Morphological Characterization of African Nightshade (S*olanum Nigrum L*) Accessions in Western Kenya

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Abstract

African nightshade, Solanum nigrum L .is one of the most important leafy vegetable rich in nutritional and medicinal value, can be used to feed people with human immune deficiency virus, HIV/AIDS in Kenya. This study was conducted to morphologically characterize thirty African nightshade accessions in order to establish the level of genetic variation amongst them. The limited information available on this species hinders their sustainable conservation and development. Existing knowledge on genetic potential is limited and also the information regarding the genomic group of Solanum nigrum complex species is scanty. The aim of this study was to evaluate the morphological characteristics in different African nightshade accessions in the field. A total of 30 samples from three counties Bungoma, Kakamega and Trans Nzoia were evaluated. The African nightshade accessions were planted at Kibabii University farm and scored for several agro morphological characters based on National Bureau of Plant Genetic Resource NBPGR descriptors on the following qualitative traits; Leaf surface as Glaborous or pubscent, Color of ripe fruit as Orange or Dark purple or Black), Stem ridge as Smooth ridges or Dented), Leaf shape as Lanceolate or Ovate or rhomboid), Leaf margin as Sinuate dented or Entire and Inflorescence orientation as Simple or Forked the plant type was scored as Semi erect or erect. Cluster analysis of morphological data was done using PASW Version 20 Statistical software. Results showed that there was phenotypic variation among accessions of African nightshade collected from the three counties since they were grouped into two major clusters A and B meaning that there exists diversity amongst the accessions.

Keywords: African nightshade, accessions, characterization, variation.

INTRODUCTION

Solanum nigrumL is the most diverse plant species within the genus *Solanum (*Matasyoh *et al.*, 2015). African nightshade is a dicotyledonous crop in the *Solanaceae* family. Even though African nightshade species have been studied broadly, their correct taxonomic identification is yet to be established. This is because of continued inter and intraspecific hybridization which occurs naturally among African nightshade species. The vulnerability of morphological traits to phenotypic plasticity have also caused problems to their taxonomic identification. Different communities use different local names to identify african nightshade species creating further confusion in the differentiation of one species from the other (Poczai and Hyvonen, 2011; Ojiewo *et al.*, 2013a).

African nightshade has as well been regarded as a weed by the relevant institution hence little studies have been done on the crop and there's also lack of enough personnel assigned the duty of evaluation and preserving of African nightshade germplasm (Zebish *et al.*, 2016).This has led to limited information, to help the researchers develop more improved varieties.

The most commonly grown African nightshade species in Kenya include *S. villosum, S. sarrachoides, S. scabrum, S. americanum* and *S. physalifolium* (Ojiewo *et al*., 2013b; Matasyoh and Bosire, 2016). Studies have shown that *S. scabrum, S. nigrum, S. villosum* were the species found in Western, Rift valley, and Nyanza regions of Kenya with *S. scabrum* being preferred in most regions except in Kisii and Nyamira where *S. nigrum* was preferred (Matasyoh and Mwaura, 2014; Matasyoh and Bosire, 2016).

African nightshade has tiny flowers comprising of five petals that are white or purple in colour with berries that turn black on maturity and grows wild on uncultivated lands, dump sites and it is also grown in kitchen gardens. African nightshade mainly requires soils that are rich in nitrogen for its growth (Atanu *et al.,* 2011; Jagatheeswari *et al*., 2013).

The leafy African nightshade contains substantial amounts of protein and amino acids such as methionine, minerals like calcium, iron and phosphorus, vitamins A and C, fat and fiber (Zebish *et al.*, 2016).

African nightshade is used worldwide for the treatment of various ailments such as ear pains, as a therapy for convulsions, pain reliever, anthrax pustules, blood pressure, heart diseases, and tonsillitis (Jagatheeswari *et al.,* 2013; Matasyoh and Mwaura, 2014).

The increase in consumer awareness on the nutritional and medicinal value of African nightshade has concurrently led to increased consumption increasing its market demand (Ojiewo *et al.*, 2013) but the supply is low because researchers have not come up with enough improved varieties to meet the increased demand.

