

## Agro-morphological characterization of horned melon (*Cucumis metuliferus*) accessions from selected agro-ecological zones in Kenya

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### Abstract

Morphological characterization of genotypes is fundamental in providing information on their genetic status to guide on their conservation and improvement. The objective of this study was to determine agro-morphological diversity within horned melon in Kenya. The study was carried out in two seasons at the University of Embu in Kenya. The study characterized 19 horned melon accessions collected from different agro-ecological zones in Eastern, Central and Western regions in Kenya. The experiment was laid out in a randomized complete block design with three replications. Morphological characterization of the accessions was based on melon descriptors from International Plant Genetic Resources Institute (IPGRI) with slight modifications. Significant differences were observed in all the quantitative traits except the number of branches and main vine length. However, qualitative variations were only observed in fruit shape, rind colour and seed shape. Principle Component Analysis (PCA) showed that fruit weight, main vine length and days to maturity had the highest contribution to the observed diversity. Cluster analysis separated the accessions into seven groups with between classes diversity of 79.20% and within classes diversity of 20.80%. The diversity observed can be exploited by plant breeders for genetic improvement of the crop.

**Keywords:** Genetic Diversity, Agronomic Variation, Qualitative and Quantitative Characters.

**Abbreviations:** ANOVA\_analysis of variance; AEZ\_agro-ecological zone; FAO\_Food and Agriculture Organization; GPS\_Global Positioning System; HCD\_Horticultural Crops Directorate; IPGRI\_International Plant Genetic Resources Institute; LH\_lower highland; LM\_lower midland; NS\_Not Significant; PCA\_principal component analysis; SE\_standard error; SNK\_Students Newman's Keuls; TSP\_triple super phosphate; UH\_upper highland; UM\_upper midland; UPGMA\_unweighted pair group method with arithmetic average.

### Introduction

Cucurbits are widely cultivated around the world and are among the most important fruits consumed in Africa since they are rich in several vitamins and minerals (Deguine et al., 2015). They are mainly annual vines belonging to the family *Cucurbitaceae*. This family comprises of about 118 genera and 825 species with their members spread mainly in regions of tropical and subtropical worldwide (Wang et al., 2007). They are ranked among the major vegetable fruits grown in Kenya and exported abroad for their nutritional value and economic significance as foreign exchange earners (Tshilidzi et al., 2016). The major species of cucurbits grown in Kenya are butternut (*Cucurbita moschata*), pumpkin (*Cucurbita maxima*), cucumber (*Cucumis sativus*), courgettes (*Cucurbita pepo*), and watermelon (*Citrullus lanatus*) (HCD, 2016). Minor species include horned melon (*Cucumis metuliferus*) or kiwano, which has high economic and nutritional value that is yet to be fully exploited (Aliero and Gumi, 2012).

Horned melon (*C. metuliferus*) belongs to the genus *Cucumis* and species *metuliferus*. It has many common names like jelly melon (English), Kiwano, Melano (Israel), bitter wild cucumber (South Africa), thorn melon and many others. Its fruit has

horn-like spines, hence the name "horned melon" (Wannang, 2011). Horned melon is a dioecious plant with male flowers appearing several days before the female flowers (Wilkins-Ellert, 2004). The flowers form in clusters of 1 to 4 and are small sized, bright yellow, funnel-shaped, axillary and opening to 5 lobes. The pistillate enlarges to form the fruit which grows above a prickly green ovary (Usman et al., 2015). The fruit is ellipsoid-cylindrically shaped and is normally light green when immature and bright orange at ripening (Bester and Condy, 2013). The skin has obscure longitudinal stripes of small pale markings, with sharp spines at the top (Usman et al., 2015). Unripe fruit is dark-green in colour. Ripe fruit has jelly-like translucent, green flesh filled with whitish seeds and with a refreshingly fruity taste and texture similar to a passion fruit (Weng, 2010).

