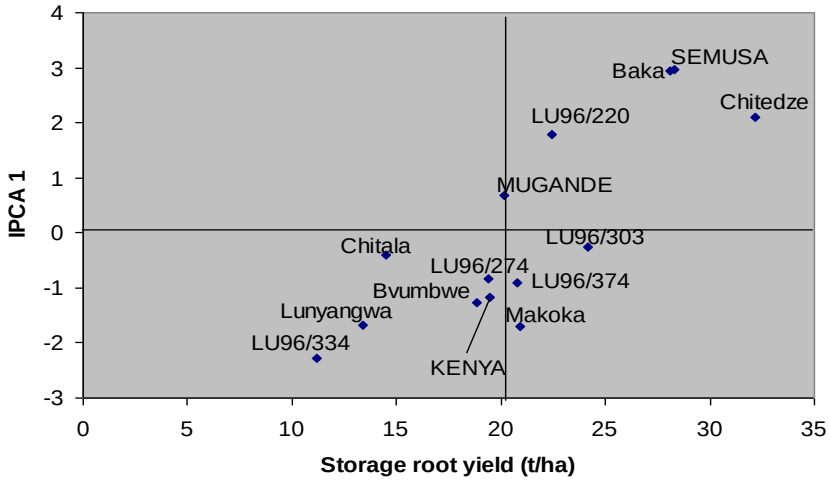

Figure 4. Biplot of eight genotypes and six locations for root yield and IPCA 1 scores.

and the remaining 30.5% was considered residual or noise not interpretable and was therefore discarded.

In order to identify genotypes adapted to specific test environments and their yield performance and stability, biplots were used (Yan, 2002). The biplot (Figure 3) was generated using genotypic and environmental scores of the first two PCA components as suggested by Vargas and Crossa (2000). Genotypes and location environments that appear almost on horizontal line (y axis) have a similar negative or positive interaction pattern for the IPCA 2 and equally those that fall along the x axis or close to it have similar interaction pattern along IPCA 1. Therefore, genotype LU96/334 is best for Lunyangwa while Kenya, LU96/274 and LU96/374 are best for Bvumbwe and Makoka and LU96/303 for Chitala. LU96/303 is located near the plot origins of both IPCA1 and 2 and therefore less responsive than the vertex genotypes, hence more stable across the testing environments since its IPCA scores are closer to zero (Figure 3) (Mwololo et al., 2009; Osiru et al., 2009; Thiyagu et al., 2013). Such a stable performance is a desirable attribute of cultivars, particularly in Malawi where environmental variations especially the rainfall pattern are very high and unpredictable as shown in Figure 2.

Semusa was unstable (Figure 4) across the six sites when variation was explained by IPCA 1 but stabilised (Figure 3) when IPCA 2 was included. In contrast, LU96/334 is a non-adaptable genotype as indicated by its large IPCA 1 and IPCA 2 scores. Mugande and LU96/220

(high yielding) are moderately stable and are not closely associated with any site (Figure 3). Other genotypes of interest are LU96/274 and LU96/374 which are stable when explained by IPCA 2. Makoka and Bvumbwe are similar environments and Kenya, LU96/274 and LU96/374 exhibit similarity in interaction patterns (Figure 3).

Figure 4 is a biplot of average yield of a genotype at different sites and PCA 1 effects. Genotype Semusa had the highest average yield because it yielded the highest at sites Chitedze and Baka, and yielded above average at all other sites.

On the other hand, the average yield of genotype LU96/334 was the least while the yield of Mugande was average. LU96/220, LU96/303 and LU96/374 yielded above average. Piepho (1996) indicated that if cultivars are selected for varying environments, stability and mean yield across all environments are more important than yield for specific environments. This is even more important in view of climate change hence farmers would prefer widely adapted cultivars (Zhang et al., 2006; Fikere et al., 2009; Khamphas et al., 2015).

In this study therefore, LU96/303 is considered the most stable genotype (Figures 3 and 4) suitable for the variable production conditions under small holder farmers in the country. It is not uncommon for stability studies to recommend one or two genotypes from a test of many genotypes. Chalwe et al. (2017) and Makunde et al. (2017) both identified two out of eight and 48 genotypes respectively as most stable for root yield and weevil damage and root yield and drought tolerance, respectively. In a study done on winter wheat genotypes in Turkey, two were also reported as stable for all environments by Altay (2012).

Erratic rains in 2004/2005 season, which resulted in low root yields across sites for most of the genotypes, exemplified the variability of cropping seasons in Malawi. Makunde et al. (2017) showed that drought (water stress) reduced sweet potato storage root yield by as much as 35%. This observation indicates that though sweet potato generally grows in marginal areas characterized by poor soils and low rainfall, differential genotypic responses are registered in varying environmental conditions (Chalwe eta l., 2017).

Chitedze and Baka were conducive for high yields (above average) and Makoka though close to Bvumbwe hence similar environments, Makoka yields were however above average (Figure 4), hence a better site of the two. Osiru et al. (2009) reported that high and positive PCA scores show that genotypes are likely to yield more in that environment and conversely high and negative PCA scores are indicative of lower yields in these environments. By implication, Figure 4 confirms that Semusa would yield highly at Baka and Chitedze and yields of Lu96/334 would be lower at Lunyangwa. These findings show that knowledge on GEI of a crop in a country helps plant breeders reduce cost of extensive genotype evaluation by eliminating redundant testing sites (Shafii et al., 1992; Kang and Magari, 1996). The ultimate goal of any breeding program is to produce genotypes that consistently yield more in different environments (Khamphas et al., 2015).

