COMPARATIVE STUDY OF MOLECULAR AND MORPHOLOGICAL METHODS FOR INVESTIGATING GENETIC RELATIONSHIPS AMONG BVUMBWE AGRICULTURAL RESEARCH STATION FIELD GENE BANK BANANA CULTIVARS

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ABSTRACT

Morphological characterisation of bananas has contributed considerably to the body of knowledge of present banana taxonomy. However, the influence of the environment on plant morphology necessitates the use of genetic characterisation to validate taxonomic classifications based on phenotypic traits. This study compared the use of microsatellite markers and morphological traits in estimating genetic relationships among banana cultivars. A total of 12 gene bank cultivars were characterised morphologically using 109 descriptors and genetically utilising 12 microsatellite loci. The two data sets were not analogous as revealed by Mantel's distance matrices comparison test (r=0.063, *p*=0.695), probably because genetic radiation detected by molecular markers is not necessarily reflected in morphological variables measured.

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However, cluster analysis of morphological and microsatellite data revealed two distinct subgroups of *Nakashuga*(AA)-*Phwiza*(AA)-*Ngelezi*(AA) and *Zomba green*(AAA)-*Zomba red*(AAA) which were analogous indicating that the two techniques can compliment each other. *Ngelezi* (AA), *Nakashuga* (AA) and *Phwiza* (AA) were confirmed by both methods as possible synonyms and somatic mutants.

Keywords: Bananas, genetic characterisation, morphological characterization, microsatellite

INTRODUCTION

Importance of Bvumbwe Research Station (BRS) banana field gene bank

Establishment of field gene banks has become a common practice among plant breeders because germplasm conserved in field gene banks is an important component of plant improvement programmes as it provides plant breeders a reservoir of useful traits (Cordeiro *et al*., 2003). The BRS field gene bank was established in 1999 and planted with different *Musa* cultivars collected from southern districts of Thyolo, Mulanje, Chikwawa, Mangochi and Zomba. The districts were chosen possibly due to their high banana cultivar diversity and proximity to the research station. Prior to the germplasm collection in 1991, a countrywide survey was conducted which determined the genome groupings of different local cultivars by using the morphological descriptors based A/B system. (Simmonds and Shepherd (1955; Stover and Simmonds (1987). On the basis of the determined genome groups, some of the cultivars were selected for conservation in the field gene bank (Laisnez, 2005). Collections were made in 1999 and 2000 with a general objective of establishing a field gene bank to conserve available banana and plantain germplasm for future use by farmers and researchers. Upon completion of collection and establishment, the germplasm was to be characterised in order to better understand classification and genome diversity of the cultivars. The field gene bank cultivars were first characterised by Laisnez (2005) using phenotypic traits in order to determine banana germplasm diversity in Malawi.

Phenotypic diversity

Phenotypic diversity also called phenotypic or morphological variation refers to the variation of the physical traits, or phenotypic characters of an organism, such as differences in anatomical, physiological, biochemical or behavioral characteristics (Williams and Humphries, 1996). Phenotypic or morphological characters represent an important measure of the adaptation of the organism to its environment because it is the phenotypic characters that interact with biotic and abiotic factors of the environment. The phenotypic diversity between individuals, populations and species is usually described in terms of variation in external morphology of individuals. Variations in physiological and biochemical characteristics of the organism are also important indicators of phenotypic diversity (Williams and Humphries, 1996). Morphological diversity, the total observable variation, is thus a product of genetic and environmental component of variations (Gemelal, 2002). Quantification of diversity in crops has been done using morphological characters by farmers since time in memorial by observing variations in visible features. Farmers use visible traits on plants to distinguish between cultivars and usually they rightly differentiate between two different cultivars. Chiwona-Karltun *et al*. (1998) observed that farmers distinguished cassava genotypes better than learned investigators when using a standard botanical key. The high accuracy of the farmers' identification of the cassava plants of each cultivar was explained by their use of more detailed morphological characters than the ones used by investigators (Msowoya, 2005). The farmers' experiences indicate that use of more detailed morphological descriptors leads to more accurate identification of species, making morphological characterisation relevant even in the presence of more precise DNA markers. Benesi *et al.* (2004) showed that the use of constant morphological descriptors as markers is reliable and can give a classification as detailed as that generated by AFLP DNA markers.

