ASSESSING MORPHOLOGICAL DIVERSITY OF MANGO GERMPLASM FROM THE UPPER ATHI RIVER (UAR) REGION OF EASTERN KENYA

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Abstract
Morphological characterization allows for the study of plant variation using visual attributes. Fruits have been the major descriptors for identification of different varieties of fruit crops. However, even in their absence, farmers, breeders and interested stakeholders require to distinguish between varieties. This study aimed at determining diversity in mango germplasm from the UAR and providing useful descriptors for the identification of different mango varieties in the absence of fruits. A total of seventeen IPGRI (2006) descriptors for mango were selected for use in visual assessment of 98 mango accessions from 15 sites of the Upper Athi River (UAR) region of Eastern Kenya. Purposive sampling was used to identify farmers growing diverse varieties of mangoes. Evaluation of descriptors was performed on site and data collected was subjected to multivariate analysis including Principal Component and Cluster (PCA) analysis. Results classified the accessions into two major groups corresponding to indigenous (17.35%) and exotic (82.65%) varieties. The PCA showed the first seven principal components accounting for 82.87% of the total variance. A strong and highly significant correlation was also found between the colors of young leaves, stem circumference, tree height, leaf margin type and fragrance strength. Four leaf descriptor traits namely pulvinus thickness, leaf pubescence, angle of secondary veins to midrib and presence of secondary veins on leaf, were discarded for presenting only one phenotypic class and hence ineffective in distinguishing between mango varieties in the UAR. These results reveal that mango germplasm in the UAR possesses significant diversity and that other morphological traits apart from fruits are useful in determining significant morphological variation that can be incorporated in mango breeding programs in Kenya.

Key words: Morphological characterization, multivariate analysis, mango, upper Athi River region

1 Introduction

Mango (*Mangifera indica* L.) a native of Southeast Asia, is one of the important fruit crops in the tropical and subtropical lowlands thought to have been introduced to East Africa in the 14th century (Duval *et al.*, 2005). Currently, mango has been listed as the third most important fruit crop after bananas and pineapples in terms of area and total production in Kenya (HCDA, 2010). The increasing demand for the fruit is due to the fruits high vitamin, mineral and fiber levels besides the value-added products made from it. Consequently, the fruit brings economic benefits from both local sales and foreign earnings upon export.

Mango has been reported to have extensive diversity due to alloploidy, outbreeding, continuous grafting and phenotypic differences arising from varied agro climatic conditions in different mango growing regions (Ravishankar *et al.*, 2000). The important commercial mango varieties introduced in Kenya from Florida, Australia, Israel and other countries remain to be fully evaluated and distributed (Griesbach, 2003). In addition, mango being highly cross pollinated, open pollination between the cultivars could have resulted in new varieties not yet documented. Subsequently, mango varieties have experienced great confusion in their nomenclature with many synonyms existing for the same varieties. Further, while geneticists and plant breeders are particularly interested with diversity at the molecular level (Hawkes, 1991; Dempsey, 1996), farmers are more concerned with how visible morphological and agronomic variations can be used for sustainable farming. They are faced with the challenge of identifying cultivars that are productive for their agro-ecological zones because they are unfamiliar with the characteristics of the many different cultivars of mango that are now grown and available in the country, resulting in lower productivity (Griesbach, 2003; Kehlenbeck *et al*, 2011 ; Wahdan *et al*., 2011).
Morphological characterization is thus the simplest of the formal, standardized, repeatable method of identifying and presenting mango's genetic diversity (Watson and Eyzaguirre, 2002). Assessment of morphological variation in fruit crops usually requires the availability of fruits. However, in the off-fruiting season, farmers, grafters, nursery managers and breeders still require to tell apart different varieties. This necessitates the provision of mango descriptors that can be used in the absence of fruits. This study's objective was to determine diversity in mango germplasm from the UAR, a region growing both local and improved varieties, using descriptors for mango plant that excluded fruit traits. This will enable the effective utilization of mango's genetic resources especially in breeding programs for sustainable improvement of this crop.

