Full Length Research Paper

Morphological and molecular characterization of cultivated yam (*Dioscorea* species) in selected counties in Kenya

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This study was conducted to characterize *Dioscorea* spp. in Kenya using morphological and molecular characteristics. Data on 22 morphological traits were subjected to cluster analysis and multivariate analysis using principal component (PCA). The dendrogram of cluster analysis revealed three main groups: Species distribution based on PC-1 and PC-2 showed the distantly related species in each quarter; *D. alata* L. (1st quarter), *D. bulbifera* L. (2nd quarter), *D. cayenensis* Lam. (3rd quarter) and *D. minutiflora* Engl. (4th quarter). In molecular characterization, one sub-cluster grouped *D. minutiflora* Engl. and *D. burkilliana* J. Miege as one genetic group. However not all *D. minutiflora* Engl. species were in one specific cluster showing that there may be variation within the species. *D. alata* L. and *D. bulbifera* were seen to be potentially related because they shared a common origin. *D. bulbifera* L. and *D. cayenensis* Lam. genotypes clustered together, indicating that the species might be closely related. Generally, the *rbcL* marker demonstrated the phylogeny of Kenyan *Dioscorea* spp L. Comparison of morphological and molecular data analysis gave almost similar results. From the study, the phylogenetic relationships of Kenyan *Dioscorea* spp. were established and morphological and molecular characterization was efficient in establishing species relatedness among *Dioscorea* spp.

Key words: *Dioscorea* spp., *rbcL*, principal component analysis, molecular characterization, morphological characterization, yams.

INTRODUCTION

Yams (*Dioscorea* spp.) are important monocotyledonous tuberous plants belonging to the order Dioscoreales, family Dioscoreaceae and the genus *Dioscorea* (Tamiru et al., 2008; APG III, 2009). The genus contains about 644 species distributed throughout the tropics in West Africa, South East Asia and Tropical America (Asiedu and Sartie, 2010; Couto et al., 2018). More than 8 species are important staples *D. rotundata* Poir. (White yam), *D. alata* L. (Water yam), *D. cayanensis* Lam. (Yellow yam), *D. bulbifera* L. (Aerial yam), *D. dumetorum* (Kunth) Pax. (Trifoliate yam), *D. esculenta* (Lour) Burk. (Chinese yam) *D. nummularia* Lam., *D. pentaphylla* L., *D. hispida* Dennst. and *D. trifida* L. (Ihediohanm et al., 2012). They are annual or perennial herbaceous vines,

with edible underground and aerial tubers (either stem or root depending on species) and are the world's second most significant tuber crop.

Yams are essential sources of food consumed as vegetables boiled, baked or fried. Yams bring food security to about 300 million people in Africa, Asia, parts of South America, Caribbean and the South Pacific Islands (Nanbol and Namo, 2019). Some species contain medicinal components useful in the pharmaceutical industries. For example, *D. nipponica, D. alata* L. and *D. zingiberensis* contain diosgenin helpful in relieving arthritis and muscle pain and lowers cholesterol levels (Chandrasekara and Kumar, 2016; Jesus et al., 2016). Purple yam contains anthocyanin that slows down lipid peroxidation and prevents the onset of cardiovascular disease (Blesso, 2019; Reis et al., 2016).

In Kenya, the diversity of yams has been evolving over the years as numerous generations in many parts of the country select and domesticate different species and types independently according to their local cultivation practices and needs. In a recent report by the Kenya National Strategy on Genetic Resources (2016-2020), yams were listed among the underutilized and neglected crops in the country. The cultivated yams in Kenya include *D. rotundata* Poir., *D. minutiflora* Engl., *D. bulbifera* L., *D. dumetorum* (Kunth) Pax., *D. alata* L. and *D. cayenensis* Lam. They are mainly cultivated by elderly farmers basically for food in counties of Eastern, Central, Western and Coastal regions of the country (Muthamia et al., 2013).

Molecular studies done on Kenyan yams have been minimal. Previous studies have investigated the genetic diversity using polymorphic Simple Sequence Repeats (SSR) markers that distinguished the landraces Muthamia et al. (2013) and ploidy levels; this revealed variable ploidy levels among the local yam landraces (Muthamia et al., 2014) and not the phylogeny of the species. Both studies recommended further work on the phylogeny of Kenyan yams. Other studies have solely utilized morphological characters to infer relationships within and between the *Dioscorea* species in Kenya (Mwirigi et al., 2009). This study aims to establish the relationships of Kenyan *Dioscorea* species using morphological and molecular characterisation, taking into account the recommendations of previous research.