The most popular system of classification of *Musa* cultivars was developed by Simmond and Shepherd (1955), based on 15 morphological diagnostic characteristics also known as descriptors (Wan *et al*, 2005). Later Ortiz *et al*. (1998) developed a phenotypic diversity index based on 16 quantitative descriptors for the classification of *Musa* cultivars from Africa. Ortiz (1997a) found considerable morphological variation among plantain landraces, particularly in inflorescence characters, some of which have been used to subdivide this germplasm (Swennen and Vuylsteke,

1987). Four broad groups have been identified on the basis of inflorescence morphology: French, French Horn, False Horn and Horn (Tezenas du Montcel, 1987). Plantains have also been subdivided on the basis of pseudostem height into giant, medium and small (De Langhe, 1964). Plant size depends on the number of leaves produced prior to flowering. Giant plantains have more than 38 leaves, while small plantains produce less than 32 leaves (Swennen *et al.*, 1995). Morphologically, there is variation even among the wild founding species of banana. For example, DeLanghe (2000) reported that *M. acuminata* has the greatest phenotypic variation and has subspecies while *M. balbisiana* has lower variation. It is evident that morphological characterisation of bananas has contributed considerably to the bulk of knowledge on present bananas. However, the influence of the environment on plant morphology necessitates the use of genetic characterisation to confirm morphological characterisation. Consequently, this study investigated the association between BRS field gene bank banana cultivars morphological and genetic characterisation data.

MATERIALS AND METHODS

Morphologocal characterisation of BRS, Gene bank banana cultivars

Morphological characterisation of BRS Gene Bank germplasm was conducted by Laisnez (2005) using 109 phenotypic traits, expressed by banana cultivars as described in the Descriptors for Banana (*Musa spp*) (IPGRI-INIBAP, 1996). Data were collected between August and September 2004 prior to drier conditions in October which influence plant conditions. Data on fully ripen fruit were recorded during the second week of October in order to complete the characterisation process. Phenotypic data was collected through visual observation. Characters were recorded when the first ripe finger (fruit) appeared on the bunch (unless otherwise specified like in the case of mature fruit characteristics). More than three plants growing next to each other were used in an appraisal of an accession as recommended by IPGRI (IPGRI-INIBAP, 1996). Each phenotype character was assigned a figure code according to the format in the Descriptors for Banana publication. In case of doubt on the attributes of a character, such a character was not scored in order to maintain high quality and reliability of the data. Two colour charts provided along side descriptors were used to help in colour scoring. A specially designed data collection

form consisting of information on accession identification and plant descriptors was used. Field plan for Bvumbwe Gene bank was used to identify cultivars by accession number and local name. Some cultivars have died since the Gene bank's establishment and at the time of the morphological characterisation work, the field Gene bank had a total of 24 different cultivars of which 14 cultivars were characterised because the others were either in bad condition or did not have inflorescence during the period of fieldwork (Laisnez, 2005). Data from 12 cultivars common to both morphological and genetic data sets were used in the comparative analysis. The specific cultivars used in the analysis are provided in Table 1.

Table 1 Characterised accessions/cultivars.

Acc. No.	Local name	Subgroup	Genotype	Use
Acc. 2518	Zandazanda	Pisang Awak	ABB	C
Acc. 2524	Zomba Red	Red	AAA	D
Acc. 2526	Khazanga	Plantain	AAB	C
Acc. 2528	Mulanje II	Cavendish	AAA	D
Acc. 2551	Nakashuga		AA	D
Acc. 2553	Zomba Green	Green Red	AAA	D
Acc. 2561	Ngerezi		AA	D
Acc. 2570	Makumbuka		ABB	C
Acc. 2581	Phwiza		AA	D
Acc. 2598	Kazanda	Pisang Awak	ABB	C
Acc. 2746	Kholobowa	Bluggoe	ABB	C
Acc. 2753	Kabuthu	Dwarf Cavendish	AAA	D

Source: Laisnez, 2005; C=cooking banana, D=dessert banana

Genetic characterisation of the BRS Gene bank banana cultivars

Collection of banana plant specimens

Banana leaf specimens were collected from 19 BRS Gene bank banana cultivars/accessions in November 2005. Three to four young and tender banana leaf discs were collected into 2mL microcentrifuge tubes by punching the discs directly into the tubes, using the lid as a punch. A total of five plants (individuals) growing next to each other were collected representing each locally named banana cultivar. The leaf samples were transferred to Chancellor College, Molecular Biology and Ecology Research Unit, DNA laboratory in Zomba, Malawi for DNA analysis.

