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Full Length Research Paper

Assessment of phenotypic diversity of macadamia (*Macadamia* spp) germplasm in Kenya using leaf and fruit morphology

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The ability to identify genetic variation is indispensable to effective management and use of genetic resources. Morphological traits are among the earliest markers used in germplasm characterization and management. Leaf and fruit morphological characteristics were recorded for 23 cultivars of Macadamia using a sample of 30 for each trait and replicated three times. The analysis of variance revealed significant differences in leaf length, width, petiole length and leaf marginal serrations. Significant differences were also revealed in fruit cluster length, number of fruits per cluster, fruit length but not fruit width. Cluster analysis using R statistics grouped the accessions into three major clusters referring to the two cultivated Macadamia species; *Macadamia integrifolia* and *Macadamia tetraphylla* displaying the highest dissimilarity, and the hybrids at the intermediate position. These markers are found to be reliable in distinguishing between the macadamia cultivars in Kenya. Among the markers, leaf petiole and marginal serrations are easily assessable and possible to use in distinguishing between the species.

Key words: Genetic diversity, macadamia germplasm, morphological markers, cluster analysis.

INTRODUCTION

Macadamia (*Macadamia* spp) is an evergreen spreading semi-hard wood tree that can grow up to 20 m high (Duke, 1983). The species belongs to the family Proteaceae of which about 1000 species exist including the Banksias and Grevilleas (McConachie, 1995). The genus consists of ten species but only two, *Macadamia integrifolia* and *Macadamia tetraphylla* are cultivated for their edible nuts (McHargue, 1996). The mature fruit consists of the edible cream to white color seed (kernel, nut) enclosed in a hard brownish seed coat (shell) which is then enclosed in a dull green pericarp (husk).

Macadamia is the most important nut crop in Kenya, with an annual production of about 10000 metric tons produced by over 100,000 small-scale farmers who depend on them for income and livelihood as it is a low-input crop (Waithaka, 2001), and about 500 large-scale growers with at least 1000 trees each. It is a growing agro-processing industry that targets niche markets in Europe and the Orient (Rotich, 2004). There are many other growing countries including Hawaii, Australia, South Africa, Malawi, Zimbambwe, Guatemala, Brazil, Costa Rica and Fiji (Kiuru et al., 2004).

The first introduction of Macadamia into Kenya was in

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Abbreviations: KARI, Kenya Agricultural Research Institute, BHL, Bob Harris Ltd., HAES, Hawaiian Agriculture Experiment Station.

1946 by Mr. Bett Wittleton a Harris family member who brought six seeds of *M. tetraphylla* as souvenirs from New South Wales in Australia and gave to Bob Harris family who planted them in Mangu, Thika in Central Kenya. Later in 1964, seeds of *M. integrifolia*, *M. tetraphylla* and hybrids of the two were also imported from Australia, Hawaii and California (Harris, 2004). In 1965 the Harris family set up a nursery (Bob Harris Ltd.) and used these two sources to mass propagate seedlings that were distributed to farmers in Central, Eastern and Coast Provinces, as an alternative cash crop to tea and coffee (Harris, 2004). In 1968, scion material from superior *M. integrifolia* varieties were imported into the country from Hawaii and grafted seedlings were produced and planted in different agroecosystems. Hence, these three sources form the Macadamia gene pool in Kenya.

Macadamia is preferentially (>75%) out-crossing (Sedgley et al., 1990) and trees originating from open-pollinated seeds (seedling trees) are varied in nut yield and quality and therefore of little commercial value (Duke, 1983). Therefore, true-to-type clones are conventionally propagated by grafting scions from selected parents onto rootstocks raised from seeds (Nyakundi and Gitonga, 1993; Gitonga et al., 2001). Old unwanted varieties can be top-worked by cutting back the tree and grafting scions from selected parents onto the remaining stump (Leigh, 1973). Trees and seedlings grafted with scions from a particular accession are genetically similar to the mother accession.