There is need that efforts are made to improve on African nightshade production through increased cultivation for commercial purposes so as to try and meet the demand in the market. Therefore, the aim of the study was to evaluate morphological characteristics of African nightshade accessions collected from Bungoma, Kakamega and Trans Nzoia counties of Western Kenya.

MATERIALS AND METHODS

Sampling criteria

Stratified random sampling was done based on prior information on species grown and sampling partnership with farmers. Consultations were done with area Agricultural officers before collection of materials. Sampling was done in a 0.5km radius in each location. Five farms where African nightshades were growing were selected at random. The selected farms ranged in sizes between 0.3ha and 2ha and production of African nightshade done at a small scale. Five mature plants having mature fruits were randomly selected in each farm and also seeds of the same accession in the farm were collected from the farmers. A total of 30 seed samples of the accessions were collected from the three selected counties constituting of 10 samples each. These materials were labelled based on the area and farm (farmer) number of collection as Bungoma (B), Kakamega (K) and Transnzoia (T) , the envelopes were placed in glass Jars with tight lids and the Seeds were kept dry using a Silica Gel Packet .(Table 1).

Table 1: **African nightshade accession used in the study**

(Accessions) Name	Local Name	Source	GPS Coordinates	
			longitude	Latitude
Bungoma 1	Namasaka	Lugulu	34.751	0.665
Kakamega1	Esucha	Ingotse	34.696	0.356
Trans-Nzoia 1	Kisoyet	Kwanza	35.003	1.163
Bungoma 2	Namasaka	Maeni	34.750	0.792
Kakamega 2	Irisuza	Lukuyani	35.103	0.711
Bungoma 3	Namasaka	Mabanga	34.619	0.601
Kakamega 3	Liisucha	Chimoi	34.826	0.580
Kakamega 4	Esucha	Navakholo	34.681	0.407
Kakamega 5	Lisutsa	Shinyalu	34.766	0.274
Bungoma 4	Namasaka	Mayanja Vitunguu	34.544	0.528
Bungoma 5	Namasaka	Chwele	34.581	0.737
Kakamega 6	Liisucha	Lubao	34.807	0.332
Bungoma 6	Namasaka	Bokoli	34.660	0.712
Trans-Nzoia 2	Managu	Kiminini	34.927	0.884
Trans-Nzoia 3	Namasaka	Sikhendu	34.830	0.884
Bungoma 7	Namasaka	Sang’alo	34.593	0.528
Bungoma 8	Namasaka	Kimilili	34.727	0.792
Trans-Nzoia 4	Managu	Kiungani	34.951	0.95
Bungoma 9	Namasaka	Ndalu	34.987	0.818
Kakamega 7	Liisucha	Malava	34.855	0.454
Kakamega 8	Liisucha	Kaburengo	34.801	0.578
Trans-Nzoia 5	Kisocheet	Saboti	34.838	0.931
Trans-Nzoia 6	Osoig	Endebesi	34.852	1.086
Kakamega 9	Liisucha	Lwandeti	34.849	0.607
Bungoma 10	Namasaka	Kamukuywa	34.784	0.799
Trans-Nzoia 7	Managu	Mucharage	34.856	0.818
Trans-Nzoia 8	Namasaka	Bidii	35.035	1.033
Kakamega 10	Liisucha	Matete	34.805	0.555
Trans-Nzoia 9	Managu	Bondeni	34.902	0.991
Trans-Nzoia 10	Ksoyo	Cherang’ani	35.234	0.988