Horned melon has high economic and nutritional value which has not been fully exploited (Aliero and Gumi, 2012). The flesh and the seeds can be eaten raw after being sprinkled with sugar or salt to enhance the flavour (Lim, 2012). The high moisture proportion in the fruit provides a good source of water for animals and human in the arid and semi-arid areas

(April et al., 2018). The fruits can be baked whole or sliced and dried for future use (Lim, 2012). The fruit can also be sliced and added to tropical fruit salads (Wilkins-Ellert, 2004). Immature fruits can also be relished like cucumber but the nutritional value is rated twice higher than that of cucumber (Lim, 2012). Young leaves can be boiled and eaten like spinach. The ripe fruit can also be used for decoration purposes due to its interesting look (Cantwell, 2011). The pulp and the seeds can also be blended in a food processor for making refreshments such as yoghurts and ice creams (April et al., 2018). It can also be used as a substitute for vinegar in salad dressing (McCormack, 2005).

Horned melon grows at an altitude range of 210 to 1800 m above sea level (Bester and Condy, 2013). It dominates both tropical and sub-tropical areas and cannot tolerate cold and mist conditions (Wilkins-Ellert, 2004). It prefers shallow or deep, well-drained, mostly alluvial sandy soil on river banks or flood plains, but has also been recorded from clay or loam soil and rocky slopes (Usman et al., 2015). Optimum growing temperature ranges between 20 - 30°C (Aliero and Gumi, 2012). However, its growth is not greatly affected by temperatures as high as 40°C although flowering seems to be affected by temperatures over 30°C and germination is greatly inhibited above 35°C (Aliero and Gumi, 2012). The plant can do well with as little as 350 to 550 mm of rainfall per season and dry air is beneficial during the harvest period (Cantwell, 2011). Semi-arid climate and warm season rainfall enhances the fruit ripening stage, enabling the fruit to develop full flavour (Aliero and Gumi, 2012).

Despite the numerous agronomic, nutritional, medicinal and economic advantages of horned melon, the crop remains less popular among farmers and consumers in most parts of the world (Wilkins-Ellert, 2004) and thus its potential remains underutilized (Aliero and Gumi, 2012). The crop is also less studied by researchers and therefore its agronomic, nutritional and economic potential is not well documented. In Kenya, some horned melon accessions have been identified in various agro-ecological zones but their genetic and agronomic traits have not been studied. Consequently, no selection or genetic improvement has been conducted on this crop despite its high economic potential. It is expected that where this plant already exists in the wild or introduced in farmers' fields, a wealth of genetic diversity that can be utilized in its improvement would be found. This study therefore targeted to harness this genetic wealth as the basis for selection, genetic improvement and promotion of horned melon production in Kenya.

## Results

### *Assessment of the qualitative traits of horned melon*

The qualitative characters that were scored on the 19 horned melon accessions were growth habit, leaf blade, leaf shape, flower biology, corolla colour, fruit shape, predominant rind colour, design produced by secondary skin colour, skin stripe colour, presence of grooves on the fruit, flesh colour, seed shape and seed colour. Variations among accessions were only observed in fruit shape, predominant rind colour and seed shape. For the fruit shape, 74% of the accessions were elliptical

in shape while 26% were cylindrically shaped. The cylindrical ones were from Maragua, Meru, Migori, Oyugis and Rongo. For the rind colour, 63.84% were light green while 36.16% were dark green. The dark green ones consisted of accessions from Kathwana, Maragua, Chuka, Kianjokoma, Migori, Kiambere and Oyugis. For the seed shape, 63.16% were elliptically shaped while 36.84% had pinonette shape. The pinonette shaped ones included accessions from Embu, Siakago, Chuka, Kathwana, Mitunguu, Machakos and Kwale. There was no variation in all the other qualitative traits namely growth habit, leaf blade, leaf shape, flower biology, corolla colour, design produced by secondary skin colour, presence of grooves on the fruit, flesh colour and seed colour. The leaves had 3-palmately shallow lobed blade. Both male and female flowers were yellow in colour for all the accessions. The fruit had a mixture of both dotted and stripe skin design with green background and grooves present in the whole fruit. The flesh colour was green with white seeds. All the accessions were runners with a main vine that was highly branched.

