Morphological Characterisation of East African AAB and AA Dessert Bananas (*Musa* spp.)

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Abstract

Within the small-fruited *Musa* AAB dessert bananas in East Africa are cultivars that have potential for export, and the diploid AA bananas known in Kenya as "Muraru" are socially valued in the country. It is important to be able to distinguish these cultivars from other similar cultivars and identify the various subgroups within the AAB and AA genome groups, and possibly come up with some recommendations for future economical considerations. Objectives of this study were: 1) to identify morphological characters that distinguish a) the various subgroups of AAB dessert bananas found in East Africa, and b) the Muraru from other cultivated AA bananas; 2) to evaluate the relationship among the AAB and among the AA Muraru dessert banana groups of East Africa in relation to other bananas. Forty-three (43) cultivars of AAB, AA groups and outgroups from a large banana collection at the Kenya Agricultural Research Institute, Kisii were characterised in 2007 using morphological traits. Morphological data were collected using 84 characters derived from a modified version of the descriptors for bananas developed by Bioversity International in conjunction with CIRAD. Techniques of multivariate analysis were employed. Based on unweighted pair group using arithmetic mean (UPGMA), two major clusters of *Musa acuminata*-derived cultivars (AAs and AAAs) and hybrids of *Musa balbisiana* and *M. acuminata* (AAB) were produced. Within the major clusters were subclusters conforming to various subgroups. Within the AAB dessert cluster, four distinct subclusters were formed, i.e. Sukari Ndizi, Prata, Mysore and Silk. Muraru also formed a well-defined cluster. Thirty-three (33) characters contributed 71% of the total variation within the 43 accessions on the first and second principal components, allowing separation of clusters corresponding to genome groups and subgroups. The analysis further revealed that morphological traits, particularly of male bud, fruit and sucker, can be used to make distinctions within genome groups and subgroups, and to isolate various subgroups within genome groups. Morphological traits can be used confidently to describe various banana subgroups.

INTRODUCTION

Cultivated bananas are predominantly derived from two wild species, *Musa acuminata* Colla and *Musa balbisiana* Colla, that contributed the A genome and B genome, respectively. Banana cultivars can be diploids, triploids or tetraploids. Genome groups are defined based on the contribution of the two wild species and on ploidy: e.g. AA, AAA, AB, AAB, ABB. Morphological characters can help in classifying cultivars in genome groups, based on whether the cultivar has more morphological characteristics of the A genome or the B genome (Simmonds, 1966). While sexual reproduction is rare in cultivated bananas, somatic mutants have occurred independently in different progenitor clones and this has made morphological classification difficult. The genome groups are further subdivided by banana scientists and banana growers into named clusters of related clones, called subgroups. Each cluster of related clones can be subdivided further before reaching the level of a clone or cultivar.

The triploid cultivars have become the most important dessert and cooking bananas worldwide, and hence a lot of work has been done towards their characterisation. In East Africa, however, a number of small-fruited dessert bananas are becoming potentially important for future exports. Although they all have similar fruit size, they are very different in other aspects. A number of these, e.g. 'Sukali Ndiizi' and 'Kamaramasenge', have been considered diploids when they are not (Pillay et al., 2001), while others have been considered triploids. Diploid bananas are important in banana breeding as parent materials and for genetic studies aimed at efficient management of crop characters. In East Africa, there has been a search for wild diploids which could be relatives of the East African highland bananas (AAA, EAHB), a group of cooking bananas important for income generation in the region. If found, such diploids could be used in producing EAHB types with for instance disease resistance characteristics.

Clarification of the identity of these important diploids and triploids in the region is very much needed. Although a substantial amount of morphological studies have been done on different groups of bananas, including in East Africa (Stover and Simmonds, 1962; Karamura, 1998; Nsabimana and van Staden, 2005; Uma et al., 2004, 2006; Ortiz, 1997; Simmonds and Weatherup, 1990), these studies did not include the East African AAB "Apple" or AA "Muraru" dessert bananas. It is the purpose of this study 1) to determine morphological characters that distinguish the AAB Apple 'Sukari Ndizi' from other AAB Apple dessert bananas, 2) to identify morphological characters that allow recognition of East African AA Muraru dessert bananas, and 3) to evaluate the relationships between East African AAB Apple, AA Muraru dessert bananas and other bananas using morphological features.