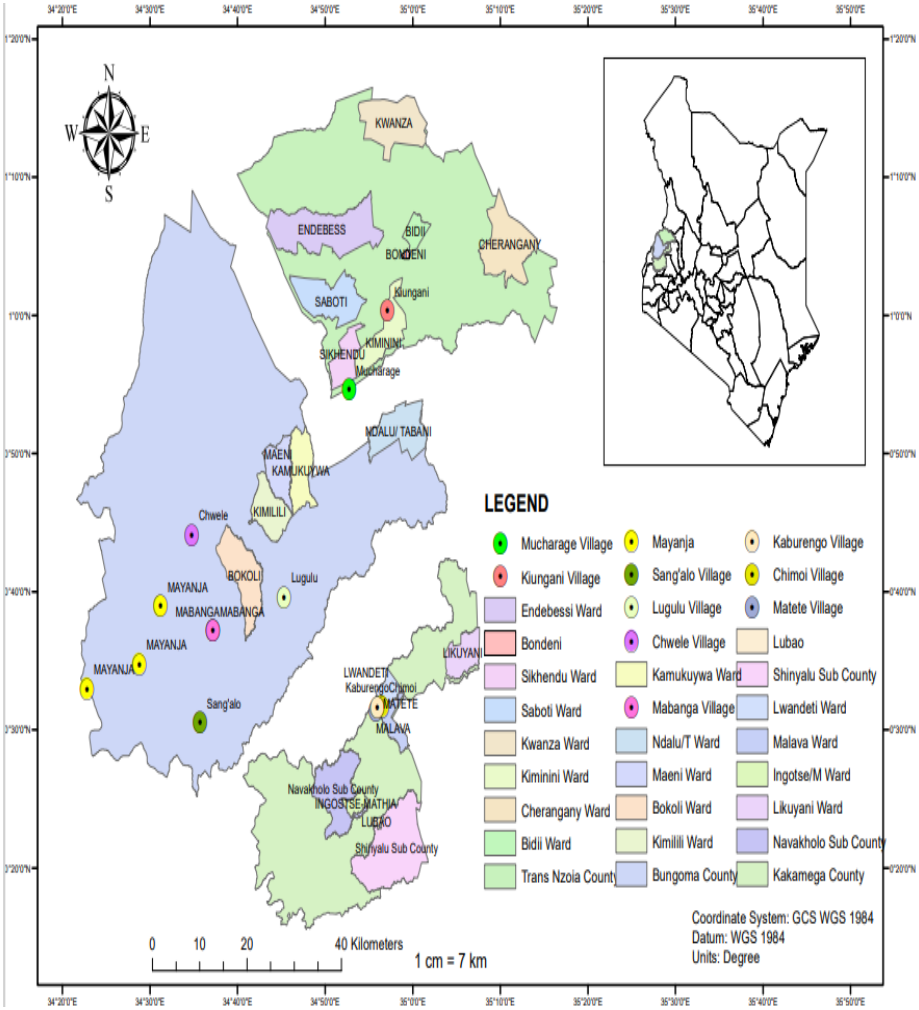

Figure 1: Map showing three counties in Western Kenya where the African nightshade samples were collected

Experimental design

The experiment was laid out in a randomized complete block design with three replications each.

Experimental layout

Land was ploughed and harrowed until fine tilth was achieved. Thirty accessions of African nightshade were then planted in a Randomized complete block design replicated three times with each accession appearing once per block, at Kibabii University Agricultural farm. Seeds were drilled at a depth of 1cm on single row plots of 3 M X 4 M spacing 30 cm between plants, 40cm between rows and 1M between blocks during the short and long rainy seasons. DAP fertilizer applied at the rate of 0.012kg/plot and thoroughly mixed with the top soil at planting time. The plant stand count (germination percentage) was taken two weeks after planting and thinning was done to ensure a desired plant stand of ten plants per plot was achieved.

Data Collection

Data was collected on four randomly selected plants from the middle of each plot as per the criterion described by Nandhini *et al*., 2014.The four seedlings were tagged and a morphology descriptor list was made and developed using the morphological features described by National Bureau of Plant Genetic Resource (NBPGR) (Singh *et al.*, 2003) as shown on Table 2.