Conclusions

Sweet potato [*I. batatas*) (L.) Lam] cultivars in the study varied significantly in yield across locations and seasons as shown by combined analysis of variance ($p<0.01$). The variance in yield was mainly attributable to environment variability (62.86%) than genotypes variation (14.25%) and G × E interactions (15.06%). Stability analysis (AMMI) revealed that LU96/303 (24.16 t/ha) was the most stable, while ANOVA identified Semusa as the highest yielding cultivar (28.33 t/ha). While Lunyangwa was a low yielding site, Chitedze was the highest; therefore Chitedze should be used to screen cultivars for maximum yield potential. In times of limited resources, Baka and Bvumbwe sites are not necessary for yield evaluation as they are similar to Chitedze and Makoka, respectively.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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Full Length Research Paper

Assessment of plantain (*Musa sapientum* L.) accessions genotypic groups relatedness using simple sequence repeats markers

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Plantains are important sources of high-calorie energy in Ghana. They are also of great socio-economic importance in the country, and very important sources of rural income. Although several species exist all over the world, plantains belonging to the AAB group are unique to West Africa and Ghanaian collections have unique features and peculiar taste. Morphological and biochemical characterization are the popular techniques used to characterize plantain genotypes in Ghana. Thus, there is limited report on molecular characterization of plantains genotypes. Characterization based on morphologic characteristics alone may be limited since the expression of quantitative traits is subjective to strong environmental influence. Alternatively, molecular characterization techniques are capable of identifying polymorphism represented by differences in DNA sequences. The objective of this research was therefore to conduct molecular characterization of Ghanaian local accessions of plantain and assess relationship amongst known genotypic groups (populations). This study sampled 40 accessions of plantains representing four popular genotypic groups. Simple Sequence Repeats (SSRs) were used to assess diversity in reference to a set of global *Musa* collections. The 40 accessions of plantain were clustered into populations as being French plantain, True Horn, False Horn, and Hybrid prior to analysis. PopGene version 32 was used to analyze the data. This revealed that the overall plantain population used have Shannon's Informative Index (I) value of 0.61±0.28 in the overall plantain population, 100% polymorphism for all loci, 2.7±0.67 and 1.81±0.45 for ne and na respectively. Average heterozygosity was 0.34±0.17, loci mMaCIR231 and mMaCIR07 were the most informative, having I values of 0.85 and 0.81 respectively. The Fis and Fit values were both negative indicating lack of inbreeding and the gene flow value was 0.533. The study also revealed relationship among the various populations (French plantain, True Horn, False Horn, and Hybrid) on basis of molecular characterization.

Key words: Characterization, false horn, french plantain, molecular, PopGene, simple sequence repeats, true horn.

INTRODUCTION

Plantains (*Musa* spp.) are major food crops widely grown across the world's tropical and subtropical regions. The fruits are highly nutritious containing high amounts of carbohydrates, minerals such as Ca, and K as well as vitamins A and B (http://healthyeating.sfgate.com/benefits-eating-plantains-3634.html). An estimated 20 million people eat banana and plantain as their major source of dietary carbohydrate.

These crops serve as important revenue for many small-scale farmers (Bioversity International, 2007). Mostly, the world's bananas and plantains are grown on small farms for local consumption (Ortiz and Vuylsteke, 1996). Banana and plantain production in sub-Saharan Africa, therefore provide a good source of income and serve as an important component of daily diet.

Two main centers of banana and plantain cultivation are found in Africa: the wet tropical zones of West and Central Africa, and the East African Highlands (De Langhe et al., 1995). In the west and central humid tropical areas, a very distinct type of cooking banana (plantain, AAB) is widely cultivated. Plantains are relatively rare in most of Asia as well as in other parts of Africa, and their origin in West Africa is shrouded in mystery. It is thought that they have been cultivated in this region for more than 3,000 years, but the identity of the people responsible for such cultivation is unknown (De Langhe, 1996). It is possible that the same proto-Polynesians that carried the banana east to the Pacific islands, also carried it to West Africa (De Langhe, 1996; De Langhe and De Maret, 1999). Such hypothesis fits with the finding that plantains must have reached Africa more than 3,000 years ago, but archaeological evidence for such voyages is unlikely to be found. Plantains constitute over 70% of the bananas and plantains grown in this area (Mbida et al., 2000). Recently, the production of plantains in West and Central Africa was saddled with diseases and this includes the black sigatoka disease (Dzomeku et al., 2016). Attempts to deal with these problems have led to the production of hybrid varieties through breeding programs to develop line with resistance or tolerance. These hybrids have been introduced into some of the West and Central African countries and their acceptability by the consumers may be based on various preferences, including taste, consistency and cooking properties. An insight of genetic make-up may contribute to information, vital for both breeders and consumers. Several methods have been used to investigate the genetic variability present in *Musa* germplasm (Silva et al., 2015; Hippolyte et al., 2010). The development and application of technologies based upon molecular markers provide the only tools that are able to reveal polymorphism at the DNA sequence level, which are adequate to detect genetic variability between individuals and within populations (Kresovich et al., 1995) which will facilitate breeding efforts to improve the crop against biotic and abiotic stresses (Rodrigues et al., 2017). Recently, several molecular tools have been used to assess the molecular make up of *Musa* species (Christelová et al., 2016). Microsatellites or simple sequence repeats (SSRs) are among several molecular markers used to characterize and assess genetic variability of the genus *Musa*, because they are highly polymorphic, multi-allelic, codominant, reproducible, easy to interpret, and amplified via polymerase chain reaction (PCR) (Crouch et al., 1999). Christelová et al. (2016) used molecular and cytological tools to characterize *Musa* germplasm collections and this provided insight into the diversity of banana. Biswas et al. (2015) conducted genome-wide computation analysis of *Musa* microsatellites and has introduced a concise procedure to SSR marker development.