MATERIALS AND METHODS

Study Site, Accessions and Sources

Morphological characterisation of 43 accessions (Table 1) was conducted at the Kenya Agricultural Research Institute (KARI) in Kisii, Kenya, where the horticultural trial had been established in October 2005. Data scoring for reference accessions was done in the collection established at the same location in 2002. The trial was located at an altitude of 1800 masl, latitude 000 41'S and longitude 340 37'E. The soils were characterised as acidic ultisol with a pH ranging from 5.6 to 6.2 at 10-15 cm deep and well drained although poor in nutrients. In the trial, each accession was represented by two plants established from suckers, planted 2 m apart with spacing of 3 m between rows.

The plants were grown under rainfed conditions of an annual average of 1700 mm; no additional irrigation was provided. The average relative humidity was 65%, the mean temperature 21°C, and there was an average of 7 hours of sunshine per day. At planting, 20 kg of well-rotted cattle manure plus 200 g of diammonium phosphate (DAP) was applied per planting hole. Later, the plants were top-dressed with 100 g of calcium ammonium nitrate (CAN) per mat every 6 months and coinciding with the rains. The orchard was kept weed free by manual weeding of the mats and chemical control (by using Roundup) on the paths. The study group consisted of the AAB Apple and AA Muraru bananas shown in the left column of Table 1. The outgroup in the right column of Table 1 consisted of bananas of other groups chosen to allow clear separation among the ingroups.

Choice of Morphological Characters and Character States

Eighty-four characters were selected from the Bioversity International banana descriptors (IPGRI-INIBAP/CIRAD, 1999). The characters selected were those which could easily be observed and are polymorphic enough to distinguish between various accessions (e.g. Fig. 1-4). Two representative plants per accession were scored for the various characters. The scoring of most characters took place at flowering, though bunch and fruit characters were scored at harvest when a bunch first showed a ripened yellow finger (a single individual banana fruit) on the first hand when the bunch was mature and ready for harvest. Data were recorded in a field notebook, transferred to Excel and subjected to multivariate analysis. All subsequent calculations were performed using the programme NTSYS-PC Version 1.8 (Rohlf, 2006). The average taxonomic distance coefficient was calculated between all pairs of accessions, based on equal weighting of all characters. The resulting distance matrix was subjected to cluster analysis. A variance-covariance matrix was also calculated and used in principal component analysis.

RESULTS

Cluster Analysis

The unweighted pair group method produced two major clusters, one consisting of AA and AAA accessions, and another consisting of AAB accessions. Banana accessions with the B genome were thus clearly separated from those lacking the B genome.

The AAB cluster was subdivided further into smaller subclusters representing commonly known subgroups (plantain, Mysore, Silk and Prata) but with an additional cluster of Sukari Ndizi (Fig. 3). The phenogram shows that Sukari Ndizi is closer to Silk than to Prata, while members of the Mysore subgroup were more distant (0.590 similarity) from the other AAB Apple bananas. The plantains, one of the major subgroups in the AABs, were closer (0.605 similarity) to the other AABs.

AA Muraru accessions formed a distinct cluster that was next (0.82 similarity) to the AAA dessert bananas cluster of the Gros Michel and Cavendish subgroups. While Muraru belongs to the AA genome group, Onyango et al. (2008a) using microsatellite markers showed that it is quite different from other AA diploids, like Sucrier and AA banksii group of bananas.