Table 2: Characters used in morphological analysis of African nightshade accessions of Western Kenya

Character	Scoring method
Leaf margin	Entire or Sinuate or Sinuate dentate
Leaf hair surface	Presence or absence
Stem ridge	Presence or absence
Berry colour	Green or purple or orange
Inflorescent type	Forked or simple
Leaf shape	Ovate or lanceolate
Plant type	Erect or Semi erect

Table 3: Qualitative traits and their scores according to the NBPGR descriptors

Accession	Colour of ripe fruit	Stem ridge	Leaf shape	Leaf margin	Inflorescence Type	Leaf surface	Plant type
Bungoma (*Solanum villosum* 1)	1	1	1	1	1	1	1
Kakamega 1(*Solanum villosum)*	1	2	1	1	1	1	1
Transnzoia 1*Solanum nigrum*	2	1	2	2	1	1	1
Bungoma 2(*Solanum nigrum*	2	2	2	2	1	1	1
Kakamega 2(*Solanum scabrum* improved)	3	2	1	1	2	2	2
Bungoma 3(*Solanum villosum)*	1	1	1	1	1	1	1
Kakamega 3(*Solanum scabrum* improved)	3	2	1	1	2	2	2
Kakamega 4(*Solanum villosum)*	1	2	1	1	1	1	1
Kakamega 5(*Solanum villosum*)	1	1	1	1	1	1	1
Bungoma 4(*Solanum nigrum)*	2	2	2	2	1	1	1
Bungoma 5(*Solanum nigrum*)	2	2	2	2	1	1	1
Kakamega 6(*Solanum nigrum)*	2	2	2	2	1	1	1
Bungoma 6(*Solanum nigrum*)	2	2	2	2	1	1	1
Transnzoia 2(*Solanum villosum)*	1	2	1	1	1	1	1
Transnzoia 3(*Solanum nigrum)*	2	1	2	2	1	1	1
Bungoma 7(*Solanum scabrum* improved)	3	2	1	1	2	2	2
Bungoma 8(*Solanum nigrum)*	2	2	2	2	1	1	1
Transnzoia 4(*Solanum scabrum* improved)	3	2	1	1	2	2	2
Bungoma 9(*Solanum villosum)*	1	2	1	1	1	1	1
Kakamega 7(*Solanum nigrum)*	2	2	2	2	1	1	1
Kakamega 8(*Solanum nigrum)*	2	1	2	2	1	1	1
Transnzoia 5 (*Solanum nigrum)*	2	2	2	2	1	1	1
Transnzoia 6 (*Solnum nigrum)*	2	1	2	2	1	1	1
Kakamega 9 (*Solanum scabrum* improved)	3	2	1	1	2	2	2
Bungoma 10(*Solanum villosum)*	1	1	1	1	1	1	1
Trans nzoia 7(*Solanum villosum)*	1	1	1	1	1	1	1

Transnzoia 8(*Solanum villosum)*	2	2	1	2	1	1	1
Kakamega 10(*Solanum nigrum)*	2	1	2	2	1	1	1
Transnzoia 9(*Solanum nigrum)*	2	1	2	2	1	1	1
Transnzoia 10(*Solanum nigrum)*	2	1	2	2	1	1	1

NBPGR Descriptors

Plant type: 1=Semi erect2=Erect; Leaf surface: 1=Glaborous (sparsely), 2=pubescent (Densely). Leaf margin: 1 =Sinuate –dentate, 2 =Entire, 3 = Lobed; Leaf shape: 1 = Lanceolate, 2 = Ovate, 3 = Rhomboid; Stem ridge: 1= Smooth ridges, 2= Dented; Fruit colour : 1= Orange, 2= Dark purple, 3=Black; Inflorescence type:1=Simple,2=Forked.

Source: Singh 2003.

Statistical analysis

Data collected was analyzed using PASW Version 20 Statistical analysis package and a dendrogram drawn using hierarchical cluster analysis procedure and Euclidian average distance.