The principal edible species are in the section *Eumusa* of the genus *Musa* and comprise *M. acuminata* Colla ($2n=2x=22$; A genome; 600 Mbp) and *M. balbisiana* Colla ($2n=2x=22$; B genome; 550 Mbp), and their hybrids, the triploid lines ($2n=3x=33$) with genome constitutions AAA (dessert or export banana), AAB (plantain) and ABB (cooking banana) (Simmonds, 1962; Gowen, 1995). Considering morphological characteristics of plantains in Ghana, they can be classified into three main subgroups; namely False Horn "Apantu group", French "Apem group", and True Horn: "Asamienu group" (Dankyi et al., 2007). The French plantains have the bunch complete at maturity, with many hands of numerous, rather small fingers. The bunch axis is covered with neutral flowers and male flowers, where the male bud is large and persistent. The False Horn plantains have incomplete bunch with no male bud at maturity. The hands consist of large fingers followed by few neutral flowers. True Horn plantain's bunch is incomplete at maturity. The hands are few in number and consist of a few but very large fingers. There are no neutral flowers or male bud and the True Horn plantain resembles the False Horn but it has no neutral flower and has larger fingers. Basically, the available cultivars in Ghana are 10 of False Horn, four of French plantain 4 and two of True Horn 2. Hence, there is the need to study diversity among local and introduced varieties at the molecular level. This study seeks to determine the genetic relationships among genotypic groups of elite local triploid (AAA, AAB, and ABB), and tetraploid hybrid (ABBB) accessions of *Musa*.

The objective of this study was to assess relatedness among the collection of *Musa sapientum* genotypic groups (population) using B-genome derived Simple Sequence Repeat (SSR) markers. This provided fingerprint for the unique plantain genotypes in Ghana within the West African sub-region. Also, this study estimated the genetic variation or genetic diversity within and among populations, estimated the genetic population structure, and determined with reference to known morphological traits, if SSR based on known selected set of microsatellites (in reference to a reference set of *Musa*

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Table 1. Plantain genotypes sampled for analysis.

Sample Code	Local Name	Genotypic group
Ghana 1	APEM	French Plantain
Ghana 2	APEM NYERITIA	French Plantain
Ghana 3	APEM	French Plantain
Ghana 4	APANTU OSOBOASO	False Horn
Ghana 5	APANTU KENTENMA	False Horn
Ghana 6	ONIABA	French Plantain
Ghana 7	ASAMIENU	True Horn
Ghana 8	ONIABA	French Plantain
Ghana 9	APANTU 1	False Horn
Ghana 10	APANTU 2	False Horn
Ghana 11	APANTU 3	False Horn
Ghana 12	APEM KESE	French Plantain
Ghana 13	APEM KETOA	French Plantain
Ghana 14	APEM NYERETIA	French Plantain
Ghana 15	APANTU 1 (N)	False Horn
Ghana 16	APANTU 2 (N)	False Horn
Ghana 17	APANTU 3 (N)	False Horn
Ghana 18	APANTU 4 (N)	False Horn
Ghana 19	APANTU 5 (N)	False Horn
Ghana 20	APEMPA (N)	French Plantain
Ghana 21	ONIABA (N)	French Plantain
Ghana 22	APANTU DICHOTOMY (N)	False Horn
Ghana 23	APANTU (N)	False Horn
Ghana 24	BRODE SEBO (E)	False Horn
Ghana 25	ONIABA (E)	French Plantain
Ghana 26	APANTU 1 (E)	False Horn
Ghana 27	APANTU 2 (E)	False Horn
Ghana 28	APEM MEDIUM (E)	French Plantain
Ghana 29	APANTU DICHOTOMY (F)	False Horn
Ghana 30	BRODEYO (F)	False Horn
Ghana 31	ONIABA (F)	French Plantain
Ghana 32	APEM MEDIUM (F)	French Plantain
Ghana 33	APANTU LONG TAIL (F)	False Horn
Ghana 34	APANTU NORMAL (F)	False Horn
Ghana 35	APANTU (RED-ASSIN FOSU) (F)	False Horn
Ghana 36	APANTU (NOT BRANCHING) (F)	False Horn
Ghana 37	OSOBOASO (F)	False Horn
Ghana 38	APEM LARGE (F)	French Plantain
FHIA 21 A	Apem Hemaa A	Introduced Hybrid
FHIA 21 B	Apem Hemaa B	Introduced Hybrid

genotype) can provide adequate information on fingerprinting Ghanaian plantain genotypes.

MATERIALS AND METHODS

Genotype sampling

Plantain genotypes used for the study were selected on the basis of known morphological classification traits and genotypic groups. The samples were collected from farmers' fields and backyard gardens in the Ashanti and Eastern Regions in Ghana. Samples collected were of the French plantain (15 entries), True Horn (1 entry), False Horn (22 entries) and introduced hybrid (2 entries). Known morphological data was used to cluster the collections genotypic groups referred to in this study as "Populations" (Table 1).