Principal Component Analysis

The principal component analysis produced comparable results to the cluster analysis with the first principal component accounting for 60% of the variation, while the

second contributed 11%. The second principal axis differentiates groups with the B genome in the upper half of the scatter diagram and those without the B genome in the lower half. Sukari Ndizi and Muraru formed their own subclusters. Of the 84 characters used in the analysis, 32 had a loading on either the first or the second principal component of more than 0.5 (Table 2). The majority of these characters are from the male bud, fruits, bunch and suckers. These characters have been useful in distinguishing Sukari Ndizi from the other AABs and Muraru from the AAAs.

DISCUSSION

Multivariate analyses have shown that Sukari Ndizi and Muraru constitute distinct subclusters. This indicates that in the AAB genome group, in addition to Mysore, Silk, Prata and plantain, Sukari Ndizi constitutes a fifth subgroup. The East African Sukari Ndizi is thus distinct from Prata, Mysore and Silk within the AAB Apple dessert banana group. The Sukari Ndizi accessions clustered together as a distinct group of AAB small-fruited dessert bananas using both microsatellites (Onyango, 2007) and morphological markers. The ploidy level of Sukari Ndizi was confirmed triploid using flow cytometry.

Sukari Ndizi has an intermediate leaf habit that may be mistaken for an erect habit especially when it is grown in very fertile soils where it may grow tall (>3 m) and slender. It has a cream compound tepal basic colour without pigmentation, in clear contrast with the pink/pink-purple pigmentation of Mysore, Silk, Prata and AB Kisubi, a beer banana mentioned in a separate study (Onyango, 2007). Sukari Ndizi has a short pedicel and the ovules are arranged in two rows, an *acuminata* characteristic. Unlike Mysore and Prata that have water suckers and or tissue-culture plantlets with purple blotches, Sukari Ndizi has no blotches on the leaves of water suckers. The plants are less robust than Prata and their pseudostem is generally straighter than that of Silk (which has a slightly arched pseudostem). Although all four AAB Apple banana groups have a bare rachis at the time of fruit maturity, the Sukari Ndizi rachis is always bare, even just a few weeks after flowering. In contrast to Prata that has longer (>20 cm) waxy silvery fruits, Sukari Ndizi has smaller fruits (≤15 cm) with short pedicels. Plants have slender to normal pseudostem of medium to tall height depending on the stage of crop growth and also on the soil fertility and rainfall. Pseudostem colour is green yellow with brown rusty brown pigmentation. The petiole margins of Sukari Ndizi are curved inwards, a *balbisiana* characteristic. Sukari Ndizi can be distinguished from Mysore by its fruits that are ≤15 cm in length with short pedicel, while Mysore has medium to long fruits (16-18 cm) that are slightly waxy. Mysore also has a darker green pseudostem with black pigmentation and purple midrib, and a purple cigar leaf, while Sukari Ndizi has green cigar leaf and a light green midrib. Sukari Ndizi has an average bunch weight of 8 kg with an average number of 8 hands per bunch and a mean of 100 fruits per bunch. Each hand has an average of 12 fruits each weighing 80 g, and the average weight of the hand is 1 kg. The fruit is green while mature but unripe, and turns yellow when ripe. Pulp colour is cream while mature green and yellow when ripe. Unlike fruits of most of the Silk subgroup that have pulp that is dry with hard brownish parts in the flesh, Sukari Ndizi has firm textured spotless uniform cream fresh pulp. Although the Sukari Ndizi fruits resemble those of Mysore at maturity and when fully ripe, the fruits of Sukari Ndizi have thicker peels and a more firm texture with an acidic taste, unlike the Mysore fruits that have a sweeter taste. Silk banana fruits are non-acidic and sweet.

In summary, key characteristics that may be used to identify the East African Sukari Ndizi from other small-fruited AAB Apple dessert bananas are the cream

compound tepal basic colour without pigmentation, lack of blotches on the leaves of water suckers and fruits ≤15 cm in length with short pedicel.

Multivariate analyses based on 84 morphological traits distinguished the same clusters as those based on the molecular analyses using microsatellite markers (Onyango, 2007) and the morphological phenogram has provided a phenotypic depiction of the relationships between the groups.