MATERIALS AND METHODS

Study area

The study was conducted in Meru, Embu, Taita Taveta, Busia and Bungoma counties. These counties were selected based on information gathered from the Kenya Agricultural and Livestock Research Organization on where *Dioscorea* species are mainly grown. *Dioscorea* specimens were collected from six farms from three sub-counties in Meru county; Imenti North (N 00° 4´32.43684´; E 37° 38´54.29688´), Imenti Central (S 0° 1´34.56264´; E 37° 38´37.65588´) and Tigania Central (N 00°

8´35.1168´; E 37° 50´52.20852´). Specimens were also collected in Embu county (S 00° 27´53.36388´; E 37° 29´56.65272´), Taita-Taveta county (S 03° 24´2.46888´), Busia county (N 00° 29´47.86548´; E 34° 12´7.0272´) and Bungoma county (N 00° 34´10.300´; E 34° 33´31.1536´). Purposive sampling was used to select representative study sites with respect to the potential of yam production. This was done with the help of agricultural officers in each county who identified farmers farming yams.

Collection of *Dioscorea* specimens

Leaves and voucher specimens of *Dioscorea* species were collected from the various geographical regions of Kenya in the year 2018 (September to November). Collected specimens were identified and voucher specimens deposited in Kenyatta University Herbarium. Silica-gel dried leaves were collected for each sample for molecular characterization.

Morphological characterization

Twenty-four yam specimens were used for this study. Morphological data were observed directly on living plants under field conditions from farms where yams were grown. Twenty-two characteristics obtained from the International Plant Genetic Resources Institute's (IPGRI) descriptors of yam (Dioscorea species) were considered (IPGRI, 1997) (Table 1).

DNA extraction

DNA was extracted from 0.2 g silica-gel dried leaves obtained from 17 randomly selected representative specimens and collected into Eppendorf tubes. Normal saline was added, and centrifuged. 400 µl lysis buffer was added and incubated for 1 h at 55-60°C with occasional mixing. The specimens were crushed and incubated again at 37°C for 3-4 h to deactivate lysozyme in the lysis buffer. They were cooled for 30 min, and afterwards centrifuged at 13,000 revolutions per minute for 5 min; an equal amount of chloroform was added gently and mixed thoroughly. The specimens were centrifuged again at 13,000 revolutions per minute for 8 min, using a large-bore pipette. The supernatant was transferred to another labelled Eppendorf tube, 600 µl isopropanol was added and mixed gently until the DNA was precipitated. The specimens were kept at -4°C for 20 min to precipitate the DNA further, centrifuged at 12,000 revolutions per minute for 5 minutes and the supernatant was discarded. The DNA pellets were washed by adding 70% ethanol and centrifuged again at 13,000 revolutions per minute for 2 min. The supernatant was discarded and the pellets were air-dried at room temperature. DNA yield was checked by running 3 µl of freshly extracted DNA specimens on 1% agarose gel stained with 3 µl loading dye and 1µl SYBR® green stain; it was visualized under an ultraviolet transilluminator at the Kenyatta University Tissue Culture laboratory. The quality and concentration of all DNA specimens were determined using Agarose gel electrophoresis.

PCR and sequencing

PCR was achieved using *rbcl* marker (H1f F: CCACAAACAGAGACTAAAGC and Fofana R: GTAAAATCAAGTCCACCGCG (Fofana et al., 1997) and synthesized from Inqaba Biotec East Africa (IBEA), SouthAfrica. This primer marker was selected as a result of ease of PCR amplification and discriminatory power among yam species (Girma et al., 2015a). *rbcl* codes for ribulose 1, 5 bisphosphate carboxylase/oxygenase. This was carried out in a 25 µl reaction

Table 1. Character and character states scored for morphological studies.

Character	Character state
Twining direction	1-Clockwise (climbing to the left)
	2-Anticlockwise (climbing to the right)
Stem colour	1-Green; 2-purplish green; 3-brownish-green; 4-dark brown; 5-purple and 6-other.
Absence/presence of spines	Absent/ Present
Absence/presence of wings	Absent/ Present
Wing position	At the base/ Above base
Spine shape	1-Straight; 2-Curved upwards; 3-Curved downwards
Leaf colour	1-Yellowish; 2-Pale green; 3-Dark green; 4-Purplish green; 5-Purple; 6-Other
Leaf margin colour	1-Green; 2-Purple; 3-Other
Vein colour	1-Yellowish; 2-Green; 3-Pale purple; 4-Purple; 5-Other
Position of leaves	1-Alternate; 2-Opposite; 3-Alternate at base/opposite above; 4-Other
Leaf type	Simple/ Compound
Leaf margin	Entire/ Serrate
Leaf shape	1-Ovate; 2-Cordate; 3-Cordate long; 4-Cordate broad; 5-Sagittate long; 6-Sagittate broad; 7-Hastate; 8-Other
Leaf apex shape	1-Obtuse; 2-Acute; 3-Emarginate; 4-Other
Petiole colour	1-All green with purple base; 2-All green with purple leaf junction; 3-All green with purple at both ends; 4-All purplish-green with purple base; 5-All purplish-green with purple leaf junction; 6-All purplish-green with purple at both ends; 7-Green; 8-Purple; 9-Brownish green; 10-Brown; 11-Dark brown; 12-Other
Flowering	1-No flowering; 2-Flowering in some years; 3-Every year
Flower colour	1-Purplish; 2-White; 3-Yellowish; 4-Other
Inflorescence type	1-Spike; 2-Raceme;3- Panicle
Aerial tuber shape	1-Round; 2-Oval; 3-Irregular (not uniform); 4-Elongate
Skin colour	1-Greyish; 2-Light brown; 3-Dark brown; 4-Other
Surface texture	1-Smooth; 2-Wrinkled; 3-Rough
Flesh colour	1-White; 2-Yellowish white or off-white; 3-Yellow; 4-Orange; 5-Light purple; 6-Purple; 7-Purple with white; 8-White with purple; 9-Outer purple/inner yellowish; 10-Other