Since most of the seedlings originally planted by farmers were from open pollinated seeds, diversity in tree characteristics is expected. Over the last two decades cultivars that are superior in yield and nut quality have been selected through selection breeding (Ondabu et al,. 1996). However cultivars adapted to various agroecosystems are still lacking. Macadamia breeders will require as much genetic diversity as possible from which to select and recombine favorable traits through cross-breeding (McHargue, 1996) so as to develop varieties that are adapted to Kenyan environment and those that can compete in the world market. Genetic diversity forms the basis of agriculture and the usefulness of a genetically diverse gene pool in plant breeding cannot be overemphasized (CGR, 2005). Moreover, genetic diversity within and among populations is the backbone of conservation of plant genetic resources for both present and future use (Quedraogo, 2001). An understanding of the level and structure of genetic diversity allows identification of populations that are worthy of conesrvation because of their diversity or distinctiveness (Thormann et al., 1994; Chamberlain, 2001). Morphological traits are among the first markers used in germplasm management (Smith and Smith, 1992). Storey and Salleeb (1966) used four sets of vegetative taxonomic characters to classify macadamia parental species and the F1 hybrids. These included the number of leaves in the nodal whorls (phylotaxy), leaf type indicating whether the leaves are petiolated or sessile, the number of marginal serrations (spines) and color of new growth. Beyene et al., (2004) used a total of 15 morphological traits to reveal morphological variability of 62 maize accessions collected from the Northern, Southern and Western highlands of Ethiopia. Morphological traits have continued to reveal extensive information on genetic diversity in various crops (Hcini et al., 2007; Mathew et al., 2007; Prabalee et al., 2007) . This study was carried out to assess the morphological diversity of selected Macadamia accessions.

MATERIALS AND METHODS

Accessions' passport data

The monitored trees included some accessions that have been covered in the Macadamia breeding program of KARI. Other accessions included new introductions from Hawaii and Australia, some accessions that were found to have peculiar but otherwise agronomically important characters during a previous survey, one accession *munyoroku* believed to be an ancestor to Macadamia germplasm supplied by Bob Harries Ltd., and another species *Macadamia ternifolia* that is maintained at KARI. In cases where the original trees that were selected by KARI existed, data was taken on these trees at their original sites in farmers' fields. In the absence of the original tree, one tree was randomly selected from a set of clones of the various accessions maintained at KARI site. Each tree was assigned an accession code and tagged for data collection. Information on passport data is shown in Table 1.

Leaf traits

Four leaf morphological traits were assessed. The monitored traits were the entire leaf length (including petiole), petiole length, leaf width (the widest part) and number of marginal serrations per leaf.

Fruit traits

Fruit traits included length of entire fruit cluster (including stalk) and number of fruits per cluster. In-husk fruit length and width were recorded for individual fruits.

All linear measurements were taken using a standard tape measure or ruler. Fruit length and width were taken using a digital Vanier caliper (Absolute Digimatic CD-20C, Mitutoyo Corp. Japan). Counting of leaf spines was done using a counter. Leaf and fruits samples for assessment were collected randomly at the mid height of the tree and covering the full circumference of the tree canopy. All measurements were recorded according to the International Board for Plant Genetic Resources (IBPGR, 1980; IBPGR, 1988) guidelines. Analysis of variance was carried out using SAS (SAS, 2001) while morphological diversity was analyzed using R statistics version 2.5.1. (R Development Core Team, 2007).

RESULTS

Leaf traits

Leaf length and width, petiole length and number of spines per leaf are significantly varied among the

Table 1. Passport data of the 23 Macadamia accessions used in the study.