RESULTS AND DISCUSSIONS

The dendrogram (Fig. 2) shows that the samples of African nightshade accessions from African nightshade growing areas in Kakamega, Bungoma and Trans nzoia county are quite homogeneous and most of samples also tend to be distributed in a homogeneous group, with the exception of a few more scattered. The dendrogram has two major clusters **A** and **B** linked at a square Euclidean distance of 25.**A** links two major clusters at a square Euclidean distance of 16, **A1** links three clusters at a square Euclidean distance of 8.**A2** links two clusters at a square Euclidean distance of 8; **A1$_1$** comprises of *Solanum nigru*m (Trans nzoia 9), *Solanum nigrum* (Trans nzoia 10), *Solanum nigrum* (Trans nzoia 1), *Solanum nigrum* (Trans nzoia 6), *Solanum nigrum* (Kakamega 10), *Solanum nigrum* (Transnzoia 3),*Solanum nigrum* (Kakamega 8).**A1$_2$** comprises of *Solanum nigrum* (Kakamega 7),*Solanum nigrum* (Trans nzoia 5), *Solanum nigrum* (Bungoma 2), *Solanum nigrum* (Bungoma 6), *Solanum nigrum* (Bungoma 8), *Solanum nigrum* (Bungoma 5), *Solanum nigrum* (Kakamega 6),*Solanum nigrum* (Bungoma 4). **A1$_3$** comprises of *Solanum villosum* (Trans nzoia 8). **A2$_1$** comprises of (*Solanum villosum* (Trans nzoia 2), *Solanum villosum* (Bungoma 8), *Solanum vilosum* (Kakamega 1), *Solanum villosum* (Kakamega 4), **A2$_2$** comprises of (*Solanum villosum* (Bungoma 10), *Solanum villosum* (Trans nzoia 7), *Solanum villosum* (Bungoma 1), *Solanum villosum* (Bungoma 3), *Solanum villosum* (Kakamega 5).**B** comprises of *Solanum scabrum* improved (Trans nzoia 4), *Solanum scabrum* improved (Kakamega 9), *Solanum scabrum* improved (Kakamega 2), *Solanum scabrum* improved (Kakamega 3), *Solanum scabrum* improved (Kakamega 7).