2 Materials and Methods

2.1 Survey and Sampling

A targeted baseline survey was conducted in 15 sites of the the UAR in the period of April to May 2011 and April to May 2012. These included; Kilala, Ikalyoni, Ikangavya, Kasikeu, Kikoko, Kilala, Kiou, Kithangathini, Kyamusoi, Kyanginywa, Malivani, Mbiuni, Sekereni, Station I, Station II and Wote with attitudes ranging from 718-1657 m asl. Purposive sampling with the help of the Agricultural Extension Officers was used to identify farmers in these areas that cultivated diverse mango varieties.

2.2 Data Collection

Mango accessions were visually evaluated on site using IPGRI (2006) descriptors for mango. Measurements on tree height, stem diameter, tree growth habit and crown shape were recorded. For the leaves, the leaf attitude, color of young and fully mature leaves, fragrance strength, leaf blade shape, leaf blade length, leaf blade width, petiole length, leaf apex shape, leaf base shape, leaf margin type, leaf texture, pulvinus thickness, leaf pubescence, angle of secondary veins to midrib and presence

2.3 Data Analysis

Data collected was subjected to Principal Component (PCA) and Cluster analysis using the XLSTAT 2012 statistical package. Cluster analyses were carried out on the principal components using the hierarchic ascendant analysis and Euclidian average distance.

Table 1: Morphological descriptors used in the characterization of mango germplasm from the UAR region of Eastern Kenya (Source; IPGRI, 2006)

TRAIT	PHENOTYPIC CLASSES	
	Qualitative	**Quantitative**
1. TREE		
Tree type	1. Grafted 2. Seedling 3. Wildtype/acquired	--
Tree height	--	(m)
Stem circumference	--	Inches
Growth habit	1. Erect 2. Spreading 3. Drooping	--
Crown shape	1. Oblong 2. Broadly pyramidal 3. Semi-circular 4. Spherical	--
2. LEAF		
Leaf attitude (in relation to branch)	1. Semi-erect 2. Horizontal 3. Semi-drooping	--
Color of young leaf (CYL)	1. Light green 2. Light green with brownish tinge 4. Reddish brown 5. Deep coppery tan	--
Color of fully mature leaf (CFL)	1. Pale green 2. Green 3. Dark green	--
Fragrance strength	1. Absent 2. Mild	--
Leaf blade shape (LBS)	1.Elliptic 2. Oblong 3. Ovate 4. Obovate 5. Lanceolate 6.Oblanceolate	--
Leaf blade length (LBL)	--	(cm)
Leaf blade width (LBW)	--	(cm)
Petiole length	--	(cm)
Leaf apex shape	1. Obtuse 2. Acute 3. Acuminate	--
Leaf base shape	1. Acute 2. Obtuse 3. Round	
Leaf margin type	1. Entire 2. Wavy	
Leaf texture	1. Coriaceous 2. Chartaceous 3. Membranous	--

3 Results

Ninety eight mango accessions (Table 2) were collected from the UAR, representing a total of twenty one different cultivars. Of these, nine varieties were local while the remaining twelve cultivars belonged to the exotic varieties, with three local but improved cultivars. The local varieties included, Dodo, Kasukari, Katili, Kitui, Mombasa, Ndoto, Sikio la punda and two local varieties whose names were not identified. The exotic varieties included Apple, Batawi, Haden, Keitt, Kent, Maya, Ngowe, Nimrod, Sabine, Sensation, Tommy Atkins and Vandyke whereas the local but improved cultivars included Apple, Batawi and Ngowe. A summary of the statistic parameters of characterization traits of mango germplasm is presented in Table 3.

The distribution of these accessions in the PCA based on the PC-1 and PC-2 shows the phenotypic variation among the accessions and how widely dispersed they are along both axes on the bi-plot (Figure 1). The two components explain a cumulative variability of 50.65% whereas the first seven principal components took into account 82.87% of the total variance (Table 4). Based on the distribution of variates, the accessions of 01K5 (Katili), 01K4 (Kitui), 01N1 (Ndoto), 026A (Apple) and 04K2 (Kent) are the most distantly related varieties to their respective clusters. Generally, most accessions clustered within the first and fourth quartiles. However, 2 distinct clusters were presented in the bi-plot, clustering on the positive end of the F1 axis were the local varieties namely Dodo, Kitui, Kasukari, Katili, Ndoto, Mombasa and the two unidentified local cultivars whereas all the exotic varieties and the local but improved cultivars clustered on the negative end of the same axis. This could be attributed to the obvious morphological differences observed between the cultivars such as tree size, leaf and petiole sizes and crown shape (Plate 1).