Genomic DNA isolation

During collection on the field, young tissues of Cigar leaf with approximate weight of 0.2 g were harvested, washed and kept in

liquid nitrogen for isolation of genomic DNA. In the laboratory, the phenol-chloroform-isoamyl alcohol-base DNA extraction protocol (Egnin et al., 1998) was used to extract genomic DNA from the samples. A spectrophotometer (Biochrom Libra S12) was used to estimate the quantity and quality of DNA at 260 nm (OD260) and 280 nm (OD280). The DNA was resolved in agarose gel at 0.8% in TAE buffer stained with ethidium bromide. The DNA in the gel was visualized with an ultra violet trans-illuminator in an alpha imager. The agarose gel electrophoresis was used for the determination of DNA quality and quantity.

Microsatellite analysis

The Simple Sequence Repeats (SSR) microsatellite based analysis was conducted using the standardized approach for the unknown samples characterization at the molecular level. Eighteen microsatellite markers were used to screen the 40 plantain genotypes, and their scores were compared with those from a reference sample set (Christelová et al., 2011). These SSR loci are well distributed within the *Musa* genome (Lagoda et al., 1998; Crouch et al., 1998; Hippolyte et al., 2010). The size and composition of the reference set defined the limits for the precision with which the unknown samples were characterized. There were only two representatives of African plantains in the reference DNA collection. The SSR patterns of each individual were analyzed following the protocol of Roy et al. (1996), as applied with the automated infrared fluorescence technology of a sequencer LICOR IR2 (LICOR, Lincoln, USA). For a given SSR locus, the forward SSR primer was designed with a 5'-end M13 extension (5'-CACGACGTTGTAAAACGAC-3'). The PCR amplification was performed in a 384 wells Eppendorf master cycler with PCR master mix containing 10 ng of *Musa* DNA in a 10 µl final volume of reaction + PCR buffer (10 mM Tris HCl (pH 8), KCl 50 mM, 0.1% Triton-X100 and 1.5 mM $MgCl_2$) + 8 pmol M13-labelled primer + 200 µM deoxynuleoside triphophates (dNTPs) + 1 U Taq DNA polymerase (Life Technologies, U.S.A.) + 0.06 µM of M13 primer-fluorescent dye IR700 or IR800 (Biolegio, Netherlands). The PCR program had initial denaturation step at 94°C for 5 min, followed by a touch-down protocol - initial decrease of annealing temperature by 1ºC for the first cycles depending on the primer pairs used. Fixed annealing temperatures for further 35 cycles was applied and denaturation at 94°C for 45 s. Annealing was at lowest primer Tm (between 43 - 52°C) for 60 s and elongation at 72°C for 60 s. A final elongation step at 72°C for 5 min was added to all the protocols. *Musa* standard was prepared with a mix of three *Musa* accessions (Pisang Jari Buaya, Popoulou/Maia Maoli and Tomolo), added in order to improve allele sizes determination. The ladder used had the range 71-367 bp. The IR700 or IR800-labeled PCR products were diluted 8-fold and 5-fold respectively prior to electrophoresis on 6.5% polyacrylamide gel. The band sizes were determined by the IR fluorescence scanning system of the sequencer. Information on the reference set of samples used is available at http://www.musagenomics.org.

Genomic data analysis

Molecular data analysis was performed in the phylogenetic package Phylip under restdist and UPGMA algorithm packages. The outtree files were visualized in any tree-building/editing program (Treeview or Figtree software). The data was treated as a co-dominant marker and although number of alleles per loci ranged from 5 to 21, to analyse genetic variation among genotypes using PopGene 3.2 (Yeh et al., 1997), the data was scored as a diploid data and hence alleles that were beyond the diploid set of alleles were ignored. Labelling each marker as a locus, the POPGENE version 32 genetic analysis packages was used to analyse the data. The absence of an amplification product with the 18 primers in an individual was considered as missing data. The genetic variation at each locus was characterised in terms of number of alleles (na), effective number of alleles (ne) (Kimura and Crow, 1964), and Shannon's Informative index (I) (Lewontin, 1972). The summary of heterozygosity was also established at each locus in terms of observed heterozygosity (Ho) and expected heterozygosity (He). The gene flow (Nm) was established from the genetic differentiation coefficient (Fst) as Nm = 0.25(1-Fst)/Fst. Data analysis was conducted by assigning populations into four genotypic groups on basis of known morphological traits as French Plantain (Pop_1), True Horn (Pop_2), False horn (Pop_3), and Hybrid (Pop_4). Also, all the plantain samples were considered as a single population Pop_{All} dendrogram to establish relatedness among populations was generated, based on Nei's genetic distances.

RESULTS AND DISCUSSION

Out of the 20 SSR markers tested, 18 provided applicable and scorable data. There were a total of 232 allele calls when the 40 Ghanaian accessions were analyzed together with a reference set of *Musa* accessions. All the 40 samples fell within the subgroup of triploid AAB African plantains (Figure 1). Considering the reference set of *Musa* genotypes used, out of the 232 allele calls, only 52 amplifications were within the Ghanaian genome, representing only 22.4% of known alleles when the selected set of SSR markers was used (Christelová et al., 2011).