DNA extraction

Total DNA was extracted following CTAB protocol as described by Gawal and Jarret (1991) with minor modifications. Briefly, three or four fresh leaf discs were ground in the presence of Carborudnum in a 2.0mL microcentrifuge tube. A total of 400µL of extraction buffer (1.5 % cetyltrimethylammonium bromide (CTAB); 100mM Tris-HCl; 20mM EDTA; 1.4mM NaCl; 0.2% ß-mercaptoethanol) at 60°C was added and the mixture incubated at 60°C for 60 minutes (min) in an automated Advantec water bath. After incubation, 400µL of chloroform: isoamylalcohol (24:1) was added to the microcentrifuge tube and the homogenate mixed on a shaker for 20 min at room temperature. This was followed by centrifugation at 15,000 rpm for 10 min in a Tomy High Speed Microcentrifuge. Subsequently, 350µL of supernatant was transferred to a 2.0mL microcentrifuge tube and the DNA in the supernatant was precipitated in 210µL of cold isopropanol at -20°C for 60 min. The precipitated DNA was separated from the suspension by centrifugation at 10,000rpm for 10 min, the supernant was decanted and the resultant pellet was washed in 500µL of 70% cold ethanol and centrifuged again for 5 min. The ethanol was decanted and the DNA pellets air dried for 10-15 min before dissolution in 50µL TE.

DNA amplification

Microsatellite amplification reactions were done using 12 primers (Table 2) that produced distinct reproducible amplification products. The reactions were completed in final Polymerase chain reaction (PCR) volumes of 13.11µL consisting of 5.7µL PCR grade water, 1µL of 10mM dNTP mix, 1.25µL of 10 X PCR buffer, 1.6 µL of 25 mM magnesium chloride ($MgCl_2$), 0.75µL of 15 pmol of both forward and reverse banana microsatellite primers, 0.06µL of 5u/µL *Taq* DNA polymerase in storage buffer A (Promega, USA) and 2µL of 25ng/uL template DNA. The amplifications were carried out in a Mastercycler gradient 5331 Eppendorf Version 2.30.31-09 with the following PCR conditions: denaturation step at 94°C for 30s, followed by 30 amplification cycles of denaturing at 89°C for 30s, annealing at an optimal temperature for a specific primer pair for 15s (Table 2) and elongation at 72°C for 30s. The final extension was at 65°C for 20 minutes, followed by a soaking temperature of 4°C.

Table 2 Microsatellite (SSR) primers used in this study.

Locus		Primers sequences (5'-3')	Primer source	T_{ann} (°C)	Allele size range observed
STMS1FP/1RP	F	TGAGGCGGGGAATCGGTA	Kaemmer et al., 1997	58 °C	116-150
	R	GGCGGGAGACAGATGGAGTT			
STMS7FP/7RP	F	AAGAAGGCACGAGGGTAG-	Kaemmer et al., 1997	54 °C	234-252
	R	CGAACCAAGTGAAATAGCG			
STMS10FP/10RP	F	ATGATCATGAGAGGAATATCT	Kaemmer et al., 1997	53 °C	116
	R	TCGCTCTAATCGGATTATCTC			
AGMI93/94	F	AACAACTAGGATGGTAA-TGTGTGGAA	Lagoda et al., 1998	57.5°C	124-144
	R	GATCTGAGGATGGTTCTGTT-GGAGTG			
Ma 1/16	F	TTTGCCTGGTTGGGCTGA	Crouch et al., 1998	58.5°C	142-166
	R	CCCCCCTTTCCTCTTTTGC			
Ma 1/17	F	AGGCGGGGAATCGGTAGA	Crouch et al., 1998	57.7 °C	106-128
	R	GGCGGGAGACAGATGGAGT			
Ma 1/18	F	TTTGCCTGGTTGGGCTGA	Crouch et al., 1998	57.5 °C	150-178
	R	CCCCCCTTTCCTCTTTTGC			
Ma 1/24	F	GAGCCCATTAAGCTGAACA	Crouch et al., 1998	54°C	158-164
	R	CCGACAGTCAACATACAATAC A			
Ma 1/27	F	TGAATCCCAAGTTTGGTCAAG	Crouch et al., 1998	54°C	116-126
	R	CAAACACATGTCCCCATCTC			
Ma 3/2	F	GGAACAGGTGATCAAAGTGT GA	Crouch et al., 1998	56.2°C	216-224
	R	TTGATCATGTGCCGCTACTG			
Ma 3/90	F	GCACGAAGAGGCATCAC	Crouch et al., 1998	53.2 °C	130-156
	R	GGCCAAATTTGATGGACT			
Ma 3/103	F	TCGCCTCTCTTTAGCTCTG	Crouch et al., 1998	54°C	140-152
	R	TGTTGGAGGATCTGAGATTG			