volume containing 2.5 µl of 10x standard Taq, reaction buffer; 0.5 µl of 10 mM dNTPs; 0.5 µl of 10 µM primer H1F; 0.5 µl of 10 µM primer Fofana; 1 µl of template DNA, 0.125 µl of Taq, DNA polymerase, 19 µl nuclease-free water and 0.5 µl of Triton X.

The PCR reaction was carried out in Techgene thermocycler FTGENE5D model (Techne- UK). The PCR reaction conditions for amplification consisted of initial denaturation at 94^{0}C for 2 min followed by 35 cycles (denaturation at 94°C for 30 s, primer annealing at 46°C for 30 s, extension at 72^{0}C for 90 s) and a final extension at 72°C for 7 min. The PCR products were stored at 4°C until used. PCR products were stained with SYBR green and separated by gel electrophoresis in 1% (w/v) agarose gel in 0.5X TBE buffer at 80 V for 30 min. After gel electrophoresis, the PCR products were visualized using an Ultra-violet trans-illuminator lamp. One hundred base pair (100bp) ladder was used for estimation of the molecular sizes of the bands. Gels were photographed using a Samsung digital camera. PCR products were then sent to South Africa for bidirectional sequencing at Inqaba Biotec East Africa (IBEA).

Data analysis

Data analysis based on morphological data

Data on morphological characteristics from 24 specimens were coded into numerical values and used for cluster analysis. The dendrogram was drawn based on a hierarchical cluster analysis using single linkage (nearest-neighbour) procedure using DARwin computer software version 6. The dendrogram obtained was used in comparison with *rbcL* phylogenetic tree. Standardized data for qualitative characters were subjected to multivariate analysis and principal component analysis to identify the most discriminating morphological character using MVSP 3.2 and Conoco 5 software, respectively.

Phylogenetic analysis

The obtained sequences were exported to Finch TV Version 1.4.0 for base-calling. A consensus sequence was then created using DNA Baser Assembler v5.15.0; then a contig was created in comparison with the reference sequence using Gene studio Professional Edition. BLAST analysis was done to find identities that match the species. *rbcL* sequences were subjected to multiple alignments using the muscle alignment method in MEGA X to identify gaps and similar and mismatch regions among the two molecular characters. Maximum Likelihood (ML) and neighbour-joining algorithms were applied in phylogeny reconstruction. UPGMA was the statistical method used. The aligned sequences after subjection to the above parameters resulted in the construction of *rbcL* maximum likelihood phylogenetic trees.

Table 2. Eigenvalues.

Parameter	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	16.392	6.558	5.222	2.534
Percentage	46.451	18.583	14.798	7.181
Cum. Percentage	46.451	65.033	79.831	87.012

Table 3. PCA variable loadings.

Traits	PC 1	PC 2	PC 3	PC 4
A-Twining direction	0.073	-0.044	0.007	0.047
B-Stem colour	**0.111**	-0.007	**-0.102**	**0.223**
C-Spines	**0.113**	0.074	-0.071	-0.029
D-Spine shape	**0.259**	0.058	**-0.160**	-0.066
E-Spines on stem base	**0.579**	**0.273**	**-0.558**	**-0.351**
F-Wings	-0.094	0.024	-0.011	-0.044
G-Wing position	-0.094	0.024	-0.011	-0.044
H-Leaf colour	0.005	-0.003	-0.049	-0.096
I-Leaf margin colour	**0.115**	0.032	**-0.154**	**0.613**
J-Vein colour	-0.063	**-0.124**	0.056	-0.038
K-Leaf position	**0.103**	-0.070	0.010	**0.223**
L-Leaf type	0.000	0.000	0.000	0.000
M-Leaf margin	0.000	0.000	0.000	0.000
N-Leaf shape	**-0.225**	0.034	0.081	**-0.398**
O-Leaf apex shape	0.000	0.000	0.000	0.000
P-Distance between lobes	**0.559**	**0.270**	**0.767**	-0.013
Q-Petiole colour	**-0.350**	**0.902**	-0.032	**0.127**
R-Flowering	-0.063	0.039	-0.039	**-0.112**
S-Tuber shape	-0.029	0.022	-0.012	**0.104**
T-Skin colour	0.100	-0.053	-0.048	0.065
U-Surface texture	**0.115**	0.024	-0.068	0.068
V-Flesh colour	-0.087	-0.013	**0.114**	**-0.416**