This study indicates that more SSR markers need to be screened to generate informative loci that can detect variation among the Ghanaian genotypes. Quain et al. (2010) used 49 *Musa* SSR primers of which 46 amplified a total of 233 alleles, giving an average of 5.09 alleles per locus within a range 1-13 alleles among 10 *Musa* accessions. Report by Brown et al. (2009) indicated that 15 decamer RAPD markers were used to screen 27 *Musa* accessions. Samarasinghe et al. (2010) used MaSSR primers and +ve AGMI primers to characterize 27 *Musa* cultivars from the AA and BB genome (Supplementary Table 1). Six of the primers used amplified a total of 38 alleles in the collections. In the current study, all the bands scores were polymorphic. Zhang et al. (2009) used ISSR markers to reveal genetic diversity among natural population of Ottelia acuminate having 79.44% polymorphic bands and the average band per genotype was 6.3. Although there were 1.3 alleles per genotype on the basis of band amplification in the current study amongst the Ghanaian samples when the total number of alleles were considered in reference to the reference set of samples that were 5.8 alleles per genotype, this was lower than value reported by Zhang et al. (2009). Resmi et al. (2011) sampled 38 banana cultivars representing AA, AB, BB, AAA, and ABB genomic groups. Using STMS, 15 primer pairs of Ma series specific to *Musa* species were screened for usefulness. Ten out of the 15 were selected for the analysis on basis of PCR amplification and allele scoring consistency. The 10 markers used revealed 27 alleles. In

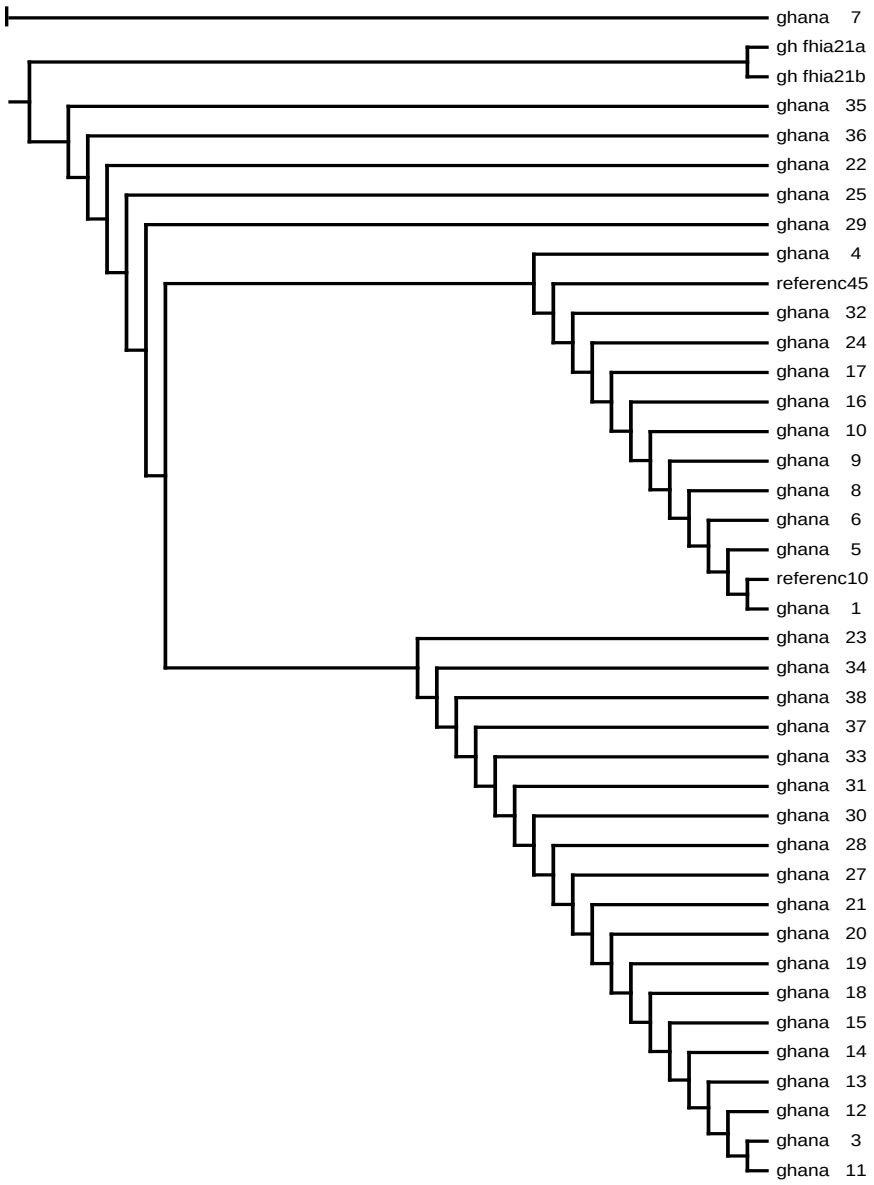

Figure 1. Dendrogram generated with Phylip phylogenetic package under the UPGMA algorithm package.

the present study, size of amplification fragments ranged from 111 to 458 bp. Resmi et al. (2011) reported amplified fragment size ranging from 50 to 290 bp. Considering all the 232 alleles called in the present study, the mean number of alleles per locus was 12.88 which is comparable to that reported by Creste et al. (2004). Similarly, Grapin et al. (1998) reported a mean number of 8 alleles per primer. Other researchers working on *Musa* genotypes reported average number of alleles as 3.32 (Ge et al., 2005), 2.7 and 8.3 respectively (Resmi et al., 2011, 2016), and 2.56 (Oriero et al., 2006).