F= Forward, R= Reverse, T_{ann} (°C) = Annealing Temperature

Detection of PCR products using Silver Staining Technique

PCR products were resolved using 6% polyacrylamide gel electrophoresis. The 6% polyacrylamide gel was poured in BIORAD Sequi-Gen® GT Nucleic Acid Electrophoresis Cell. A total of 6µL of STR 3X Loading Solution (10mM NaOH, 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol FF) was added to the PCR products in 0.2 mL PCR tubes and denatured in Mastercycler gradient at 95ºC for 5 minutes. Then 6µL of denatured PCR products was loaded on the 6% polyacrylamide gel and ran at 50W. The gel plates were fixed,

stained and developed following the direction reported in the Promega Silver Sequence™ DNA Sequencing System Technical Manual. The microsatellites bands were scored over a light box using pGem DNA marker (Promega, USA) and ϕ X174 DNA/*Hinf* 1 (Promega, (USA) as band size standard markers.

Data handling and analysis

Morphological data and PCR amplified products from individual plants were scored as either present (1) or absent (0) to create binary matrices, which were used to determine genetic similarity among banana cultivars. The morphological characters were scored using a method developed by Benesi (2002), in which traits with more than two categories of description for instance style shape were coded by considering the whole range of diversity of that trait and scored against that particular class. For example, the style shape ranges from 1 = straight, 2 = curved under stigma, 3 = curved at the base, 4= curved twice 5= other. If an accession had straight style shape, 1 was scored and the rest of the attributes were scored as 0 (Benesi, 2002).

The binary matrices from both methods were analysed using NTSYSpc version 2.11c (Rolhf, 2001). Pair wise similarity matrices were computed using simple matching coefficient (SM) (Sokal and Michener, 1958). The similarity matrices were used to construct dendrograms from the Sequential Agglomerative Hierarchical and Nested (SAHN) clustering method using the Unweighted Pair-Group Method with Arithmetical averages (UPGMA) (Sneath and Sokal, 1973). Bootstrap analysis, which is a method for determining confidence limits of clusters produced by UPGMA-based dendrograms, was performed using Win Boot program (Yap and Nelson, 1996).

Mantel's test was undertaken to determine correlation between Gene bank morphological and microsatellite data distance matrices (Table 3a; Table 3b). The matrices were based on 12 banana cultivars common to both data because though both data were collected from the same field gene bank, data collection years differences and condition of the cultivars during data collection resulted in the two data sets comprising of different number of cultivars. The Gene bank genetic data had 19 cultivars while the morphology data had 14 cultivars of which 12 were common in

both data sets. The MXCOMP programme of NTSYS-pc was used to compute a product-moment correlation coefficient (i.e. normalized Mantel's statistics Z) for the two matrices (Rohlf, 2001). In order to determine if the correlation was significant, actual coefficient was compared to the values produced by randomly permuting the matrix pair 2000 times.

Table 3a BRS GB morphological characterisation distance matrix (Nei, 1972) done in NTSYSpc 2.11c programme.