Accession	Year planted	Current Location	Year selected for study by KARI	Source of planting material	Country where originally bred or selected	Type of material maintained*
K-3	1968	Inoi, Kerugoya,	1982	Bob Harries Ltd	Kenya	Original seedling tree
K-4	1968	Inoi, Kerugoya,	1982	Bob Harries Ltd	Kenya	Original seedling tree
K-5	1968	Inoi, Kerugoya	2006[S]	Bob Harries Ltd	Kenya	Original seedling tree
K-15	1968	Inoi, Kerugoya	1987	Bob Harries Ltd	Kenya	Original seedling tree
EB1	1968	Ngandori, Embu	1979	Bob Harries Ltd	Kenya	Original seedling tree
EBA	1969	Municipality, Embu	1984	Bob Harries Ltd	Kenya	Original seedling tree
M-20	1968	KARI, Thika	1979	KARI	Kenya	Grafted tree
M-25	1970*[1] [1987}]*[2]	KARI, Thika	1978	KARI	Kenya	Grafted tree
HAES-333	1984	KARI, Thika	1984	KARI	Hawaii	Grafted tree
HAES-508	1984	KARI, Thika	1984	KARI	Hawaii	Grafted tree
HAES-660	1984*[1] [1982] [TW]	KARI, Thika	1984	KARI	Hawaii	Grafted tree
KB-3	1966*[1] [1987]*[2]	KARI, Thika	1979	KARI	Kenya	Grafted tree
KB-4	1972*[1] [1982][TW]	KARI, Thika	1979	KARI	Kenya	Top-worked tree
KB-25	1968	Gituamba, Thika	1992	Bob Harries Ltd	Kenya	Original seedling tree
M-2	1968*[1] [1982]*[2]	KARI, Thika	1979	KARI	Kenya	Grafted tree
A-4	1994	Kenyatta ATC Maragua	1994	KARI	Australia	Grafted tree
A-16	1994	Kenyatta ATC Maragua	1994	KARI	Australia	Grafted tree
EBT1	1968	Ngandori, Embu	2006[S]	Bob Harries Ltd	Kenya	Original seedling tree
EBT2	1968	Ngandori, Embu	2006[S]	Bob Harries Ltd	Kenya	Original seedling tree
EBT3	1968	Ngandori, Embu	2006[S]	Bob Harries Ltd	Kenya	Original seedling tree
EBT4	1968	Ngandori, Embu	2006[S]	Bob Harries Ltd	Kenya	Original seedling tree
MYK	1965	Kalamaini, Thika	2007[S]	Bob Harries Ltd	Kenya	Original seedling tree
M T	1965	KARI, Thika	2007s	-	Australia	Original seedling tree

*=Information given by farmer or extracted from Ondabu et al. (1996), *[1] = Year which original tree was planted by farmer; *[2] =Year which grafted clone was planted or Top worked (TW) at current site and tree from which data was taken. [S]= Year which tree was selected for study during a previous survey

different accessions. Leaf length ranged from 13.50 cm in accession M-25 to 24.85 cm in accession KB-25. Leaf forms differed in the monitored accessions and were either sessile or petiolated (Figure 1). The leaf forms are further classified into three categories *viz* completely sessile, semi sessile (petiole length < 0.5 cm) and petio-

Figure 1. Leaves of Macadamia showing (A) *M. tetraphylla* (sessile, long and numerous marginal serrations), (B) (*M*. *integrifolia** *M*. *tetraphylla*) hybrid (petiolate, intermediate leaf length, fewer marginal serrations) and (C, D, E) *M. integrifolia* accessions (petiolate, shorter and no serrations).

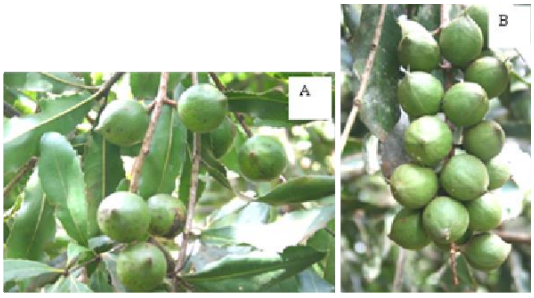

Figure 2. Fruit clusters of Macadamia showing (A) A4 with fewer nuts per cluster and (B) A16 with more nuts per cluster.

lated (petiole length 0.50 cm). Accessions are classified into four major groups according to number of marginal serrations per leaf; those with serrations ranging from 1 - 12, more than 12 to 40, between 41 to 50 and over 70. Leaf width was generally uniform and ranged from 4.09 to 6.37 cm (Table 2).