### *Assessment of the quantitative traits of horned melon*

Quantitative characters that were evaluated include vine length, number of branches on the main vine, fruit number, fruit weight, seed size and rind thickness. There were no significant differences ( $p>0.05$ ) among accessions in main vine length, number of branches and fruit weight. However, significant differences ( $p<0.05$ ) among accessions were recorded in fruit number, seed size and rind thickness (Table 1). Rongo accession produced the highest number of fruits (17) per plant while Kangundo accession had the least (7) fruits. Maragua accession had the largest seed size (4.10mm) while Kiambere accession had the smallest seed size (3.17 mm). Lastly, Machakos accession had the biggest rind thickness (5.37mm) while Nyakoe accession had the smallest rind thickness (3.01 mm). All accessions had more than 100 seeds per fruit (data not analysed). Seasonal variations were not significant for all the quantitative variables (data not shown) indicating that different variables responded in a similar way to different production seasons.

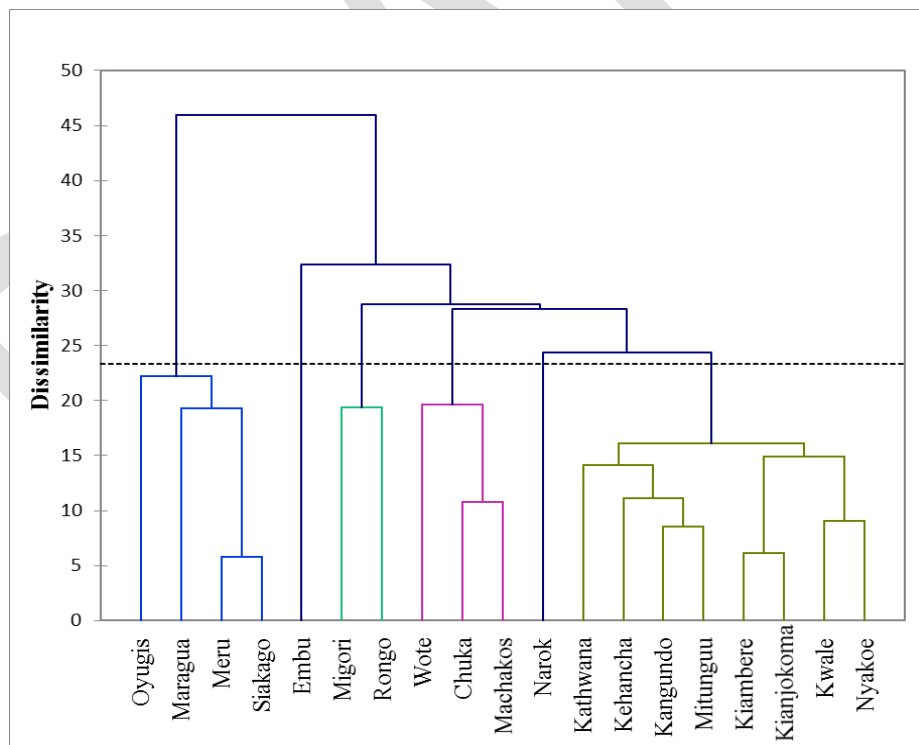
### *Assessment of the agronomic traits of horned melon*