	Zandazanda	Zombared	Khazanga	Mulanje2	Nakashuga	Zombagreen	Ngerezi	Makumbuka	Phwiza	Kazanda	Kholobowa	Kabuthu
Zandazanda	0.00											
Zombared	0.54	0.00										
Khazanga	0.44	0.53	0.00									
Mulanje2	0.53	0.40	0.45	0.00								
Nakashuga	0.59	0.37	0.51	0.48	0.00							
Zombagreen	0.50	0.17	0.49	0.39	0.34	0.00						
Ngerezi	0.46	0.39	0.49	0.43	0.22	0.34	0.00					
Makumbuka	0.39	0.44	0.42	0.50	0.63	0.51	0.49	0.00				
Phwiza	0.54	0.32	0.32	0.43	0.14	0.26	0.20	0.47	0.00			
Kazanda	0.39	0.43	0.44	0.44	0.53	0.43	0.44	0.33	0.48	0.00		
Kholobowa	0.28	0.42	0.34	0.46	0.59	0.44	0.59	0.23	0.53	0.33	0.00	
Kabuthu	0.53	0.38	0.39	0.30	0.46	0.33	0.37	0.46	0.33	0.45	0.45	0.00

Table 3b BRS GB genetic characterisation distance matrix (Nei, 1972) done in NTSYSpc 2.11c programme.

	Kabuthu	Khazanga	Kholobowa	Makumbuka	Mulanje2	Nakashuga	Ngelezi	Phwiza	Zandazanda	Zombagreen	Zombared	Kazanda
Kabuthu	0.00											
Khazanga	0.05	0.00										
Kholobowa	0.11	0.09	0.00									
Makumbuka	0.09	0.07	0.08	0.00								
Mulanje2	0.07	0.05	0.07	0.09	0.00							
Nakashuga	0.09	0.07	0.08	0.03	0.05	0.00						
Ngelezi	0.10	0.08	0.08	0.03	0.05	0.00	0.00					
Phwiza	0.08	0.06	0.09	0.03	0.05	0.00	0.02	0.00				
Zandazanda	0.13	0.07	0.16	0.07	0.09	0.07	0.07	0.07	0.00			
Zombagreen	0.12	0.08	0.14	0.08	0.14	0.08	0.09	0.08	0.08	0.00		
Zombared	0.12	0.07	0.13	0.08	0.13	0.08	0.09	0.07	0.11	0.03	0.00	
Kazanda	0.10	0.08	0.08	0.07	0.05	0.07	0.10	0.08	0.11	0.09	0.12	0.00

RESULTS AND DISCUSSION

Comparison of morphological and genetic cluster analysis and cultivar relationships

A dengrogram of 12 BRS Gene bank cultivars generated using 109 morphological characters is shown in Figure 1. It divides into two clear clusters based on genome composition at 54% morphological similarity. The first cluster has ABB genome banana except one (*Khazanga*), which is a plantain (AAB) while the second cluster has dessert banana of AA and AAA genome composition. The bootstrap values confirm the validity of *Zomba red* and *Zomba green* (*p*= 96.1), *Nakashuga*, *Phwiza* and *Ngelezi* (*p*= 97.9) clusters at significant *p* values higher that 95% (Felsenstein, 1985). *Nakashuga* and *Phwiza* both AA bananas have a highest phenotypic similarity of 82% and *p* value of 68.1%, which is high enough though insignificant at 95% level.