RESULTS

Principal component analysis

The PCA results established that the first four principal components together described 87.01% of the overall variance present in the data set (Table 2). Scores on the first principal component (PC-1) which explained 46.45% of the total dissimilarity were vastly correlated to stem colour, presence of spines, spine shape, spines on stem base, leaf margin colour, leaf position, the distance between lobes and surface texture (Table 3).

The second principal component (PC-2) described 18.58% of the overall dissimilarity and was vastly correlated to spines on stem base, the distance between lobes and petiole colour (Table 3). The third component (PC-3) which described 14.78% of the dissimilarity was primarily related to the distance between lobes and flesh colour. The fourth principal component (PC-4) described

7.18% of the total distinction and was determined by leaf margin colour, stem colour, leaf position, petiole colour and shape of the tubers. The distribution of species based on the first and second principal components shows dissimilarity among the species and how extensively dispersed they are along both axes (Figure 1). The two components explain a cumulative variability of 65.03%. Based on the distribution of specimens in the first quarter, *D. alata* L. is the most distantly related to that group; whereas in the second quarter *D. bulbifera* L. is the least similar in the group. The most distant in the third quarter is *D. cayenensis* Lam. The last quarter is made up of a *D.minutiflora* Engl. that is least similar to the group (Figure 1).

Correlation between the variables related to the first and second principal components are presented in Figure 1. From the correlation circle in Figure 1, petiole colour has a significant effect on the variables as a result of the arrow being long. There is a positive correlation between
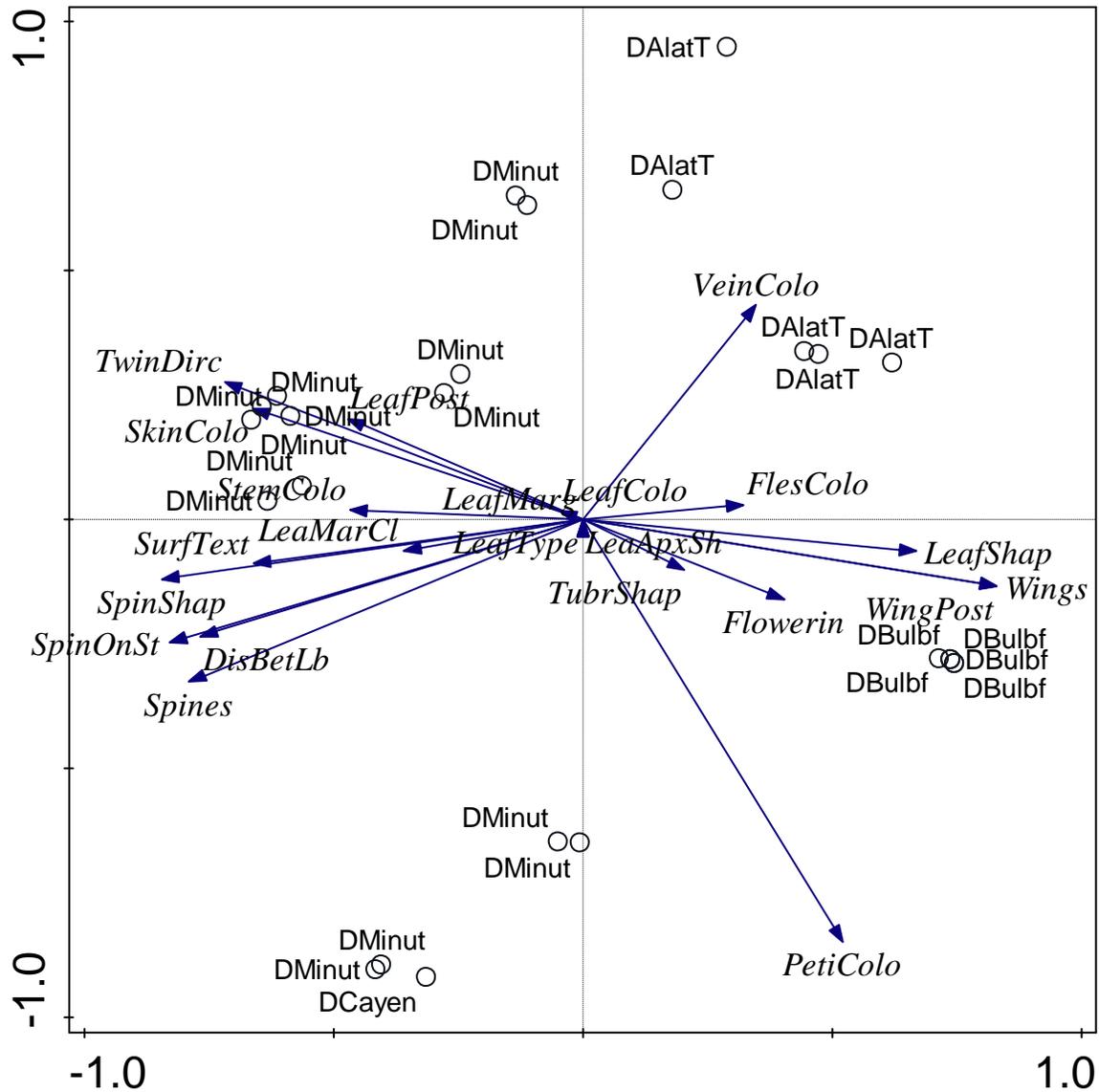
Figure 1. Correlation circle of the first two principal components (PC1 and PC2).