The correlation among descriptor traits showed several clusters (Figure 2). The highlighted cluster contains some of the most descriptive traits of the mango accessions and included: the color of young leaves (CYL), stem circumference, tree height, leaf margin type and fragrance strength. Further, the relationship among the 98 accessions was illustrated by the agglomerative hierarchical clustering dendogram that was divided into 4 main branches; C1, C2, C3 and C4 based on the major morphological characters associated with them (figure 3). The first cluster represented seventeen accessions, all being local cultivars (Dodo, Kasukari, Mombasa, Sikio la Punda, Katili, Ndoto, Kitui and two unidentified local varieties), the second one contained twenty five accessions (Batawi, Kent, Haden, Ngowe and Maya), the third one contained twenty nine accessions (Apple, Tommy Atkins, Keitt, Kent, Sabine, Sensation, Ngowe, Haden and Van dyke) and the fourth one contained twenty seven accessions (Apple, Nimrod, Sabine, Tommy Atkins, Keitt and Van dyke). Batawi and Maya only appeared in the first cluster, Sensation appeared only in the third cluster and Nimrod featured in the fourth cluster only. Kent, Haden and Ngowe featured in both clusters 2 and 3 while the remaining i.e Sabine, Apple, Tommy Atkins, Vandyke and Keitt appeared in both clusters 3 and 4.

Mango accessions collected from the UAR

Table 2: Mango accessions collected from the UAR region of Eastern Kenya (Total of 98 accessions representing 21 cultivars).

Variety	Location	Accesion code given	Variety	Location	Accesion code given
Apple	Ikalyoni	013A, 014A, 017A, 018A, 020A, 035A, 036A	**Kent**	Ikalyoni	06K2, 07K2, 09K2
	Ikangavya	01A, 06A, 011A		Kilala	08K2
	Kasikeu	015A		Kiou	05K2
	Kilala	03A, 04A, 09A, 029A,		Kyamusoi	02K2
	Kiou	023A, 025A, 026A, 027A, 034A		Station II	01K2, 03K2, 04K2
	Kithangathini	012A,	**Kitui**	Mbiuni	01K4
	Kyamusoi	02A	**Maya**	Kilala	01M1, 02M2,
	Kyanginywa	016A, 019A, 021A, 022A	**Mombasa**	Ikangavya	01M2
	Malivani	05A	**Ndoto**	Ikalyoni	01N1
	Mbiuni	028A, 030A,031A, 032A,		Kilala	01N2

		033A			
	Sekereni	024A	**Ngowe**	Ikalyoni	07N3, 08N3, 014N3
	Station I	07A		Kasikeu	06N3
	Station II	010A		Kilala	02N3, 010N3
	Wote	08A		Kiou	04N3, 05N3, 012N3, 013N3
Batawi	Kilala	01B,02B		Mbiuni	09N3, 011N3
Dodo	Kyanginywa	02D, 03D		Station I	03N3
	Wote	01D		Station II	01N3
Haden	Kilala	01H, 02H	**Nimrod**	Wote	01N0
Indigenous 1	Kiou	01L	**Sabine**	Kilala	02S2, 03S2
Indigenous 2	Sekereni	02L		Wote	01S2
Kasukari	Ikalyoni	04K1	**Sensation**	Kilala	01S3
	Ikangavya	02K1	**Sikio la punda**	Kyanginywa	01S1
	Kasikeu	06K1	**Tommy**	Kilala	03T, 04T, 05T
	Kikoko	03K1		Station II	02T
	Kilala	01K1		Wote	01T
	Kiou	05K1	**Vandyke**	Ikalyoni	03V
Katili	Kilala	01K5		Kilala	01V, 02V
Keitt	Kilala	01K3, 02K3			