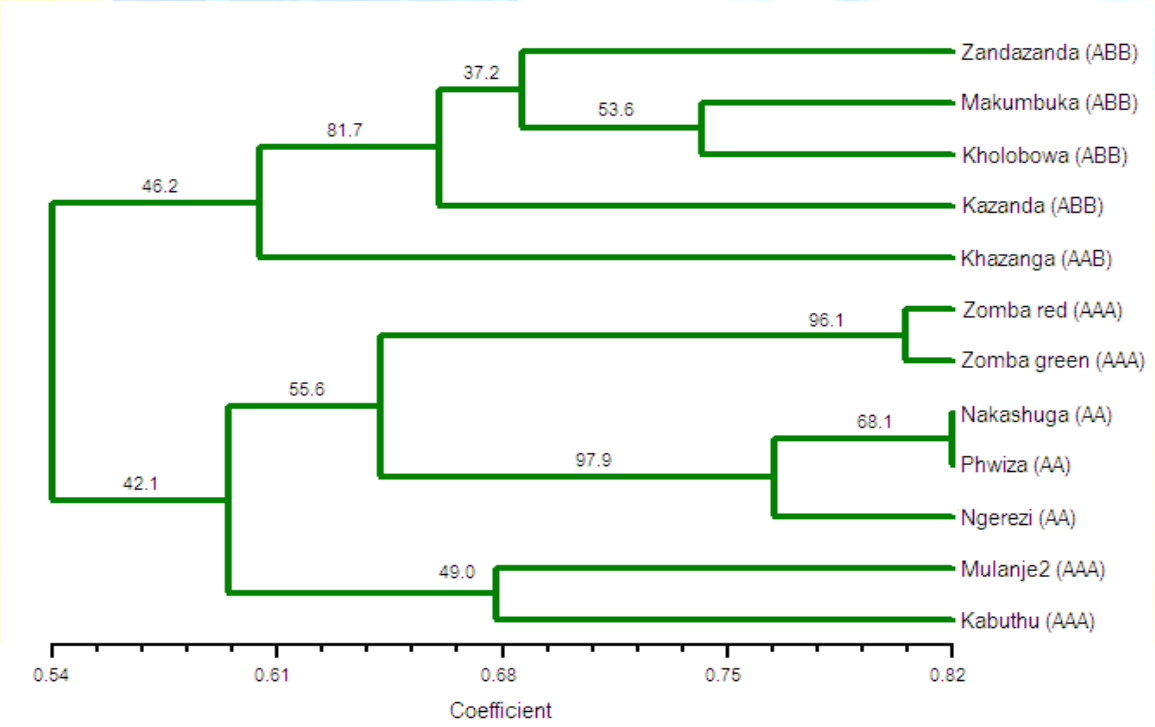

Figure 1 Morphological relationships among 12 BRS Gene Bank banana cultivars analysed using 109 morphological characters.
Number at node or side indicate bootstrap values in percentages.

In Figure 2 a dendrogram of 12 BRS gene bank cultivars based on microsatellite DNA data is presented. The major clusters in the dendrogram are not based on genomic composition of the

cultivars but the dendrogram replicates the *Nakashuga* (AA)-*Ngelezi* (AA)-*Phwiza* (AA) (*p*=80.3) and *Zomba green* (AAA)-*Zomba red* (AAA) (*p*=81.8) clusters observed in the dendrogram based on morphological characters. Highest genetic similarity is observed at 100% between *Nakashuga* (AA) and *Ngelezi* (AA) followed by *Phwiza* (AA)-{*Nakashuga* (AA) and *Ngelezi* (AA)} and *Zomba green* (AAA)-*Zomba red* (AAA) pairs at 99.05% and 96.4% respectively. Bootstrap analysis found no significant *p* values at 95 % level.