The East African Muraru dessert banana is a distinct group of AA dessert bananas, separate from Sucrier, banksii and other AA accessions studied (Onyango, 2007), the microsatellite and morphological markers were able to separate them as a distinct group of A genome accessions. In a separate study (Onyango et al., 2008b), Muraru ploidy level was confirmed as diploid by flow cytometry. The low (0.640) similarity with EAHB compared to that with AAA dessert bananas is an indication that Muraru is more closely related to the AAA desert banana than to the EAHB. Muraru bananas are medium-to-long fruited AA diploids. Most AA bananas have small fruits, but Muraru has fruits that may surpass those of some Cavendish and Gros Michel accessions in size. The plants have an erect leaf habit that shows their ploidy level, and they grow tall (>3 m) with a normal pseudostem. They have a cream-coloured compound tepal with brown pigmentation and a green ovary basic colour. The fruits have short pedicels. Unlike the EAHB, they have purple blotches on the leaves of water suckers, just like the Cavendish and Gros Michel in this study. For most Muraru, the rachis has few neutral flowers, but some have persistent withered bracts on the rachis. Muraru fruits are long and slender (>20 cm) relative to other diploid bananas, and have gradually tapering apices. The mature unripe fruits are green and shiny, and remain green-yellow to yellow when ripe, unlike the Gros Michel and the Cavendish dessert bananas which become golden yellow. The pseudostem colour is medium green with a little rusty brown to black pigmentation. A bunch of Muraru weighs on average 16 kg, with a mean 8 hands that weigh 2 kg. There is an average of 16 fruits per hand, each weighing 125 g. The pulp colour is cream while mature green, and ivory to cream when mature ripe. In a separate study (Onyango, 2007), it was shown that unlike the sugary fruits of the Musa AA Sucrier subgroup Pisang Mas; AA Muraru subgroup fruits have a mild, slightly tasty or bland predominant taste. The fruits of Muraru have thicker peels and a firmer texture than those of Pisang Mas. Daniells et al. (2001) have placed some of the cultivated AA bananas into four subgroups: (1) Sucrier, e.g. 'Pisang Mas' (2) Pisang Lilin, e.g. 'Pisang Lilin', (3) Inarnibal, e.g. 'Inarnibal', and (4) Pisang Jari Buaya, e.g. 'Niukin'. Simmonds (1962) also recorded several distinct populations within *M. acuminate*, namely *microcarpa*, *siamea*, *malaccensis*, *banksii* and *burmannica*. Since *M. acuminata* is a variable species with several different subspecies (Simmonds and Weatherup, 1990), the different edible diploids may have originated independently and more than once. Jenny et al. (2003) noted that despite the large number of cultivated diploid clones known, no further subgroups have been established.

In summary, key characteristics that may be used to identify the East African Muraru include an erect leaf habit and edge of petiole margin with pink-purple colour line. The fruit is lengthily pointed and shiny green and ≥20 cm long. The fruit colour when ripe is green to green-yellow. They have normal pseudostem aspect with medium green colour having brown-black pigmentation bract external surface is purple brown wax and no blotches on the leaves of water suckers.

Literature Cited

Daniells, J., Jenny, C., Karamuara, D. and Tomekpe, K. 2001. Diversity in the genus *Musa*. INIBAP Musalogue. A catalogue of *Musa* germplasm. INIBAP, France.

IPGRI-INIBAP/CIRAD. 1999. Descriptors for banana (*Musa* spp). IPGRI, Rome, Italy / INIBAP, Montpellier, France / CIRAD, Montpellier, France.

Jenny, C., Carreel, F., Tomekpe, K., Perrier, X., Dubois, C., Horry, J.P. and du Montcel, H.T. 2003. Banana. p.99-124. In: P. Hamon, M. Seguin, X. Perrier and J.C. Glaszmann (eds.), Genetic Diversity of cultivated tropical plants. Science publishers, Inc. Enfield (NH) Plymouth, UK.