Fruit traits

Length of cluster and number of fruits per cluster are shown in Figure 2. Fruit length demonstrated significant differences among the various accessions. Alternatively, the fruit width showed no differences (Table 3). Unlike leaf characteristics, the fruit traits could not be consistently assigned to groups. For cluster analysis, leaf and fruit data was subjected to R version 2.5.1. (R-DCT, 2007) . The monitored 23 accessions grouped into three major clusters referring to the two macadamia species namely *M. integrifolia* in group 1 and *M. tetraphylla* in group 3 and the hybrids at the intermediate position in group 2 indicating morphological diversity between the three major groups. The highest diversity as evidenced by longer branches joining the accessions was between the two species (Figure 3). Within the groups, diversity was high amongst the *M. tetraphylla* followed by M. hybrids with the *M. integrifolia* displaying lowest dissimilarity.

The *M. integrifolia* forms the bulk of the germplasm consisting 12 out of the 23 accessions. The group also forms two sub-clusters but with low dissimilarity. The accessions HAES 333, HAES 508 and HAES 660 are clustered together in one of the sub-cluster with HAES 333 and HAES 508 displaying almost similar traits. Accessions A4 and A16 are clustered together in group 2 as *M*. hybrids but in different sub- clusters. Differences particularly in leaf traits are evident (Figure 4). The fruit traits are not consistently assigned to groups, while leaf traits are more consistent.

DISCUSSION

Morphological diversity was observed between and within the species. The accessions that were used in this study covered 16 cultivars currently being used in KARI's breeding program. We demonstrate here the usefulness and the reliability of morphological markers in cultivar identification and the range of relationship between the

Table 2. Mean and standard error of the leaf morphological traits in the 23 Macadamia accessions.

Accession	Leaf length (cm)	Petiole length (cm)	Number of marginal spines	Leaf width (cm)
A16	23.63±0.34^{a}	0.60±0.02^{g}	19.55±0.83^{g}	5.40±0.08^{b}
A4	15.73±0.16^{g}	0.88±0.02^{def}	37.07±0.63^{e}	4.28±0.03^{fg}
EB1	16.92±0.20^{f}	0.91±0.03^{de}	10.23±0.63^{hij}	4.04±0.05^{g}
EBA	18.59±0.34^{e}	0.85±0.03^{ef}	10.64±0.84^{hi}	4.43±0.08^{ef}
EBT_1	21.72±0.24^{c}	0.00±0.00^{j}	83.66±1.43^{a}	4.91±0.05^{d}
EBT_2	23.64±0.44^{a}	0.00±0.00^{j}	72.66±1.13^{c}	5.45±0.10^{b}
EBT_3	23.80±0.32^{a}	0.42±0.02^{h}	55.51±1.49^{d}	5.15±0.08^{c}
EBT_4	21.14±0.26^{cd}	0.17±0.02^{i}	76.10±1.39^{b}	4.30±0.06^{efg}
HAES-333	17.13±0.19^{f}	0.76±0.03^{f}	10.12±0.67^{hij}	4.94±0.07^{d}
HAES-508	17.50±0.21^{f}	0.80±0.03^{ef}	11.09±0.61^{hi}	4.86±0.08^{d}
HAES-660	14.53±0.18^{h}	0.88±0.03^{def}	11.27±0.66^{hi}	4.28±0.06^{fg}
K-15	15.78±0.18^{g}	1.17±0.02^{a}	7.10±0.55^{jk}	4.58±0.07^{e}
K-3	18.65±0.20^{e}	0.78±0.02^{f}	21.41±0.62^{g}	5.41±0.06^{b}
K-4	19.33±0.29^{e}	0.80±0.03^{ef}	12.34±0.69^{h}	4.10±0.05^{g}
K-5	24.13±0.34^{a}	1.04±0.03^{bc}	33.06±0.97^{f}	4.40±0.06^{ef}
KB-25	24.37±0.48^{a}	0.50±0.02^{h}	77.63±1.76^{b}	6.26±0.11^{a}
KB-3	20.82±0.28^{d}	0.81±0.03^{ef}	32.21±0.91^{f}	4.83±0.07^{d}
KB-4	14.78±0.18^{gh}	1.03±0.03^{bc}	3.41±0.45^{lm}	4.10±0.06^{g}
M-2	22.51±0.31^{b}	0.50±0.02^{h}	55.36±1.03^{d}	4.80±0.07^{d}
M-20	14.38±0.16^{hi}	0.79±0.02^{ef}	1.34±0.20^{m}	4.45±0.05^{ef}
M-25	13.65±0.15^{i}	0.82±0.03^{ef}	2.73±0.42l^{m}	4.41±0.07^{ef}
MT	14.90±0.15^{gh}	0.97±0.03^{dc}	7.67±0.50^{ijk}	4.38±0.05^{ef}
MYK	15.74±0.18^{g}	1.10±0.05^{ab}	5.32±0.47^{k}l	4.49±0.07^{ef}
Mean	18.84	0.72.	28.63	4.71

Means followed by the same number in a column are not significant at = 0.05 (SNK Test).