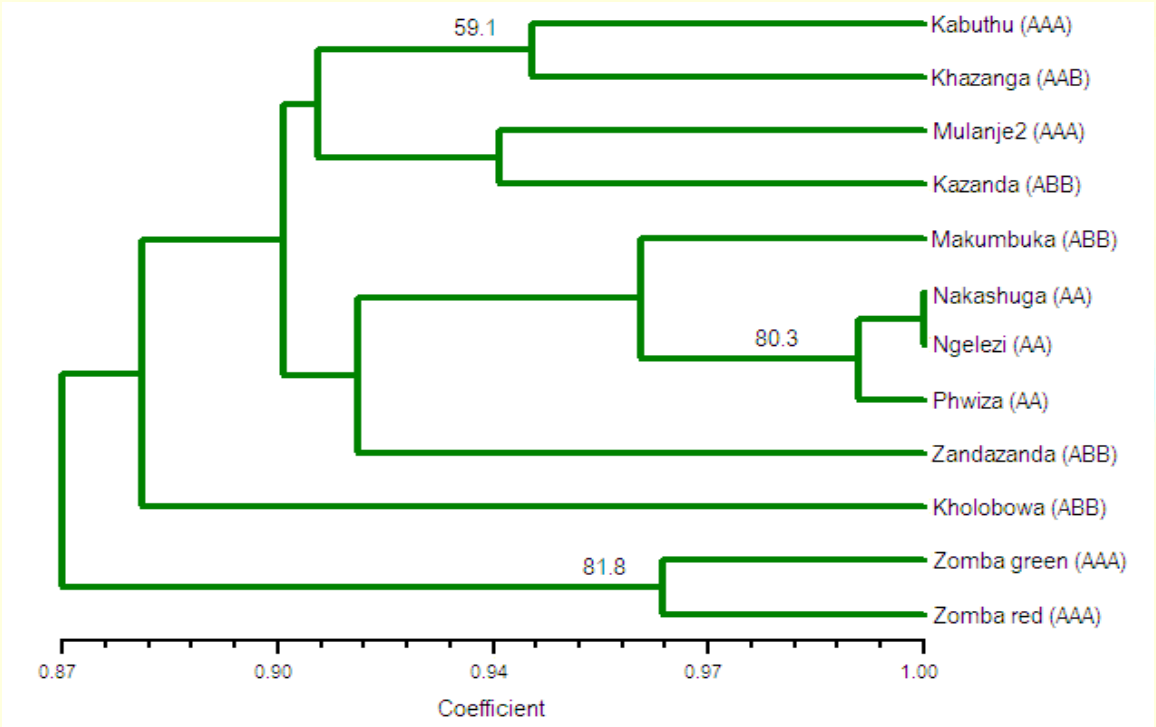

Figure 2 Genetic relationships among 12 BRS Gene Bank banana cultivars analysed using 12 microsatellite primers.
Number at node or side indicates bootstrap values in percentages.

Genetic characterisation showed that most genotypes were different except *Nakashuga* (AA) and *Ngelezi* (AA) which clustered at 100% similarity. *Nakashuga* (AA) and *Ngelezi* (AA) are possible synonymous and somatic mutants. Morphological characterisation also revealed that most genotypes were different from each other, implying high degree of morphological polymorphism. *Nakashuga* (AA) and *Phwiza* (AA) showed the highest similarity (82%). In contrast with genetic cluster analysis, morphological cluster analysis grouped cultivars into distinct genomic groups (Figure 1).

This observation is consistent with the fact that the initial classification of bananas into genomic groups was based on morphological features (Simmonds and Shepherd, 1955). Incongruence results obtained by molecular and morphological approaches have also been reported in other species such as pomegranates (Sarkhosh *et al*., 2006) and olives (Zitoun *et al*., 2008). The reason is that genetic radiation detected by molecular markers is not necessarily reflected in morphological variables measured (Kjaer *et al*., 2004). miccrosatellite markers are part of "junk DNA" which does not code for any phenotype therefore is not reflected in morphological variables which are expressions of coding DNA.

However, the ability of morphological characterisation to accurately group all cultivars at genomic level unlike genetic characterisation, reemphasizes the discriminatory power and usefulness of morphological data. Nsabimana and Staden (2006) showed in their study that determined ploidy of 89 accessions of the National Banana Germplasm Collection at Rubona in Rwanda using chromosome counting, that 65 accessions of highland banana clones were triploids as was reported previously using morphological characteristics.

Both morphological and genetic characterisation established the existence of the *Nakashuga*, *Phwiza* and *Ngelezi* and *Zomba green* and *Zomba red* subgroups (Figure 1; Figure 2). This observation confirms that genetic characterisation can be used to validate morphological characterisation in classification of *Musa* cultivars given the inadequacies of morphological characterisation (Onguso *et al.*, 2004).

Since genetic markers are not readily applied in Malawi because of a associated expenses, the complimentary link drawn between both characterisation methods can be exploited. Martinez *et al*. (2003) and Beyene *et al*. (2005) suggested that despite limitations associated with morphological descriptors compared to DNA fingerprinting, morphological characterisation is useful for preliminary evaluation because it is inexpensive and can be used as a general approach for assessing genetic diversity among phenotypically distinguishable cultivars.

The present validation of the *Zomba green* (AAA)-*Zomba red* (AAA) subgroup by morphological analysis confirms the field observation of Laisnez (2005) which showed

characterisation data conformable with expected values of the Red and Green sub group rendering *Zomba green* and *Zomba red* as members of the Red subgroup. *Zomba green* also referred to as Green red is known to be a mutant of the red sub group.

Genetic cluster analysis indicates that *Nakashuga* is closer to *Ngelezi* than *Phwiza* (100% similarity, bootstrap *p*=80.3) while morphological cluster analysis shows *Nakashuga* closer to *Phwiza* than *Ngerezi* (82% similarity, bootstrap *p* value 97.9%). Laisnez (2005) reported that *Ngerezi* and *Phwiza* had very similar characterisation data and are probably the same cultivar though they remarkably differ in the colour of the midrib dorsal surface with *Phwiza* showing pink-purple while *Ngerezi* being light green contributing to its local name which literally means being white as in "white man". *Phwiza* is probably a coloured mutant of *Ngelezi.* Therefore *Ngelezi*, *Nakashuga* and *Phwiza* could as well be somatic clones.