Karamura, D.A. 1998. Numerical taxonomic studies of East African highland banana in Uganda (*Musa* AAA, East Africa). PhD Thesis. University of Reading, UK.

Nsabimana, A. and van Staden, J. 2005. Characterization of the banana germplasm collection from Rubona - Rwanda. Scientia Horticulturae 107:58-63.

Onyango, M.A. 2007. Characterization of East African accessions of *Musa* AAB "Apple" and *Musa* AA "Muraru" dessert bananas. PhD thesis. University of Hawaii at Manoa, USA.

Onyango M. A., Haymer D., Keeley S., and Manshardt, R. 2008a. Analysis of genetic diversity and relationships in East African AAB "Apple" and AA "Muraru" dessert bananas using microsatellite markers. Paper presented at the international banana conference October 2008, Mombasa, Kenya

Onyango M., Manshardt R., Haymer D., Karamura D. and Paull R. 2008b. Assessing nuclear DNA content of East African *Musa* AAB "Apple" and AA "Muraru" dessert bananas and other banana groups using flow cytometry. Paper presented at the 11th KARI biannual conference, November 2008, Nairobi, Kenya.

Ortiz, R. 1997. Morphological variation in *Musa* germplasm. Genetic Resources and Crop Evolution. 44:393-404.

Pillay, M., Ogundiwin, E., Nwakanma, D.C., Ude, G. and Tenkouano, A. 2001. Analysis of genetic diversity and relationships in East African banana germplasm. Theor. Appl. Genet. Theor. 102:965-970.

Rohlf, J.F. 2006. NTSYS-pc 2.2 Numerical Taxonomy and Multivariate Analysis System. Version 2.2. Department of Ecology and Evolution, State University of New York, Stony Brook.

Simmonds, N.W. 1962. The evolution of bananas. Longman, London.

Simmonds, N.W. 1966. Bananas. 2nd ed. Longman, London.

Simmonds, N.W. and Weatherup, S.T.C. 1990. Numerical taxonomy of the cultivated bananas. Tropical Agriculture 67(1):90-92.

Stover, R.H. and Simmonds, N.W. 1962. Bananas. 3rd ed. Longman, New York, USA.

Uma, S., Sudha, S., Saraswathi, M.S., Minickavasagam, M., Selvarajan, R., Durai, P., Sathiamoorthy, S. and Siva, S.A. 2004. Analysis of genetic diversity and phylogenetic relationships amongs indigenous and exotic Silk (AAB) group of bananas using RAPD markers. Journal of Horticultural Science and Biotechnology 79(4):523-527.

Uma, S., Siva, S.A., Saraswathi, M.S., Minickavasagam, M. and Durai, P. 2006. Variation and intraspecific relationships in Indian wild *Musa balbisiana* (BB) population as evidenced by random amplified polymorphic DNA. Genetic Resources and Crop Evolution 53:349-355.

Tables

Table 1. Banana accessions used in morphological characterisation study.

Local names of study groups	Local names of outgroups
AAB "Apple" Mysore	**AAA Cavendish**
Sukari red, Wangae red	Poyo, Pekera
AAB "Apple" Prata	**AAA Gros Michel**
Manyatta, Kifutu -Kisii, Exera, Kifutu -Thika, Soth	Kampala, Pelipita Kisii, Bogoya, Pelipita Thika, Gros Michel
AAB "Apple" Silk	**AAA EAH cooking**
Manjano, Mboki Msukari, Ungoye sweet	Kimuga, Ngombe
AAB "Apple" "Sukari Ndizi"	**AAB Plantain**
Sweet yellow, Sweet white, Wangae, Kamaramasenge, Sukari Ndizi, Wangae, Embu, Kakamega	Horn plantain, Spambia
AAAB "Apple" tetraploid	**AAAB FHIA hybrid**
GT	Gold finger
AA "Muraru"	**ABB bananas**
Muraru, Muraru Mshare, TT2, Njuru, Majimaji, Mraru Mlalu, Kamunyilya, Muraru white green bell, Muraru red bell, Makyughu	Ngoja, Kayinja

Table 2. Character loadings PC1, PC2 and PC3.