The data generated was handled as a diploid co-dominant data set and the allelic frequency was calculated for all genotypes at each locus. The overall allelic frequency for the 18 loci determined using PopGen 32 is presented in Table 2. The highest frequency value (0.9868) was obtained in allele B of locus mMaCIR39, although allele A of loci mMaCIR24 and mMaCIR150 also had high frequencies of 0.9744 and 0.9865 respectively. The lowest frequency value of 0.0132 was obtained in allele C of Locus mMaCIR03, D of Locus mMaCIR231, C of Locus mMaCIR01, and C and D of Locus mMaCIR07.

The Phylip phylogenetic package under the UPGMA algorithm packages was used to develop the tree. The clustering according to the dendrogram generated has two major groups (Figure 1) and seven separate clustering of individuals. One of the seven clusters was

Table 2. Overall allele frequency.

Locus	Total number of alleles in respect to reference set of *Musa* genotypes	Alleles and allelic frequency in Ghana plantain genotypes			
		A	B	C	D
Ma-3-90	15	0.0278	0.5000	0.472	
mMaCIR45	6	0.500	0.500		
mMaCIR40	12	0.4730	0.0270	0.500	
mMaCIR39	10	0.0132	0.9868		
mMaCIR307	5	0.5000	0.5000		
mMaCIR03	6	0.5135	0.4865	0.0132	
mMaCIR264	18	0.4868	0.5000		
mMaCIR260	14	0.5000	0.5000		
mMaCIR24	13	0.9744	0.0256		
mMaCIR231	15	0.5000	0.0263	0.4605	0.0132
mMaCIR214	8	0.0256	0.5000	0.4744	
mMaCIR196	12	0.0256	0.5000	0.4744	
mMaCIR195	14	0.0135	0.9595	0.0270	
mMaCIR164	16	0.8243	0.0270	0.01486	
mMaCIR152	18	0.5143	0.0286	0.4571	
mMaCIR150	10	0.9865	0.0135		
mMaCIR01	21	0.4868	0.5000	0.0132	
mMaCIR07	19	0.4868	0.4868	0.0132	0.0132

the introduced hybrid. The true horn plantain was alone in a group and its group clustered close to the tetraploid Fhia hybrid. The two African representatives in the reference set of samples with reference numbers 45 and 10 (Christelová et al., 2011), respectively, clustered within the groups of French plantain and false horn. Although there were some distinct clusters of French plantain and false horn, some clusters had those two genotypes interlacing. Similarly, Amorim et al. (2008) did not get complete separation among improved, wild and cultivated hybrids of diploid genotypes using SSR markers. Rodrigues et al. (2017), however, reported that in investigating genetic variability in banana diploids, there was no separation of genotypes based solely on geographic origin, although genotypes were grouped based on their genomic constitution.

Genetic heterozygosity analysis at all the loci revealed the extent of polymorphism. These results are presented in Table 3. The genetic polymorphism ranged from 33.33% in Pop2 (True Horn) to 94.44 in Pop 3 (False Horn). In Pop3, 17 out of the 18 loci were polymorphic, and Pop2 had 6 out of the 18 loci being polymorphic. When all the genotypes were analyzed together, all the 18 loci were polymorphic resulting 100% polymorphism. The loci sample size was least in Pop2 (0-2), in Pop1 (French Plantain) it was 26-30, and Pop 4 (Introduced Hybrid) recorded the highest range at 38 to 42. When all the genotypes were analyzed together, the Loci sample size ranged from 70 to 78. The corresponding observed number of alleles was determined and in Pop1, the alleles ranged from 1 to 2 and 12 of the 18 loci had two effective alleles. The Pop1 mean effective number of alleles was 1.77±0.44. In Pop2, nine of the loci did not have alleles in the used sample; the average effective number of alleles was 1.67±0.5. In Pop3, loci mMaCIR07 registered the highest value (2.19) for the mean effective number of alleles. However, on the average, Pop4 had the highest number effective number of alleles' value of 1.88±0.32. When all the genotypes were analyzed, the number of observed alleles ranged from 2 to 4 and the locus m231 recording the highest value of 2.16 as the effective number of alleles. On the average 1.81±0.45 alleles were effective. Resmi et al. (2011) reported that 90% of ten loci used to screen 38 *Musa* samples were polymorphic, where highest polymorphism was observed with primers Ma 1 to 17 and Ma 3 to 60 with four alleles. Their percentage polymorphic loci ranged from 60 to 80% among the 5 major genomic groups.

The Shannon's information index (Lewontin, 1972) was calculated to provide a relative estimate of the degree of variation within each population, as well as within the collected genotypes as presented in Table 4. The mean measure of genetic diversity was 0.52±0.20, 0.46±0.35, 0.57±0.28, and 0.61±0.22 for Pops 1, 2, 3 and 4 respectively. When all genotypes were assessed, the mean Shannon's information index was 0.61±0.22. Resmi et al. (2011) reported an I value of 0.70±0.38, which is higher than the value reported in the present study. Locus mMaCIR24 gave no measure of genetic diversity on Pops 1 and 3. Resmi et al. (2011) reported average genetic diversity among 5 groups ranging from 0.20 to 0.42, Shannon's informative index ranged from

Table 3. Summary statistics of population genic variation statistics for all loci.