the shape of leaves and the presence of wings. However, there is a negative correlation between the shape of leaves and the presence of wings on one hand and twining direction and tuber skin colour. Petiole colour and spines are not correlated as well as petiole colour and vein colour.

Dendrogram based on morphological characters

The 24 yam specimens included in the morphological study were grouped into three clusters (Figure 2). Cluster 1 grouped *D. minutiflora* Engl. species collected from different areas; Teso North, Embu and Meru. This cluster had two sub-clusters; 1a and b respectively. Sub-cluster

1a is a group of *D. minutiflora* Engl. characterised with many spines on stem base, spines curved upwards, leaf veins yellow, yellow leaf margins, leaves alternate at base/ opposite above, green petioles and rough tuber surface texture. Sub-cluster 1b is a group of *D. minutiflora* Engl. with many spines on the stem base, spines curved downwards, vein green, leaf margin green, leaf position opposite, petioles all green with a purple base and tuber surface texture rough. This suggested a close relationship between the *D. minutiflora* Engl species collected from the different areas (Teso North, Embu and Meru) based on similar morphological traits.

Cluster 2 contained three sub-cluster groups Cluster 2(I), Cluster 2(II) and Cluster 2(III). Sub-cluster 2(I) is a group of *D. alata* L. species from Taita taveta and Busia
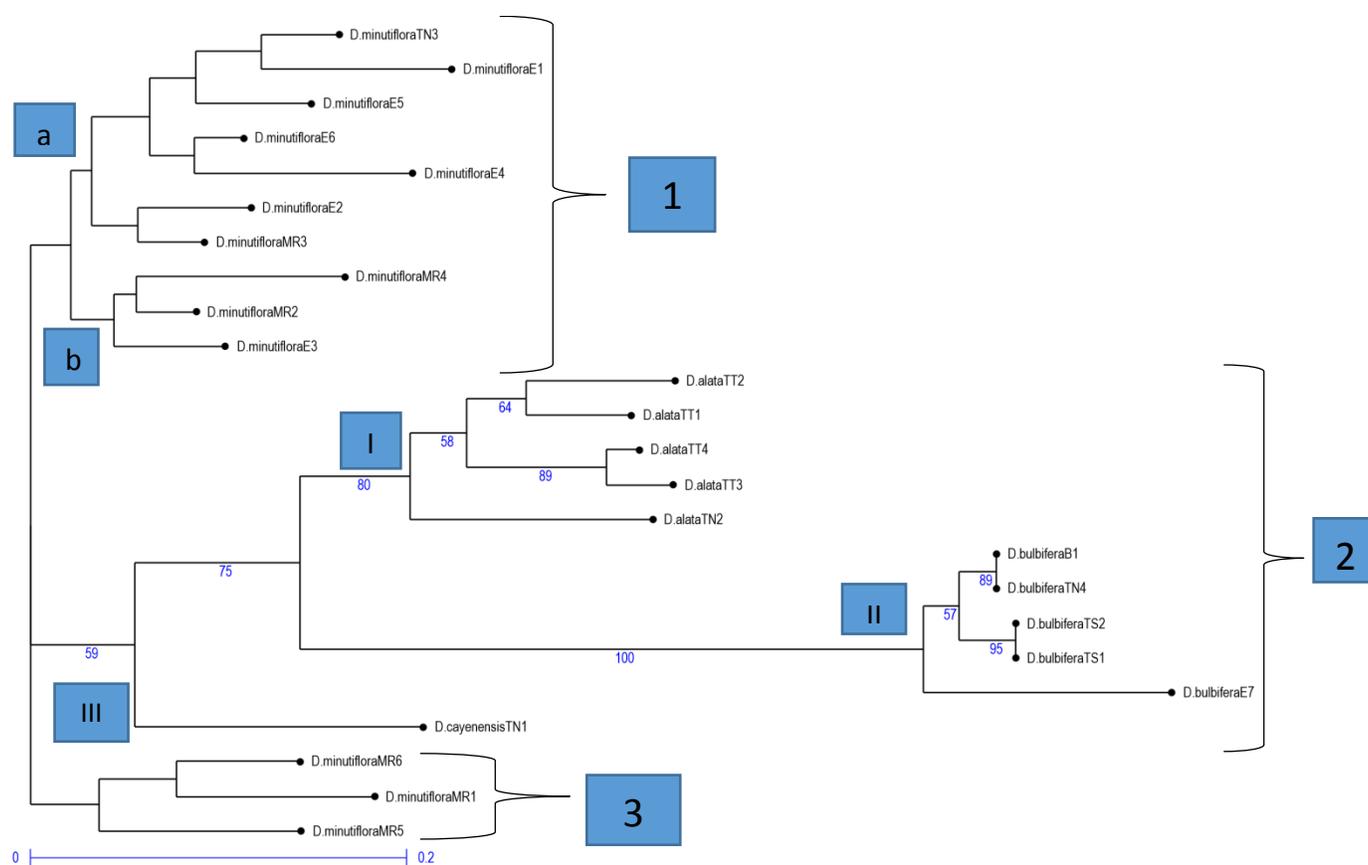
Figure 2. Dendrogram showing the relationship in yams (*Dioscorea* s) species based on morphological characteristics.