Statistic parameters of characterization traits of mango germplasm from UAR

Table 3: Showing the statistic parameters of morphological traits used in characterization of mango germplasm from the UAR

Variable	Minimum	Maximum	Mean	Std. deviation
Leaf Blade Shape	1.000	8.000	3.102	2.870
LBL	9.140	31.500	16.957	4.991
LBW	2.586	9.300	4.593	1.350
Petiole Length	2.000	8.250	4.682	1.299
Leaf Apex Shape	2.000	3.000	2.429	0.497
Leaf Base Shape	1.000	2.000	1.796	0.405
Leaf Margin	1.000	2.000	1.296	0.459
Leaf Attitude	1.000	2.000	1.633	0.485
Leaf Texture	1.000	3.000	1.755	0.499
CYL	1.000	5.000	2.531	1.333
CFL	1.000	3.000	2.551	0.558
Fragrance	1.000	2.000	1.255	0.438
Height	2.000	10.000	5.619	2.341
Stem circumference	20.800	105.800	42.812	24.060
Growth habit	1.000	2.000	1.694	0.463
Crown shape	1.000	4.000	2.980	0.885

Principal Component Analysis

Table 4: Principal component analysis (PCA) performed using the XLSTAT (2012) statistical package showing the correlations of the first fifteen principal components with the variables observed on mango accessions

Principal Component	Variate	Eigen value	Percentage Variation (%)	Cumulative Variation (%)
PC1	Color mature leaf	6.11	38.2	38.2
PC2	Color young leaf	1.99	12.45	50.65
PC3	Crown shape	1.39	8.68	59.33
PC4	Fragrance	1.18	7.35	66.68
PC5	Tree growth habit	1.01	6.28	72.96
PC6	Tree height	0.81	5.09	78.05
PC7	Leaf apex shape	0.77	4.82	82.87
PC8	Leaf blade length	0.59	3.67	86.54
PC9	Leaf blade width	0.52	3.23	89.77
PC10	Leaf attitude	0.5	3.13	92.9
PC11	Leaf base shape	0.38	2.36	95.26
PC12	Leaf margin	0.33	2.03	97.29
PC13	Leaf texture	0.26	1.61	98.9
PC14	Petiole length	0.08	0.51	99.41
PC15	Stem circumference	0.07	0.44	99.85

Eigen vectors of the most descriptive traits

Table 5: Eigen vectors of the most descriptive traits measured in mango germplasm from the UAR
[a] Values with asterisk indicate the most descriptive traits

Descriptor	Principal Component [a]		
	1	2	3
LBS	-0.026	-0.084	0.657*
LBL	0.369	0.814*	0.341
LBW	0.402	0.821*	0.161
Petiole Length	0.204	0.346	-0.274
LAS	0.447	-0.276	0.603*
Leaf Base Shape	-0.688	0.048	0.104
Leaf Margin	0.790*	-0.075	0.291
Leaf Attitude	0.397	0.059	-0.326
Leaf Texture	0.358	-0.501	0.036
CYL	0.724*	0.009	0.111
CFL	0.133	0.315	-0.348
Fragrance	0.758*	-0.177	-0.230
Height	0.853*	-0.062	-0.151
Stem circumference	0.926*	-0.023	-0.163
Growth habit	0.372	-0.043	0.165