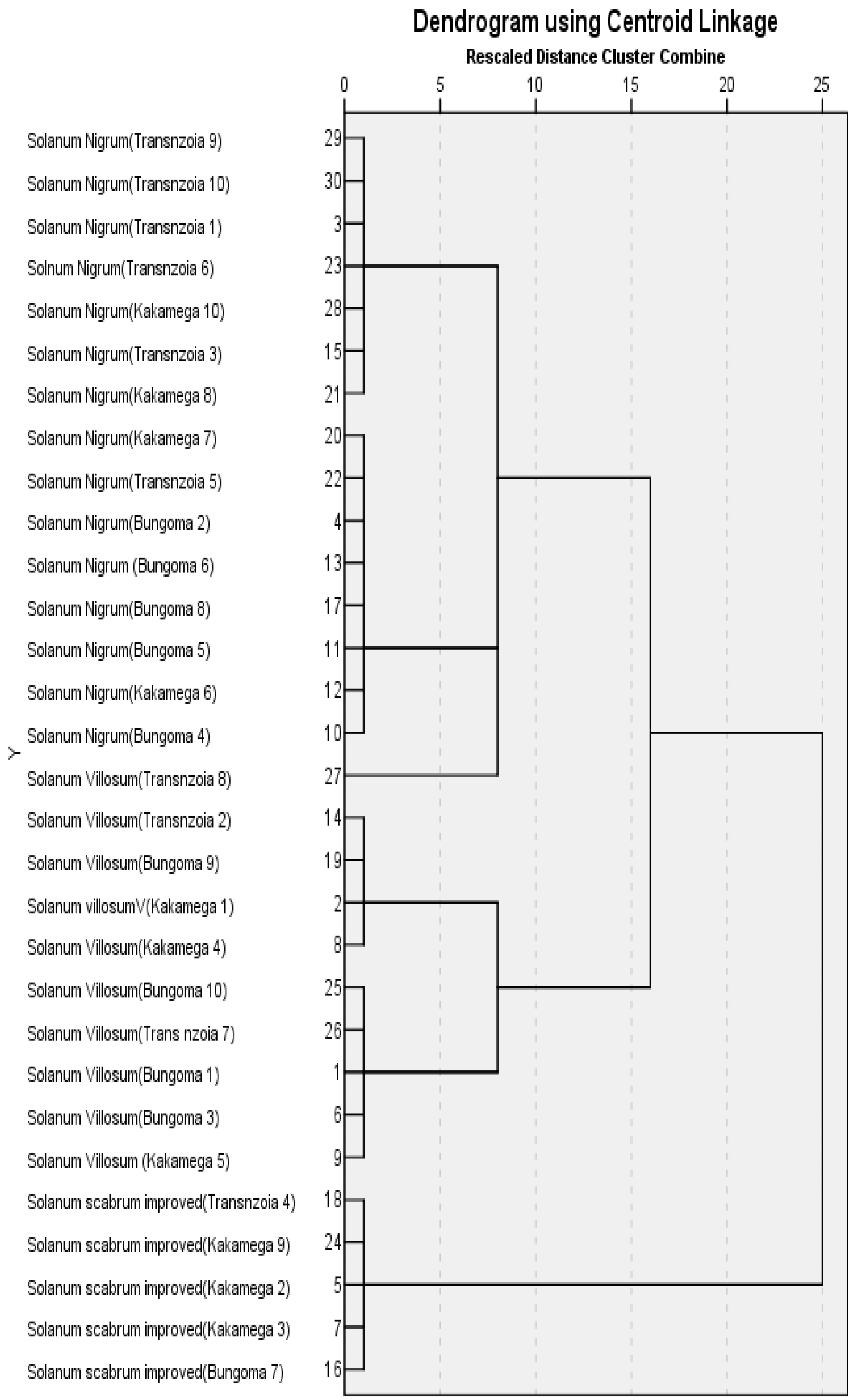

Figure 2: Hierarchical cluster analysis dendrogram displaying relationship among 30 African nightshade accessions using qualitative traits.

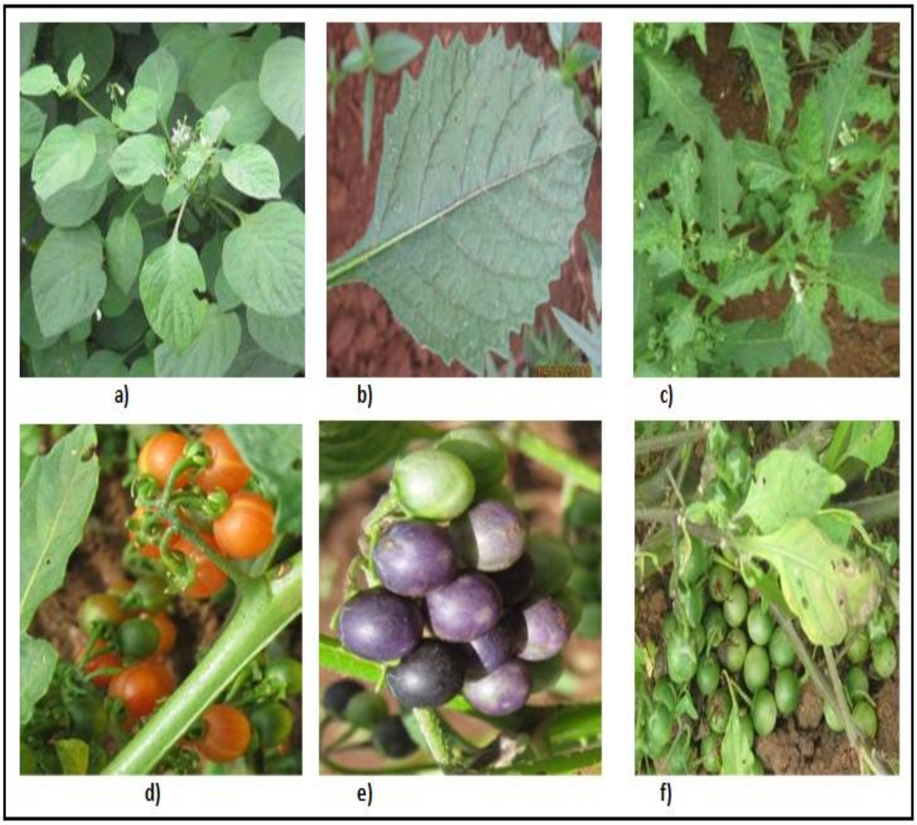

Plate 1: African nightshade accessions exhibiting diversity in leaf shape, leaf margin, leaf pubescent and fruit colour; a-Ovate leaves with entire margins, b-Rhomboid leaf with sinuate-dentate margins, c- Lanceolate leaves with sinuate to dentate margins, d- Orange berries, e- Purple berries, f- unripe Green berries