Character	**PC1**	**PC 2**	**PC 3**
Anther colour	**0.9282**	0.1902	0.0743
Dominant colour of male flower	**0.9241**	-0.2668	-0.1404
Compound tepal basic colour	**0.8509**	0.0551	-0.2791
Compound tepal pigmentation	**0.8369**	-0.4621	0.2349
Immature fruit peel colour	**0.8341**	0.2410	-0.2531
Bract apex shape	**0.7989**	0.1480	-0.1086
Anther exsertion	**0.7980**	-0.4668	0.2144
Blotches on leaf of water suckers	**-0.7928**	0.0123	-0.0224
Number of suckers	**0.7799**	-0.0631	0.4694
Fruit pedicel length	**0.7768**	0.1843	-0.4356
Immature fruit peel appearance	**0.7418**	0.5913	-0.4518
Empty nodes on peduncle	**0.6641**	0.0445	-0.4936
Petiole canal leaf III	**0.6430**	0.1451	0.4173
Bunch position	**0.5902**	0.2746	0.0287
Blotches at the petiole base	**-0.5893**	-0.1555	**-0.6798**
Fruit apex	**0.5823**	**-0.6921**	**-0.5173**
Colour of the bract internal face	**0.5217**	-0.0632	0.1432
Fruit position	**0.5072**	**-0.6374**	0.3118
Bract scars on rachis	0.3142	**0.7943**	0.0495
Male bud shape	-0.2379	**-0.7276**	-0.2841
Bract lifting	0.2197	**0.7098**	-0.2608
Free tepal apex shape	0.3572	**0.6759**	**-07932**
Predominant taste	0.4445	**0.6593**	0.4345
Male flower behaviour	0.3114	**0.6264**	-0.1703
Free tepal shape	-0.1849	**0.5516**	-0.3827
Male bract shape	-0.3256	**0.5513**	0.2421
Pseudostem height	0.1887	**0.5489**	-0.4407
Wax on leaf sheaths	-0.0881	**0.5360**	0.2049
Colour of leaf upper surface	0.0049	**-0.5347**	-0.4454
Fruit length	-0.1934	**0.5336**	-0.1159
Pseudostem colour	0.3856	**-0.5180**	-0.4800
Color of the bract external face	0.1241	**-0.5099**	0.3672

Figures

Figure 1: Inner color of pseudostem trait of various banana accessions used in scoring, from top clockwise ABB Kayinja, AAA Cooking, AA "Muraru" and AAA Cavendish

Fig. 1. Inner colour of pseudostem, used in morphological characterisation of banana accessions.

Left to right; ABB and AAA bananas. Data of petiole base pigmentation, bract colour. 2 weeks after flower emergence best time to check on arrangement of ovules

arrangement).

Figure 3: Morphological traits of various banana accessions used in the scoring of bananas; from top clockwise bunch and fruit traits, male bud and flower traits, petiole canal and cigar leaf

Fig. 3. Morphological traits of various banana accessions used in the scoring of bananas; from top left clockwise: bunch and fruit traits, male bud and flower traits, petiole canal and cigar leaf.

Figure 4: Ripe fruit traits used in scoring morphology; clockwise from top left AAB "Sukari Ndizi", AAB 'Silk', Silk pulp and AAB 'Prata' and AA 'Muraru'

Fig. 4. Ripe fruit traits used in scoring morphology; from top left clockwise: AAB 'Sukari Ndiizi', AAB 'Silk', 'Silk' pulp and AAB 'Prata', and AA 'Muraru'.