(Teso North) that twine to the right in an anticlockwise direction, stem purplish-green, leaf margin purple, leaf shape cordate long, petioles purplish-green with a purple base, tuber flesh purple and white. *D. alata* L. species collected from Teso North had sagittate long leaves, and tuber flesh purple in colour whereas *D. alata* L. tuber flesh colour from Taita Taveta was white.

Sub-cluster 2(II) is a group of *D. bulbifera* L. species from Bungoma, Busia (Teso North and South) and Embu counties with stem twining to the left in a clockwise direction, spines absent, wings present on the stem, flowering in some years and presence of aerial tubers. However, only *D. bulbifera* L. from Bungoma and Busia showed flowering and clustered together whereas that from Embu did not.

Sub-cluster 2(III) had *D. cayenensis* Lam. from Busia (Teso North) characterised by yellowish veins on the leaves, cordate broad leaf shape and a cylindrical tuber with tuber flesh colour yellow. Cluster 3 is a group of *D. minutiflora* Engl. from Meru County. Few spines on stem base, green stems, pale green and dark green leaves, brown leaf margins, green leaf veins, cordate leaves and white tuber flesh colour characterised this cluster. These characters were key in distinguishing this cluster from Cluster 1.

Molecular characterisation of Kenyan yam (Dioscorea species)

Six species' identities were used to construct the dendrogram among the 17 selected genotypes.

The dendrogram based on *rbcL* markers distinguished the seventeen yam genotypes into two main cluster groups (Figure 3)

Cluster 1 consisted of two main subclusters (a) and (b). Subcluster 1(a) and cluster 2 comprised *D. minutiflora* genotypes. This is similar to the cluster 1 and 3 of the morphological analysis which consists of *D. minutiflora* species clustered together (Figure 2). However, *D. minutiflora* genotypes were in different clusters; 1(a) and cluster 2, showing that there may be variation in the genotypes (Figure 3). Subcluster b(I) consisted of *D. alata* genotypes similar to the cluster 2(i) of the morphological analysis. Subcluster b(II) consisted of *D. bulbifera* and *D. cayenensis* genotypes, indicating that the two species might be closely related as shown in morphological studies in cluster 2(II and III) (Figure 3).