Macadamia cultivars.

The accessions HAES 333 (Ikaikia), HAES 508 (Kakea) and HAES 660 (Keaau) are clustered together in one sub-cluster as *M. integrifolia* confirming previous reports that they were developed in Hawaii as *M integrifolia* (Duke, 1983). This is also in agreement with the results of Peace et al. (2005) obtained from South African selections which proved that Hawaiian selections are closely related to *M. integrifolia*. Accessions A4 and A16 are clustered together as an intermediate cluster. Cultivar A4 is a distinct (*M. integrifolia* * *M. tetraphylla*) hybrid named Hidden Valley A4 and described by Bell et al. (1991). It was discovered and propagated at Hidden Valley Plantations, Beerwah, Queensland, Australia. Bell et al., (1991) stated that among the distinct characteristics of this tree is a combination of the increased leaf serrations per unit distance. This observation has also been made by Aradhya et al. (1998) who indicated that genetic diversity is greater among the more recent selections particularly those bred in Australia.

The accession MYK (a short form of *munyoroku* – a vernacular name for 'smooth') was included in the study as one of the ancestral trees from Bob Harries Limited. The accessions are clustered with *M. integrifolia* suggesting that it could be the ancestor of most smooth-shelled macadamias supplied by Bob Harries Limited.

The reliability of morphological markers coupled with use of standard selection criteria for macadamia was demonstrated by Ondabu et al. (1996) and Tominaga and Nyaga, (1997). Results of cluster analysis performed in the present study mostly agreed with the previous classification and description of *M. integrofolia* and *M. tetraphylla* by Storey and Salleeb (1966) and Duke (1983) indicating *M. integrifolia* to have shorter leaves (10-30 cm), long petiole and fewer serrations (less than 14) than *M. tetraphylla* with longer leaves (25 - 50 cm), short or no petiole and numerous serrations (20 - 45). Group 1 is associated with short leaves (leaf length less than 20 cm), long petiole (petiole length more than 0.7 cm) and few marginal spines (less than 20). It consists of six Kenyan selections (M-20, M-25, K-15, EB1, K-4 and EBA) that were previously classified as *M. integrifolia* (Ondabu et al., 1996; Wasilwa et al., 1999). Group 3 is associated with long leaves (21.14 - 24.37 cm), short or no petiole (0.0 – 0.5 cm) and numerous marginal spines (55-85) with the exception of K-5.

However, slight deviations from the stated ranges were observed in the present study suggesting some