Crown shape	-0.688	0.227	0.104

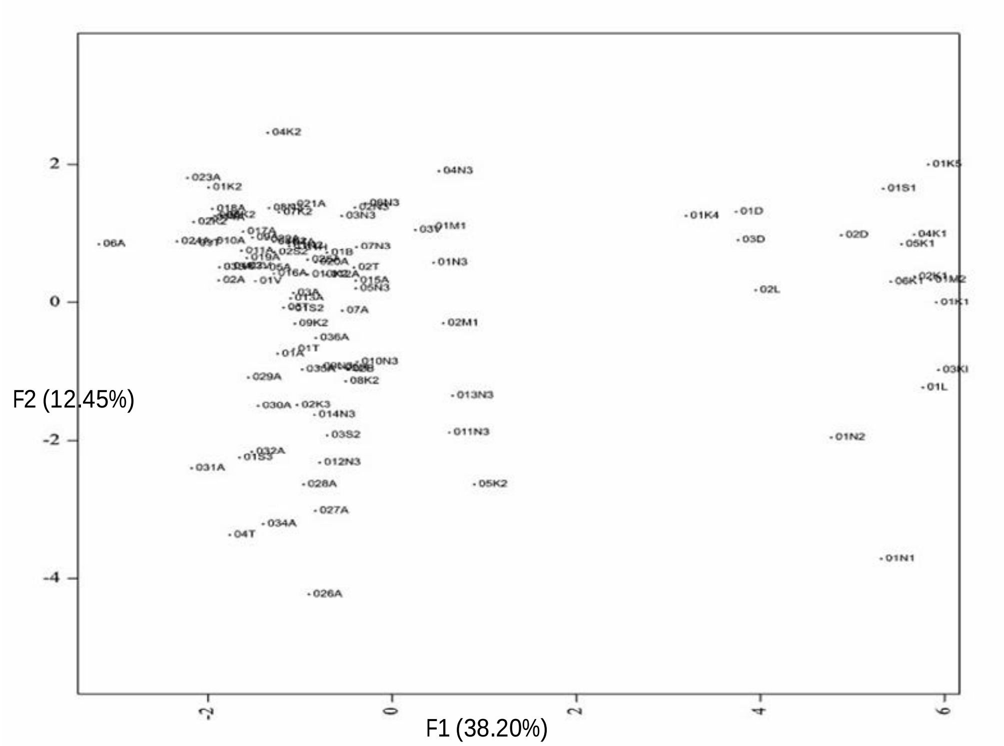

Figure 1: Distribution of variates in PC1 and PC2. PC1 accounts for 38.20% of the variation while PC2 accounts for 12.45%)

Plate 1: Morphological diversity presented leaf habit, leaf shape and color of young leaves in mango accessions of the UAR