The results showed a high correlation between the morphological and molecular data in the study of Kenyan yams (Figures 2 and 3). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and
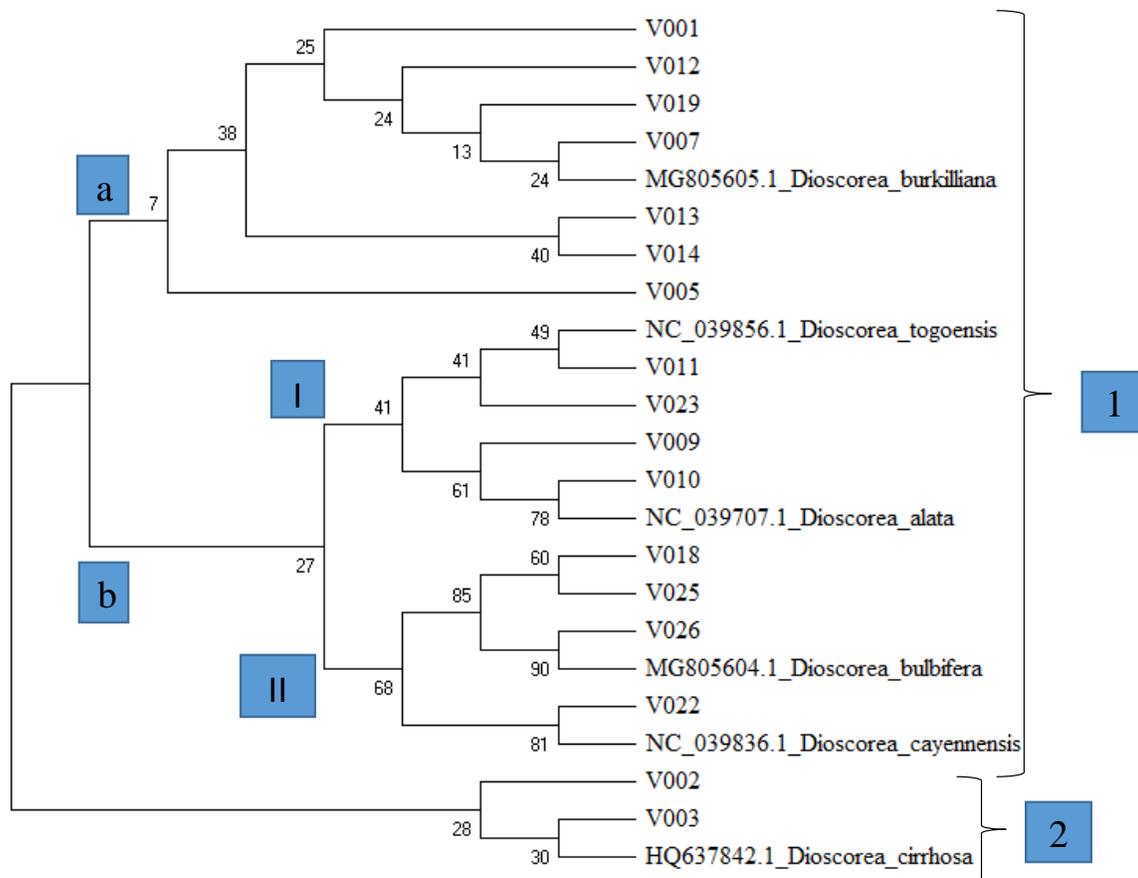
Figure 3. Evolutionary relationships of taxa.

Nei, 1987) (Table 4). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. The analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 593 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018) (Table 5).

DISCUSSION

Morphological traits that had a paramount role in discriminating between the yam species in this study were stem colour, leaf margin colour, leaf position, the distance between lobes, petiole colour, tuber shape, tuber surface texture and tuber flesh colour. These results are in congruence with results obtained by Jyothy et al. (2017). They revealed that morphological variability score on the first principal component (PC-1) was highly correlated with characters related to tuber shape and tuber flesh colour. Similarly, Mwirigi et al. (2009) reported that PC-2, PC-3 and PC-4 were mainly correlated with characters related to leaf position and tuber flesh colour similar to the results of PC-4 and PC-3 from this study. Results obtained from Sheikh and Kumar (2017) revealed that variability scores on the first principal component (PC-1) were highly correlated with characters related to stem colour. This was also similar with the results obtained in this study on the first principal component (PC-1) being highly correlated with stem colour. From the dendrogram, morphological characterisation of Kenyan yams from 5 geographical regions indicated that most species from the Eastern area (Meru and Embu) are closely related despite their geographic location being widespread and some showing a few morphological variations. This is as a result *D. minutiflora* Engl. from the

Table 4. Gene bank species identities.

Lab designation	Species identification	The accession number of nearest neighbour	Percentage identity (%)
V005, V007	*Dioscorea burkilliana*	MG805605.1	98.83
V001,V012,V013, V014, V019	*Dioscorea togoensis*	NC_039856.1	98.83
V009,V010,V011, V023	*Dioscorea alata*	NC_039707.1	99.63
V018, V025, V026	*Dioscorea bulbifera*	MG805604.1	99.82
V002, V003	*Dioscorea cirrhosa*	HQ637842.1	98.83
V022	*Dioscorea cayennensis*	NC_039836.1	99.46

Table 5. Laboratory species identities.