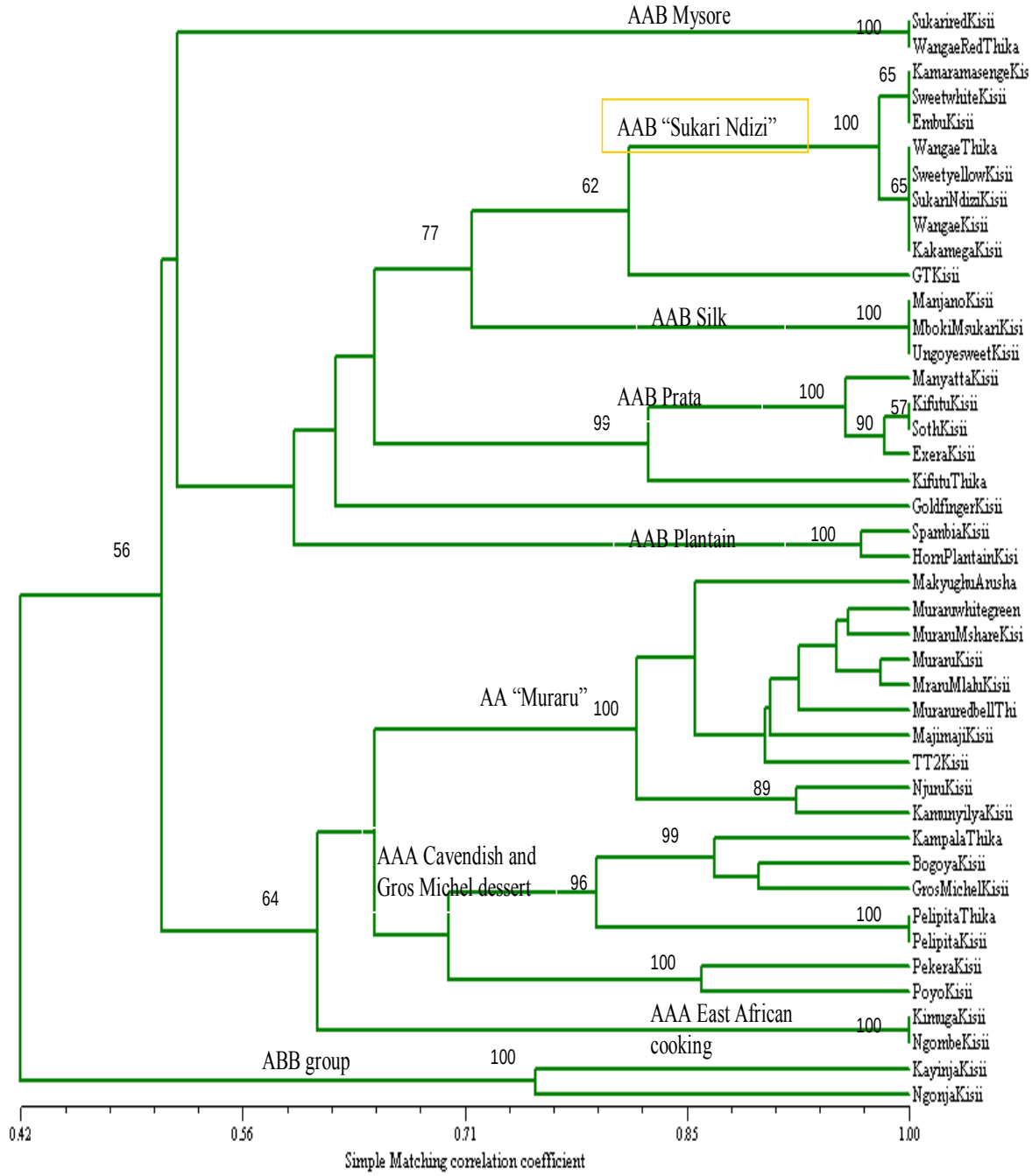

Figure 3.7: Phenogram from UPGMA between 43 East African accessions of AAB "Apple" and AA "Muraru" and outgroups based on morphological traits. Cophenetic value = 0.889