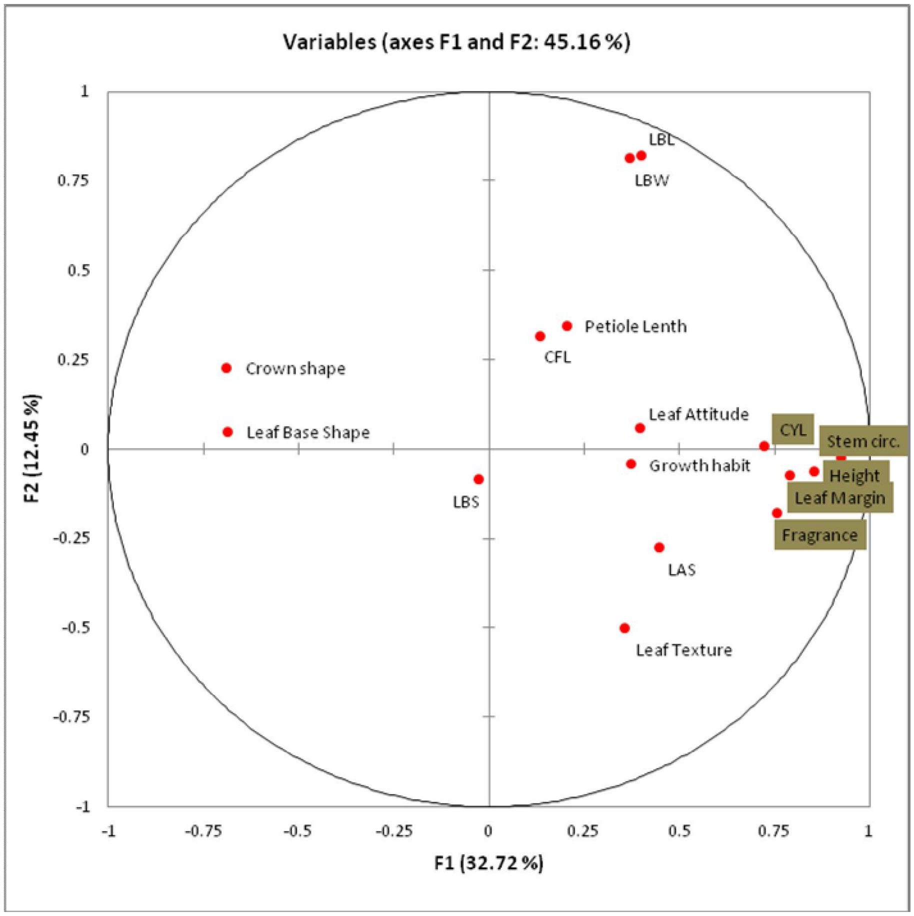

Figure 2: Correlation among characters associated with the first and second Principal Components (XLSTAT 2012). The closer the attributes are to each other in the PCA plot, the higher the correlation (i.e. the smaller the angle between the attributes, the higher the correlation)