	Lab designation	Species identities	Area of collection
1	V001	*Dioscorea minutiflora*	Meru
2	V002	*Dioscorea minutiflora*	Meru
3	V003	*Dioscorea minutiflora*	Meru
4	V005	*Dioscorea minutiflora*	Meru
5	V007	*Dioscorea minutiflora*	Meru
6	V009	*Dioscorea alata*	Taita-Taveta
7	V010	*Dioscorea alata*	Taita-Taveta
8	V011	*Dioscorea alata*	Taita-Taveta
9	V012	*Dioscorea minutiflora*	Embu
10	V013	*Dioscorea minutiflora*	Embu
11	V014	*Dioscorea minutiflora*	Embu
12	V018	*Dioscorea bulbifera*	Embu
13	V019	*Dioscorea minutiflora*	Embu
14	V022	*Dioscorea cayennensis*	Teso North
15	V023	*Dioscorea alata*	Teso North
16	V025	*Dioscorea bulbifera*	Teso North
17	V026	*Dioscorea bulbifera*	Bungoma

two regions clustering together. This indicates a likelihood of numerous exchange of planting materials among and between farmers from different zones. It is also likely that constant vegetative propagation and selection have contributed to the wide phenotypic variability of *D. minutiflora* Engl. (Mwirigi et al*.,* 2009). However, there are four accessions of *D. minutiflora* Engl. in Meru and Embu distinguished by the size of the tuber and spiny stem base. It can be seen that *D.alata* L. (Taita Taveta and Busia) and *D. bulbifera* L. (Embu, Bungoma and Busia) are very closely related and distant to *D. cayenensis* Lam (Busia).

The dendrogram from molecular data was prepared by using the neighbour-joining method. In the cluster analysis *D. minutiflora* Engl. and *D. burkilliana* J. Miege from West Africa were grouped, indicating that they might be considered as one genetic group, as stated by Chaïr et al. (2005). In another study, Magwé-Tindo et al. (2018) identified Guinea Yam wild relatives using the whole plastome phylogenetic analyses which clearly showed

that *D. minutiflora* Engl. and *D. burkilliana* J. Miege formed two strongly supported groups and clustered together. This is in agreement with results obtained by Ramser et al. (1997) who found them in the same habitat. Miège (1968), in his study, established *D. burkilliana* J. Miege and *D. minutiflora* Engl. as two morphologically similar species that differ only by the characteristics of their below-ground parts. These results are in agreement with the results of this study as a result of *D. burkilliana* J. Miege and *D. minutiflora* Engl. clustering together.

D. alata L. and *D.bulbifera* L. are seen to be potentially related from Figure 2 because they share a common origin. This, however, contradicts established taxonomy as well as earlier molecular studies involving both species stating that *D. alata* L. and *D.bulbifera* L. are not closely related (Malapa et al*.,* 2005). On the other hand, the fact that some cultivars of *D. alata* L. produce aerial tubers may support the observed closeness of the species to *D. bulbifera* L. (Tamiru et al*.,* 2007). The input of both morphological and molecular data is critical in

producing well-resolved species delimitation. In this study, results showed a correlation between morphological and molecular data analysis, indicating that molecular data supported morphological species delimitation. Caddick et al. (2008) in his study stated that higher sampling of taxa and morphological and molecular characters for Dioscoreales had produced resolved topologies that corroborate the circumscription that was proposed by APG (1998). His study also concluded that increased bootstrap support in analysis indicated high congruence between independent morphology and molecular data sets and demonstrated that both morphological and molecular data are essential in resolving the relationships within Dioscoreales.

Sartie et al. (2012) in their study on genotypic and phenotypic diversity of cultivated tropical yams using phenotypic and SSR markers established an improved understanding about the genetic and phenotypic relatedness among *D. rotundata* Poir., *D. cayenensis* Lam., *D. alata* L. and *D. dumetorum* (Kunth) Pax. This is similar to what was done in this study using phenotypic and molecular markers to establish phylogeny of *Dioscorea* in Kenya. Girma et al. (2015b) in their study of morphological and SSR analysis of *D. alata* L. indicated that combining SSR markers and phenotypic data were useful for identification of *D. alata* L. accessions likewise to combining morphological data and molecular markers in characterizing Kenyan *Dioscorea* species.

Conclusion

Dioscorea species grown in Kenya exhibited morphological variations. Phylogenetic relationships of Kenyan *Dioscorea* species were established with *D. alata* L. and *D. bulbifera* L. seen to be closely related and *D. minutiflora* Engl. and *D. burkilliana* J. Miege from West Africa grouping together as one genetic group. Molecular and morphological characterization was efficient in establishing species relatedness among *Dioscorea* species. Future studies should consider collections from other localities in addition to Meru, Embu, Taita-Taveta, Busia and Bungoma counties and more than one molecular marker should be used.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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