Population	Number of polymorphic loci	Polymorphic loci (%)	Range of loci sample size	Observed number of alleles – range (na*)	Observed number of alleles - mean (na*)	Effective number of alleles – range (ne*)	Effective number of alleles – mean (ne*)	Shannon's information index (I) - Range	Shannon's information index (I) - mean
Pop1	14	77.78	26 – 30	1 – 2	1.78±0.43	1 – 2	1.74±0.44	0 – 0.69	0.52±0.29
Pop2	6	33.33	0 – 2	0 – 2	1.67±0.50	0 – 2	1.67±0.50	0 – 0.69	0.46±0.35
Pop3	17	94.44	38 – 44	1 – 4	2.22±0.65	1 – 2.19	1.78±0.44	0 – 0.88	0.57±0.28
Pop 4	16	88.89	4	1 – 2	1.88±0.32	1 – 2	1.88±0.32	0 – 0.69	0.62±0.22
Pop_{All}	18	100	70 – 78	2 – 4	2.72±0.67	1.03 – 2.16	1.81±0.45	0.11 – 0.86	0.61±0.28

Table 4. Summary of heterozygosity statistics for all loci.

Population	Mean observed heterozygosity (Ho)	Mean expected heterozygosity (He)	Mean Nei's expected heterozygosity (Nei**)	Mean of average heterozygosity (Ave_Het)
Pop1	0.73±0.44	0.38±0.22	0.37±0.21	0.34±0.16
Pop2	0.67±0.50	0.67±0.50	0.33±0.25	0.37±0.21
Pop3	0.75±0.42	0.40±0.21	0.39±0.20	0.34±0.17
Pop 4	0.89±0.32	0.59±0.22	0.44±0.16	0.34±0.17
Pop_{All}	0.75±0.42	0.41±0.20	0.40±0.20	0.34±0.17

0.46 to 0.61. In Pop3, locus m07 had the highest value of 0.88 as the Shannon's information index ('I'). Considering all the plantain genotypes, locus m231 recorded the highest 'I' of 0.86, whereas m39 recorded the lowest value of 0.07. The I value gives a measure of population diversity. It was highest in Pop4 and lowest in Pop2, indicating that breeding efforts need to be intensified to create variation within population 2 which is constituted by the True Horn collections, locally known as "Asamienu". It will be necessary to select for genotypes that can withstand various biotic and abiotic stresses in the face of changing weather patterns. Christelová et al. (2016), while assessing banana collections with SSR reported the highest major allele frequency (0.584) at loci mMaCIR307, highest allele number (42), observed heterozygosity (0.623) and PIC (0.933) at loci mMaCIR01.

Zygosity refers to the similarity of alleles for a trait in an organism. If both alleles are the same, the organism is homozygous for the trait. If both alleles are different, the organism is heterozygous for that trait. Heterozygosity is a measure of genetic variation in natural populations. High heterozygosity indicates lots of genetic variability, whereas low heterozygosity means little genetic variability. Usually, the observed level of heterozygosity (Ho) is compared with the expected level, under Hardy-Weinberg equilibrium. If the observed heterozygosity is lower than expected, it is attributed to the forces such as inbreeding. If observed heterozygosity is higher than expected, it might be suspected that the genotypes have an isolate-breaking effect (the mixing of two previously isolated populations). The expected heterozygosity (He) is defined as the estimated fraction of all individuals who would be heterozygous for any randomly chosen locus. The 'He' differs from the 'Ho' because it is a prediction based on the known allele frequency from a sample of individuals. Deviation of the observed from the expected can be used as an indicator of important population dynamics. In this study, heterozygosity was determined at all the loci and the averages are presented in Table 4. In all the populations, the average expected heterozygosity was lower than the observed

Table 5. Nei's original measures of genetic identity and genetic distance.

Pop ID	1	2	3	4
1	****	0.7300	0.9995	0.7961
2	0.3146	****	0.7277	0.5487
3	0.0005	0.3178	****	0.7934
4	0.2281	0.6003	0.2315	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

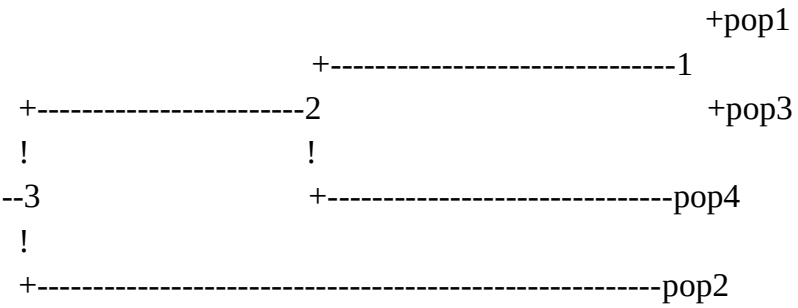

Figure 2. Dendrogram Based Nei's (1972) Genetic distance: Method = UPGMA.

heterozygosity. Observed mean value of heterozygosity was highest in Pop4 (0.88±0.32) and lowest in Pop2 (0.66±0.5). The overall observed heterozygosity was 0.75±0.41. The average genetic diversity in terms of Nei's expected heterozygosity ranged from 0.33±0.25 in Pop2 to 0.44±0.16 in Pop4, the overall Pops value was 0.39±0.20. Remsi et al. (2011) reported an average genetic diversity computed in terms of Nei's expected heterozygosity of 0.42±0.22 which is higher than that reported in the present study (0.39±0.20), although Pop4 value recorded was similar to that reported by Resmi et al. (2011). The report indicated that the Ghanaian accessions were heterozygous, and this may be responsible for the morphological variations among the popular genotypes in Ghana. Oriero et al. (2006) reported average observed heterozygosity of 0.63 among 40 *Musa* accessions which is lower than the value recorded in this present study. In a population, heterozygosity is measured by determining the proportion of genes that are heterozygous and the number of individuals that are heterozygous to each particular gene. Average expected heterozygosity indicates genetic diversity in a population. Resmi et al. (2011) reported average genetic diversity among 5 groups ranging from 0.20 to 0.42, whereas Shannon's informative index ranged from 0.46 to 0.61.