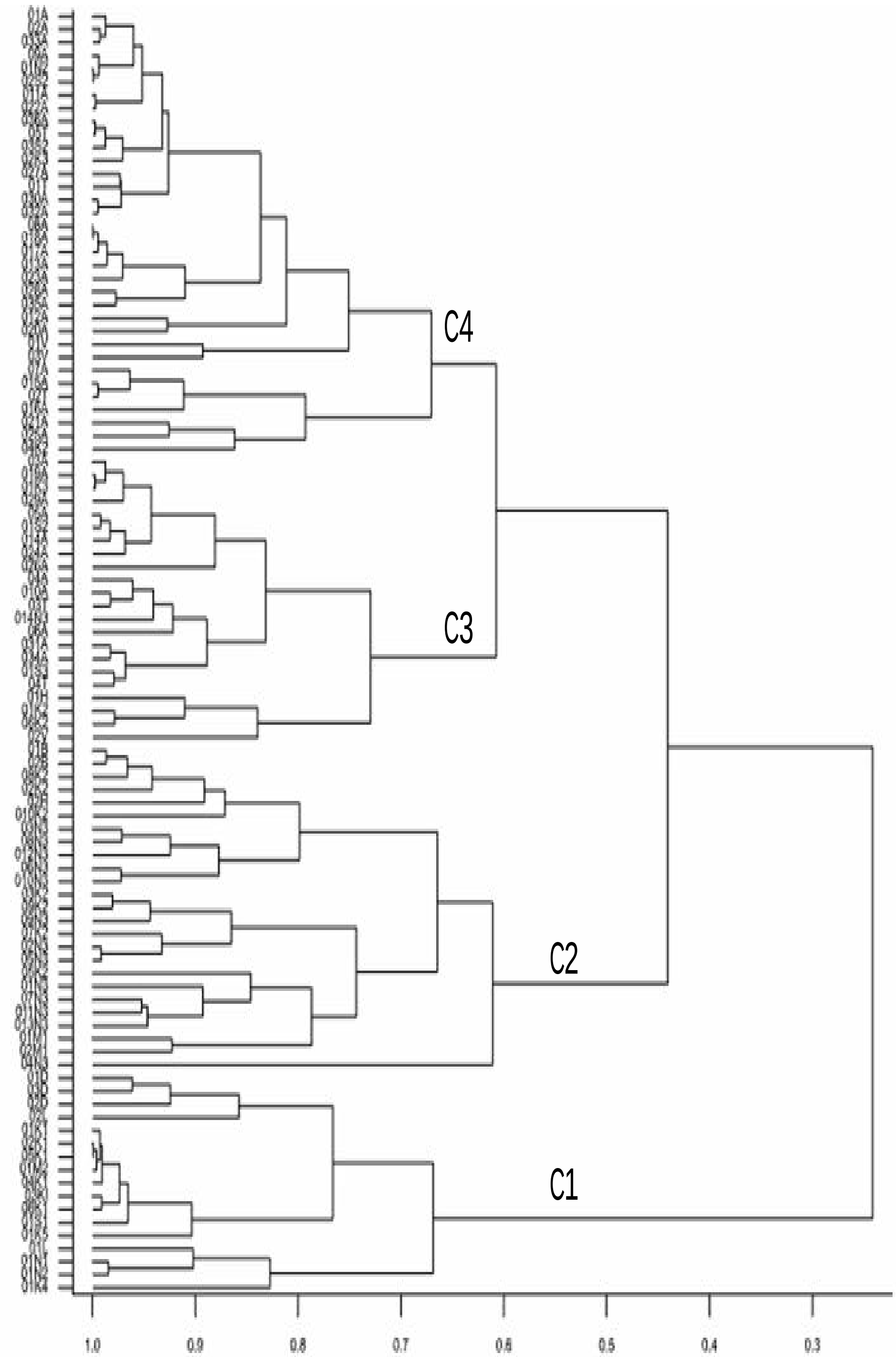

Figure 3: Dendrogram constructed based on morphological characters of 98 mango accessions from the UAR region of Eastern Kenya using the Neighbor Joining method and Euclidian average distance

4 Discussion

IPGRI descriptors allow for the use of visual assessment tools of morphological traits to characterize mango germplasm. Complex plant characters such as yield are quantitatively inherited and are influenced by genetic effect as well as genotype/environmental interaction. This poses the need to identify and use highly correlated characters (Asudi *et al.,* 2010). The color of young leaf, tree height, leaf margin type, stem circumference, fragrance strength and leaf blade length and width were some of the most descriptive traits with highly strong correlation. Galvez-Lopez *et al*., (2010) reported a high correlation that was observed between characters related to fruit and leaf and petiole size from mango accessions collected from Chiapas, Mexico. Leaf width and petiole length eigen vectors from his study were 0.74 and 0.89 respectively, whereas germplasm in the UAR had eigen vectors of 0.82 and -0.27 for similar traits.

Open pollination, continuous grafting, and use of seeds for propagation among factors such as mutations and changing environmental conditions may all contribute to the development of new traits in mango germplasm (Krishna and Singh, 2007). IPGRI descriptors which are developed after a continuous evaluation of mango germplasm grown globally may fail to capture these variations. In this study, different leaf blade shapes were identified that had not been reported by the IPGRI (2006) descriptors. These included leaf shapes that combined the characters of lanceolate and oblong leaf outlines and a second shape that combined the characters of elliptic and oblong outlines of the IPGRI (2006) leaf shape descriptors. Galvez-Lopez (2010) also identified novel morphological traits in the mango accession from Chiapas, these included accessions exhibiting hexamerous flowers, a combination of pentamerous and hexamerous flowers and a combination of tetramerous, pentamerous and hexamerous flowers in the same tree. IPGRI (1989) and IPGRI (2006) descriptors had only reported tetramerous and pentamerous flowers in mango. The study also revealed four new fruit skin colors not reported before. Continous evaluation and documentation of mango accessions in all mango growing regions in Kenya and globally will thus contribute immensely to the data bank of mango traits that are important for breeding efforts.

The results presented in this study are particularly important because they represent morphological traits that are available all year round, some of which remain the same even at the seedling stage of the mango tree. However, not all the IPGRI (2006) descriptors that were selected for analysis of variations were useful for discrimination purposes. Some descriptors proved redundant as they presented only one phenotypic class. Pulvinus thickness, leaf pubescence, angle of secondary veins to midrib and presence of secondary veins on leaf were all discarded. Other descriptors such as the type of crown shape and leaf texture were also not favorable for differentiation purposes because they may be influenced by the human activities such as pruning or top working and development age of the plant where younger trees may possess leaves that are softer and vice versa. This explains results such as Apple, Haden, Kent, Ngowe and Tommy Atkins varieties possessing all the three classes of crown shapes while varieties such as Dodo and Ngowe varieties possessed all three leaf texture classes.

Nevertheless, this study established that mango germplasm in the UAR possessed significant morphological variation among the studied accessions. We suggest in the absence of fruits, traits such as the color of young leaf, leaf margin type, leaf fragrance strength, tree height and stem circumference can be used to distinguish between cultivars particularly between the indigenous and exotic varieties. Each of the clusters generated by the dendogram possessed varieties that can be used as parents in breeding efforts. These should focus on development of disease, pest, and drought resistance, sentiments also put forward by Njuguna *et al*., (2009).

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