Table 5 shows Mantel's test statistics. The results indicate an insignificant positive correlation between Gene bank genetic and morphological distance matrices data (Table 3a; 3b) ($p \geq 0.05$) (Table 5).

Table 5 Mantel's statistics.

Normalised mantel's statistics Z (Matrix correlation, r)	**Z = 0.06**
Mantel's t-test	t = 0.51
Probability (random Z< observed Z)	*p*= 0.70

Clearly, distance matrices from morphological and SSR genetic characterisation are not analogous (Z=0.06, *p*=0.70). Similar correlation is obtained using similarity matrices (Z=0.10, *p*=0.82). Nonetheless distinct subgroups of *Nakashuga* (AA)-*Phwiza* (AA)-*Ngelezi* (AA) and *Zomba green* (AAA)-*Zomba red* (AAA) were distinctively comparable in characterisation dendrograms (Figures 1 and 2). Crouch *et al*. (2000) observed that studies that reported good positive correlation between morphological and genetic data were characterised by use of relatively small number (10-15) of morphological characters. In contrast, poor correlation was reported when a relatively large number (43) of morphological traits were used (Ortiz, 1997). In the present study, the number of morphological characters did not change the correlation

considerably. For instance, use of 15, 22, 40 morphological characters yielded Z values of 0.27, 0.21 and 0.25, respectively. Confusion about the effect of number of morphological characters on correlation values exists. Martínez de Toda and Sancha (1997a, b) and Martinez, *et al*. (2003) hypothesized that correlation between morphological and genetic data would improve if more morphological markers were used yet in this study' 109 morphological characters gave low correlation. Similarly, Engelborghs *et al*. (1999) reported that good correlations can be obtained between molecular and morphological diversity when a small number of genotypes are screened. However, a study of 16 Argentine grapevine varieties by Martinez *et al*. (2003) using morphological and AFLP data registered insignificant low correlation (Z= 0.33, *p*= 0.97). The results of the present study agreed with those of Crouch *et al.* (2000) study which reported RAPD analysis of large proportion of plantain landrace germplasm (76) screened with a relatively large number of genetic markers (11 RAPD primers). A general poor correlation between RAPD-based dissimilarity estimates and phenotype index was found, suggesting that the use of limited datasets in terms of number of variables or genotypes may lead to misleading simplistic interpretation (Crouch *et al*. 2000).

The low association between molecular and phenotypic markers distance matrices results from a range of factors. Molecular analysis samples wider genome area than morphological analysis since molecular markers such as microsatellies are abundant and widely distributed throughout the genome and also when both techniques are used, they are rarely the same number. The variations detected by molecular markers are non adaptive because they are not from coding DNA therefore, not subject to natural or artificial selection while phenotypic characters are adaptive and influenced by the environment, natural and artificial selection. Given these factors, data from molecular and morphological markers cannot be equivalent. The correlations would only be high when there is an association between the targeted loci (quantitative trait loci) and morphological traits evaluated (Dillmann *et al*., 1997; Schut *et al*., 1997; Lefebvre *et al*., 2001; Maric *et al*., 2004; Roy *et al*., 2004).

The question of predictability of characters through crosses involving Gene bank cultivars as parents is unresolved, since this study has observed no strong and significant correlation between morphological data and genetic data, It seems that candidates for crosses should be carefully

chosen not only on the basis of phenotypic diversity as a way of maximising heterozygosity in the resultant progeny. Other criteria should be used as transmission and prediction of epigenetic characters is unknown (Noyer *et al*., 2005).

CONCLUSIONS

Morphological descriptors have considerable discriminatory power and can be useful for preliminary evaluation. Morphological characterisation is inexpensive and should therefore be used for assessing genetic diversity among phenotypically distinguishable cultivars. However, genetic characterisation can be used to validate morphological characterisation in classification of *Musa* cultivars because of the inadequacies of morphological characterisation and also since the two techniques are complimentary.

The majority of BRS cultivars are different from each other. The absence of a strong correlation between phenotypic and genotypic diversity implies that hybridisation candidates chosen on the basis of morphological characters diversity would not necessarily maximize genetic heterozygosity in the resultant progeny.

Ngelezi (AA), *Nakashuga* (AA) and *Phwiza* (AA) are possibly synonymous and somatic mutants. *Zomba green* and *Zomba red* are members of the Red subgroup and *Zomba green* is mutant of the red sub group.

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