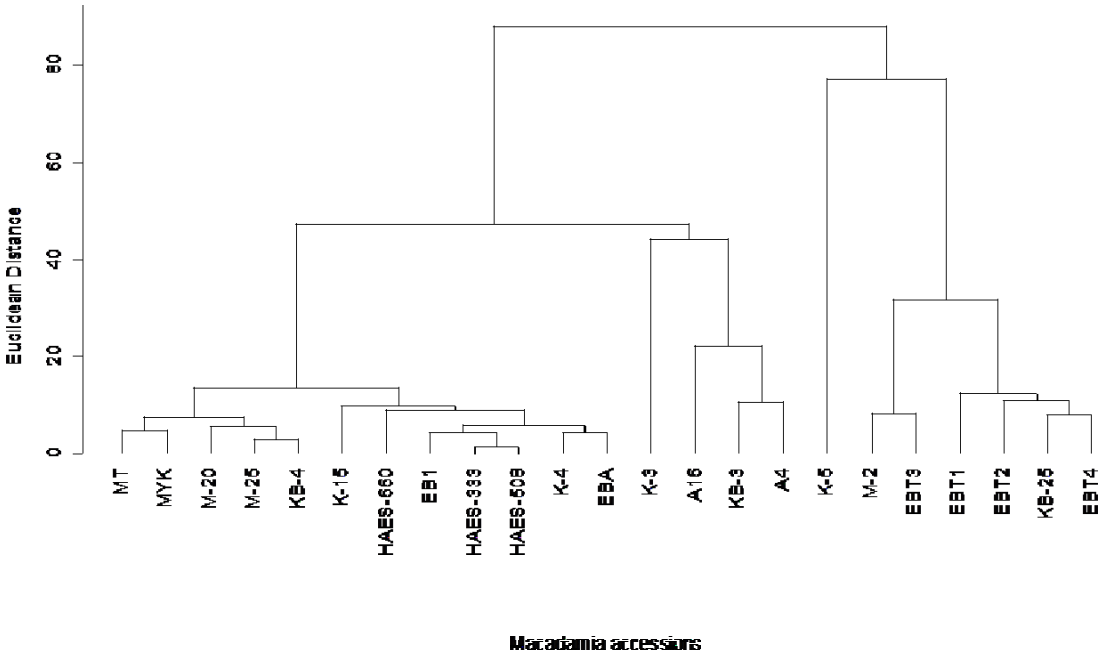

Figure 3. Dendrogram showing the morphological diversity of 23 Macadamia accessions as based on leaf and fruit morphological data.

Table 3. Mean and standard error of the fruit morphological traits in the 21 Macadamia accessions

Accession	Cluster Length* (cm)	No. of Fruits*	Fruit Length* (mm)	Fruit Width* (mm)
A16	19.21±0.60^{a}	4.50±0.45^{cb}	34.42±0.30^{g}	27.99±0.18^{a}
A4	14.68±0.64^{cd}	2.16±0.22^{d}	38.58±0.27^{c}	31.02±0.14^{a}
EB1	12.12±0.53^{ef}	1.66±0.13^{d}	37.94±0.27^{cd}	31.28±0.18^{a}
EBA	15.32±0.62^{cd}	2.51±0.12^{d}	34.15±0.21^{g}	30.37±0.15^{a}
EBT1	16.56±0.73^{cb}	2.43±0.15^{d}	35.72±0.40^{f}	28.18±0.35^{a}
EBT2	19.33±0.67^{a}	2.04±0.13^{d}	34.00±0.33^{g}	29.65±0.32^{a}
EBT3	17.83±0.66^{ab}	4.02±0.37^{c}	36.17±0.38^{ef}	31.42±0.28^{a}
HAES-333	13.91±0.58^{de}	4.04±0.20^{c}	37.93±0.27^{cd}	32.30±0.18^{a}
HAES-508	13.91±0.54^{de}	4.40±0.21^{cb}	37.66±0.23^{cd}	33.00±0.15^{a}
HAES-660	8.58±0.27^{g}	2.72±0.15^{d}	36.58±0.21^{ef}	32.00±0.21^{a}
K-15	15.09±0.62^{cd}	4.78±0.38^{cb}	41.09±0.34^{b}	31.20±0.26^{a}
K-3	13.53±0.72^{de}	6.27±0.39^{a}	37.23±0.28^{de}	32.26±0.16^{a}
K-4	14.49±0.54^{cd}	5.26±0.41^{b}	36.33±0.30^{ef}	30.60±0.22^{a}
K-5	10.49±0.51^{fg}	2.19±0.14^{d}	42.97±0.36^{a}	31.63±0.24^{a}
KB-25	15.29±0.68^{cd}	3.86±0.37^{c}	38.40±0.49^{cd}	35.91±0.33^{a}
KB-4	10.26±0.34^{fg}	3.64±0.21^{c}	32.82±0.33^{h}	28.54±0.23^{a}
M-2	16.48±0.59^{cb}	1.87±0.14^{d}	41.98±0.35^{b}	35.51±0.31^{a}
M-20	14.01±0.47^{de}	3.88±0.28^{c}	34.39±0.44^{g}	29.97±0.34^{a}
M-25	8.98±0.35^{g}	4.46±0.28^{cb}	33.33±0.26^{gh}	30.51±0.21^{a}
MT	11.88±0.34^{ef}	2.71±0.18^{d}	36.25±0.30^{ef}	29.15±0.23^{a}
MYK	13.71±0.45^{de}	2.14±0.13^{d}	34.37±0.24^{g}	31.83±0.23^{a}
Mean	**14.08**	**3.41**	**36.78**	**32.88**