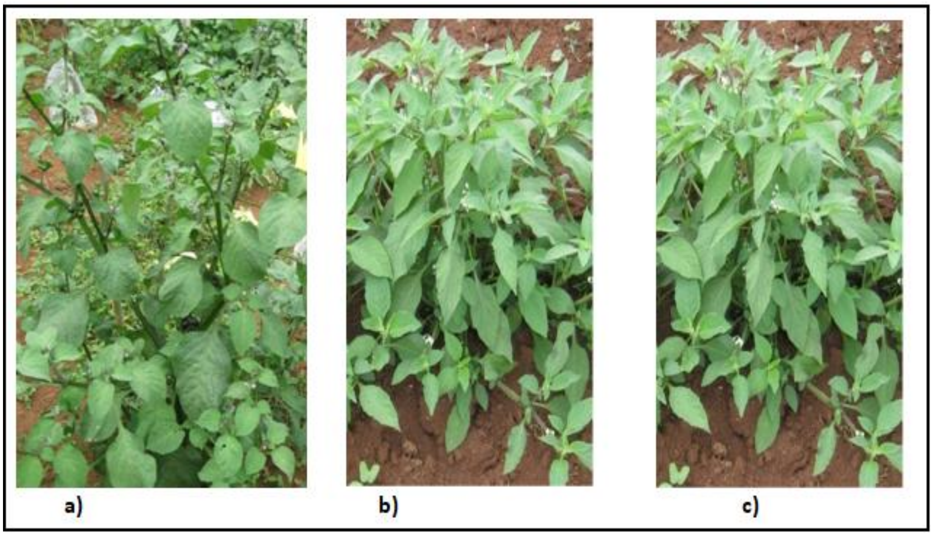

Plate 2: African night accessions exhibiting diversity in plant type; a- Erect. b-Semi erect (c) Semi erect

DISCUSSION

Morphological characterisation of plant species is an important step in crop improvement programmes as it permits researchers to identify and select superior lines for further crop advancement (Adebola and Morakinyo 2006; Das and Kumar 2012; Julia *et al.* 2016; Peratoner *et al*., 2016; Ngomuo *et al.* 2017) as it allows for the study of plant variation using visual attributes.

Significant variation was observed for all the qualitative traits among the 30 African nightshade accessions and this implies that qualitative traits can be used as a measure of diversity among African nightshade cultivars. These results are in line with those of Olet (2004) who stated that qualitative traits are more reliable in the identification of genetic relationship among African nightshade than quantitative traits. Nandhini *et al*., (2014) also observed considerable variation in qualitative traits among African nightshade cultivars. This variation observed could either be genetic or as a result of the effect of the environment of the genes of the cultivars.

For instance, the different fruit colours expressed by different African nightshade accession could be as a result of anthocyanin concentration in the plants and could be influenced by environmental factors (Manoko, 2008). A cluster dendrogram is a good measure of diversity among and within species as it groups similar entries under one cluster (Malek *et al.*, 2014). The cluster analysis demonstrated the existence of variability among the 30 African nightshade accessions for the morphological traits studied (Figure 2). A similar strategy was applied by Zhang *et al*., 2012 and Mekonnen *et al.*, 2014 on the morphological characterization of *Cucumis melo* and lentils accessions, respectively.

The clustering pattern shows that *Solanum Scabrum* accessions were genetically distant from the *Solanum villosum* and *Solanum nigrum* accessions and can be used to improve one another. Accessions from the same counties were grouped together but there was also sub-clustering of the major clusters, suggesting that there was still variation within clusters. The clustering also revealed some singletons (*Solanum villosum* Transnzoia 8). Singletons are those accessions that are placed separately from the rest of the accessions in a cluster. They are more diverse and are to be given special attention during selection because of their superiority over other accessions as suggested by Choudhary *et al.* (2013). Singletons were also observed in other genetic characterization studies (Corchurus by Dube *et al*.,2018, Chickpea by Chowdhury *et al.*,2015, Amaranthus by Gerrano *et al*.,2015).