The fixation index (F_{ST}) which is a measure of population differentiation is also referred to as the F-statistics. In this study, the F-statistics and gene flow at all the loci was determined and the average F_{ST} value was 0.319. The average Fis (average inbreeding coefficient with all genotypes) value was -0.97, and this is indicative on absence of inbreeding among the populations. In a population, gene flow or gene migration is the transfer of alleles of genes from one population to another. The average gene flow (Nm) value for all the populations at the various loci was 0.533. As *Musa* species are pathenocarpic, the study has revealed that there has not been inbreeding among the genotypes, and this was expected; consequently the low level of gene flow was 0.533. To promote genetic diversity, gene flow should be encouraged and can be achieved by utilizing *in vitro* micro-propagation to introgress genes from varieties that produce seeds into the cultivated plantains in West Africa. Oriero et al. (2006) reported negative Fis and Fit values and also, observed heterozygosity was higher than the expected.

The population's similarity indices based on Nei's original measures of genetic identity and genetic distance is presented in Table 5. The similarity measure revealed similarity among populations. The Populations 2 (True Horn) and 4 (introduced improved hybrid) were least similar at 0.54, whereas populations 1 (French Plantain) and 3 (False Horn) were very similar, at 0.9995. Similarly, the dendogram generated on basis of Nei's (1972) genetic distance using UPGMA, modified from neighbor procedure of PHYLIP version 3.5, dendrogram based on Nei's genetic distance is shown in Figure 2, and confirmed the relationship among the various populations. At least two main clusters were identified on the dendrogram, with Pop2 clustered as an outlier. Within the second cluster, Pop4 differentiated from Pops 1 and 3 which clustered together.

Conclusion

This work, to the best of our knowledge, is one of the first ever reports on application of SSR markers to study molecular diversity in genotypes of plantain in Ghana. The SSR markers used in this study have limitations as they could not adequately group the collections into their genotypic groups. There is thus the need to develop SSR markers that will detect more variation among our genotypic groups. The data generated would thus contribute to the development of a database for Ghanaian Plantain Germplasm. Informative Molecular markers identified in this study and be used to support plantain germplasm fingerprinting.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Supplementary Table 1. List of SSR primers used for the study.

Name	Forward sequences (5'-3')	Reverse sequences (3'-5')	Tm	Expected size (bp)
mMaCIR03	TGACCCACGAGAAAAGAAGC	CTCCTCCATAGCCTGACTGC	55	110-147
mMaCIR07	AACAACTAGGATGGTAATGTGTGGAA	GATCTGAGGATGGTTCTGTTGGAGTG	53	136-195
mMaCIR39	AACACCGTACAGGGAGTCAC	GATACATAAGGCAGTCACATTG	52	329-390
mMaCIR40	GGCAGCAACAACATACTACGAC	CATCTTCACCCCCATTCTTTTA	54	164-247
mMaCIR45	TGCTGCCTTCATCGCTACTA	ACCGCACCTCCACCTCCTG	57	272-318
mMaCIR152	CCACCTTTGAGTTCTCTCC	TTTCCCTCTTCGATTCTGT	54	147-195
mMaCIR195	GAATCGCCTTAGTCTCACC	TCATGTGCTCCCATCTTT	54	239-306
mMaCIR214	CCATTGAGAGATCAACCC	CTATTTGACGTTGGTGGTC	53	115-238
Ma3_90	GCACGAAGAGGCATCAC	GGCCAAATTTGATGGACT	53	147-191
mMaCIR01	TTAAAGGTGGGTTAGCATTAGG	TTTGATGTCACAATGGTGTTCC	55	241-440
mMaCIR150	ATGCTGTCATTGCCTTGT	GAATGCTGATACCTCTTTGG	54	253-376
mMaCIR164	AAGACAAGTTCCATTGCTTG	GTTCGGGCTTTCGGT	55	255-458
mMaCIR196	GCTCCAAACCTCCCTTT	CGATGCCACACTGGAC	55	163-201
mMaCIR231	GCAAATAGTCAAGGGAATCA	ACCCAGGTCTATCAGGTCA	55	236-286
mMaCIR24	ATCTTTTCTTATCCTTCTAACG	ATTAGATCACCGAAGAACTC	48	237-297
mMaCIR260	GATGTTTGGGCTGTTTCTT	AAGCAGGTCAGATTGTTCC	55	194-264
mMaCIR264	AGGAGTGGGAGCCTATTT	CTCCTCGGTCAGTCCTC	53	234-383
mMaCIR307	AGACTTGTATCGCTTGGTAAA	ACGCTGCACCAGTCAA	54	143-172

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