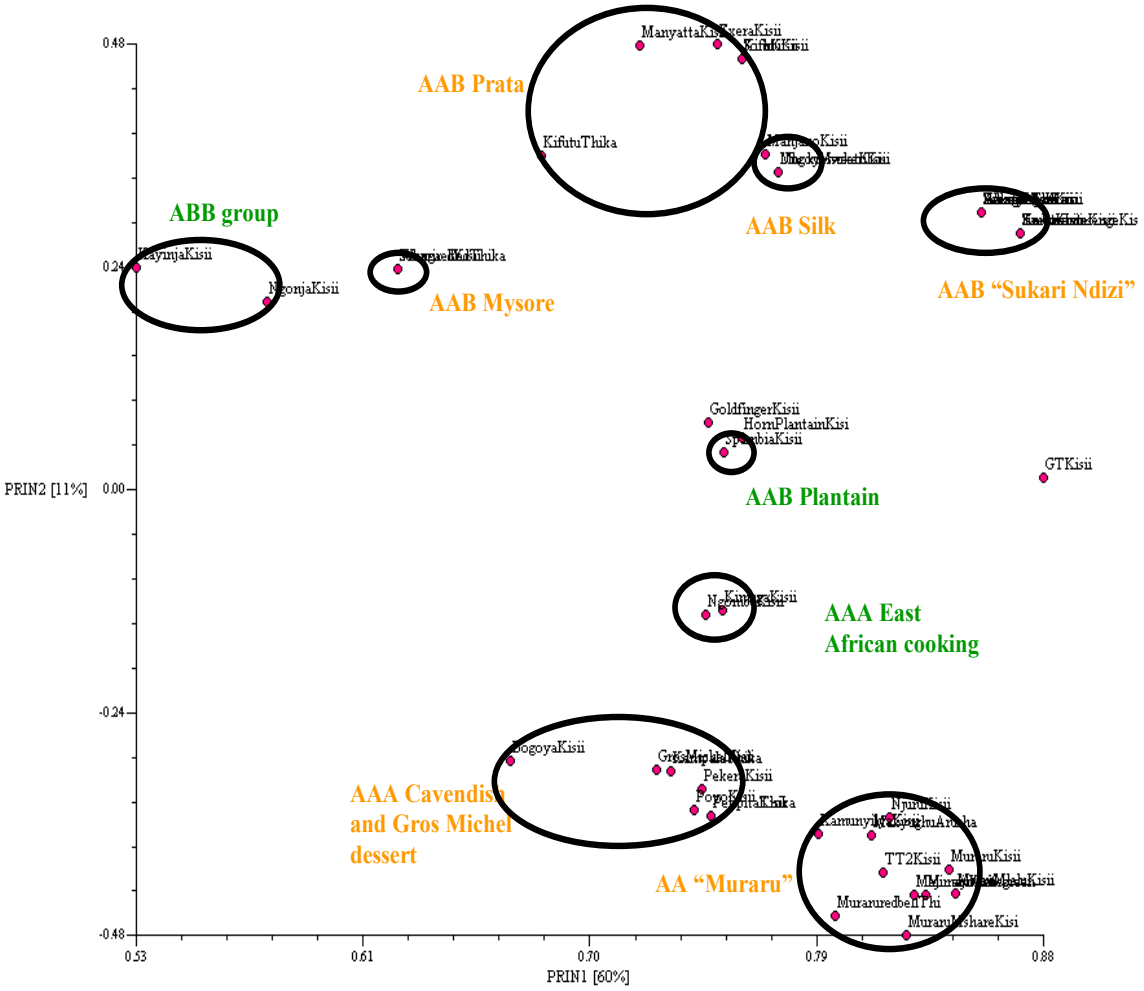

Figure 3.8: PCA based on morphological traits showing relative positions on the 1st and 2nd PC of 43 banana accessions at KARI-Kisii, Kenya