The observed complex clustering with the dendrogram was suggestive of a rich diversity within the African nightshade cultivars assessed since the shorter the Eucleadean distance of the branches of a dendrogram the more similar the cultivars are while the longer the branches the more genetically diverse the cultivars are (Kalinowski, 2009). These indicates that the cultivars in a given cluster are more genetically similar than cultivars across cluster groups. These results are in agreement with those of Nyadanu *et al.,* (2014) working on agro morphological characterization of eggplant.

African nightshade accessions with lanceolate leaves such as cultivars belonging to *Solanum nigrum* L, and *Solanum villosum* species have been shown to tolerate water stress more than cultivars with ovate leaves (Ojiewo *et al.,* 2013). This is because lanceolate leaves are narrow hence have a smaller surface area exposed to the atmosphere for transpiration and therefore they lose less water as compared to ovate leaves which are broad, have a large surface area exposed for transpiration and lose a lot of water loss when exposed to water stress conditions and also plant orientation has been indicated to determine the exposure of leaves and other vegetative parts to sunlight for photosynthesis, and that might affect the yield obtained.

The upright and erect African nightshade accessions are also thought to be better yielding due to their plant orientation (Nandhini *et al.,* 2014). In addition, Sekyere *et al*., (2011) working with okra reported that genotypes with erect plant type had high dry matter production leading to an increase in yield.

All accessions belonging to African nightshade species *Solanum scabrum* had leaves with pubescent surfaces (hairs). Cultivars with pubescence have been shown to be tolerant to pests and insects (War *et al*., 2012) since trachomes (hairs) hinder insects and pests from laying eggs, feeding and also interferes with their larval feeding (Steinite and Ievinsh, 2003). The trachomes also interfere with the movement of insects and pest on the plant surface thereby decreasing their contact with the leaf epidermis hence preventing leaf damage (War *et al*., 2012). Pest and diseases have been indicated to be the main challenge encountered by farmer during production of African nightshade (Onyango *et al.,* 2016).

These two traits (leaf shape and leaf pubescence) are important traits that can be exploited in developing African nightshade cultivars that are resistant to drought and pest and also disease tolerant.

The existing intra specific and inter specific diversity between cultivars is the key to crop improvement (Nyadanu *et al.,* 2014; Ojiewo *et al*., 2013) and this is because cultivars with superior yield traits can be developed through breeding for improved vegetable productivity. Different communities prefer different African nightshade species for instant the Abagusii community prefer genotypes with spreading plant type, producing small leaves (lanceolate) with mild bitterness such as *S. sarrachoides* species while the Abaluhya prefer genotypes that have an erect plant type producing broad leaves with bitter taste such as the *S scabrum* species (Onyango *et al*., 2016). This is an indication that there are variations in terms of the preferred African nightshade species from one community to the next hence when breeding African nightshade for improved productivity, specific community interests should also be put into consideration.

For qualitative traits, most variation observed were across species and not within species for instance all cultivars belonging to *S. villosum* had semi erect plant type, and produces mature orange berries *S. scabrum*s producing mature purple berries. This may imply that the variation seen are genetical and not environmental and hence do not change from one location to another since the same qualitative traits observes on the accessions within a certain species long rain were the very same once observed in the short rain for example, the leaf shape of cultivars within *S. scabrum* species was ovate both in the short and long rain.

The evaluation of African nightshade accessions using morphological traits showed that there was significant variation recorded for the qualitative traits among the 30 African nightshade accessions. African nightshades accessions in this study clustered into different sub groups representing different species which included *S. scabrum, S. villosum, and S. nigrumL* based on their distinct morphological features.

The significant variation seen among African nightshade accessions indicated that there exists diversity within cultivars belonging to the same species as well as among accession across different species and the variation observed could either be genetic or environmental.

This study also revealed that qualitative traits were important in the evaluation of genetic diversity within African nightshade accessions and could be used in the identification of important accession that could be incorporated into breeding program for further crop improvement. This study recommends that further morphological characterization be carried out with both qualitative and quantitative traits and be narrowed down to studying accession belonging to the same species in Western Kenya after which superior cultivars within particular African nightshade species could be identified and documented to be accessed by breeders.

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