Means followed by the same number in a column are not significant at = 0.05 (SNK Test)

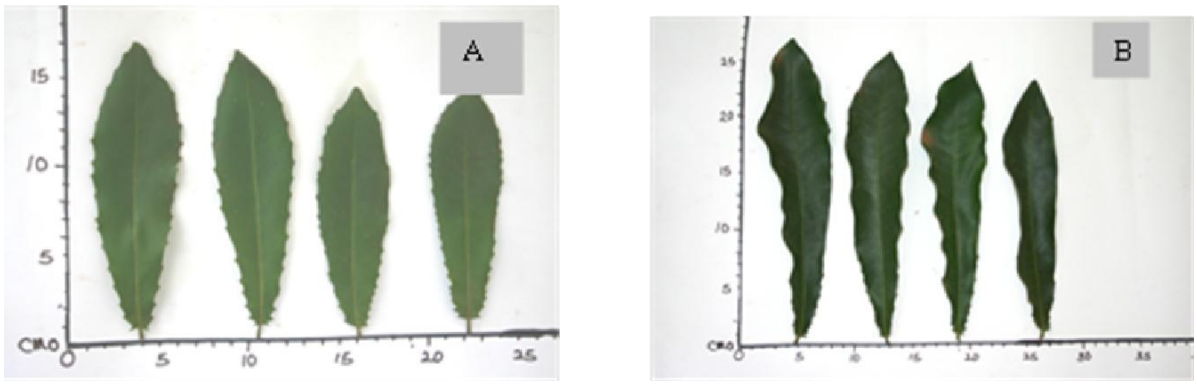

Figure 4. Leaves of Macadamia showing differences in morphology between (A) A4 with shorter leaves and more serrated margins, and (B) A16 with longer leaves and sparingly serrated margins.

adaptational or evolutionary changes leading to genetic differentiation into distinct varieties (Muluvi et al., 1999). Other exceptions include accessions KB-4, KB-25 and M-2 previously characterized as (*M. integrifolia * M. tetraphylla*) hybrids but clustered differently in the present study. KB-4 clustered with *M. integrifolia* and KB-25 and M-2 are clustered together with *M. tetraphylla*. This may be attributed to differences in species composition within the accessions (Peace et al., 2005).

Accession MT, which is maintained at KARI-Thika and believed to be a different Macadamia species; *M. ternifolia*, is clustered together with *M. integrifolia*. These deviations are attributed to the use of leaf and fruit data only. Moreover, the genetic information provided by morphological characters is often limited and expression of quantitative traits is subject to location (Ito et al., 1991; Ito, 1995; Sacramento et al., 1995) and the strong environmental influence (Allan, 1989; Stephenson and Gallagher, 1987; Rao, 2004).

The results of this study show that leaf and fruit characteristics can be used as taxonomical traits for macadamia germplasm and as an important data base in the breeding programs of Macadamia. A survey of farmers' knowledge on macadamia genetic diversity (Gitonga et al., 2007) also revealed leaf and fruit traits to be the most important makers used by farmers to differentiate between macadamia species. This confirms the importance of understanding how individual traits or a group of traits are used to identify different genotypes by farmers. Morphological characterization of crop varieties is of direct relevance to local farmers as well as plant breeders in the conservation and use of germplasm (Jarvis et al., 2000). However, it is worth noting that morphological traits have a number of limitations, including low polymorphism, low heritability, late expression and vulnerability to environmental influences (Smith and Smith, 1992). On the other hand, DNA-based markers do not have such limitations and can be used to distinguish between closely related genotypes (Beyene et al., 2005), where the presence of the same DNA in the living cells of plant allow genotypic tests on any tissue at any stage of growth (Morell et al., 1995). This may lead to the conclusion that morphological characterization is complementary to the molecular characterization. This will enable the distinction between different but otherwise morphologically close genotypes.

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