Agronomic traits that were evaluated include percentage germination, days to emergence, days to male and female flowering and days to maturity. There were significant variations ( $p<0.05$ ) among accessions in all the agronomic characters as shown in Table 2. On seasonal average, Kathwana, Siakago, Kangundo and Embu accessions recorded 100% germination while Kehancha accession recorded the lowest germination of 54.33%. Mitunguu accession took the shortest time to emerge (9 days) while Kathwana accession took the longest period of 11 days to emerge. Kehancha accession recorded the least days to male and female flowering (59 and 62 days respectively), while the Rongo accession took the most days (74 and 75 days respectively). In addition, Kehancha accession took the shortest period (102 days) to mature while Embu accession took the longest period

**Table 1.** Variation in quantitative traits of horned melon accessions.

Accessions	Main Vine Length (cm)	Branch Number	Fruit Number	Fruit Weight (gm)	Seed Size (mm)	Rind thickness (mm)
Kathwana	261.83	15.48	10.00 <sup>abc</sup>	226.33	4.00 <sup>abcd</sup>	4.24 <sup>b</sup>
Meru	250.58	13.75	13.17 <sup>abc</sup>	259.33	3.75 <sup>bcd</sup>	4.28 <sup>b</sup>
Siakago	249.42	16.00	14.88 <sup>abc</sup>	256.00	3.87 <sup>abcd</sup>	3.94 <sup>bcd</sup>
Maragua	264.17	15.25	14.81 <sup>abc</sup>	246.00	4.10 <sup>a</sup>	3.61 <sup>cde</sup>
Chuka	258.67	14.67	11.03 <sup>abc</sup>	194.67	3.46 <sup>ef</sup>	3.12 <sup>ef</sup>
Kehancha	253.33	16.58	10.97 <sup>abc</sup>	214.67	3.67 <sup>de</sup>	4.26 <sup>b</sup>
Narok	236.75	14.33	12.18 <sup>abc</sup>	207.00	3.73 <sup>cde</sup>	3.46 <sup>def</sup>
Kianjokoma	249.17	13.83	10.79 <sup>abc</sup>	215.33	3.2 <sup>f</sup>	3.16 <sup>ef</sup>
Kangundo	263.25	16.00	7.41 <sup>c</sup>	218.00	3.69 <sup>de</sup>	4.12 <sup>bc</sup>
Kwale	245.83	15.67	9.50 <sup>abc</sup>	227.67	4.00 <sup>abcd</sup>	3.19 <sup>ef</sup>
Rongo	254.75	14.75	16.50 <sup>a</sup>	204.00	3.35 <sup>f</sup>	3.72 <sup>cde</sup>
Machakos	267.92	15.58	12.85 <sup>abc</sup>	195.00	3.98 <sup>abcd</sup>	5.37 <sup>a</sup>
Migori	240.83	14.83	13.38 <sup>abc</sup>	196.67	3.44 <sup>ef</sup>	3.12 <sup>ef</sup>
Mitunguu	261.58	15.00	12.33 <sup>abc</sup>	214.33	4.07 <sup>ab</sup>	3.89 <sup>bcd</sup>
Wote	277.08	16.21	16.07 <sup>ab</sup>	207.00	3.71 <sup>de</sup>	3.66 <sup>cde</sup>
Kiambere	254.17	14.88	12.33 <sup>abc</sup>	214.67	3.17 <sup>f</sup>	3.20 <sup>ef</sup>
Embu	265.25	15.00	10.96 <sup>abc</sup>	230.67	3.86 <sup>abcd</sup>	3.68 <sup>cde</sup>
Nyakoe	252.00	15.91	14.11 <sup>abc</sup>	226.00	4.05 <sup>abc</sup>	3.01 <sup>f</sup>
Oyugis	252.42	14.42	8.88 <sup>bc</sup>	259.33	3.45 <sup>ef</sup>	3.310 <sup>ef</sup>
P value	0.949 (NS)	0.987 (NS)	0.005	0.470 (NS)	<0.0001	<0.0001
SE	9.823	1.021	1.477	14.410	0.076	0.128

Means followed by the same letter are not significantly different based on Students Newman's Keuls (SNK) test at  $p \leq 0.05$ . NS = Not Significant; SE = Standard Error.

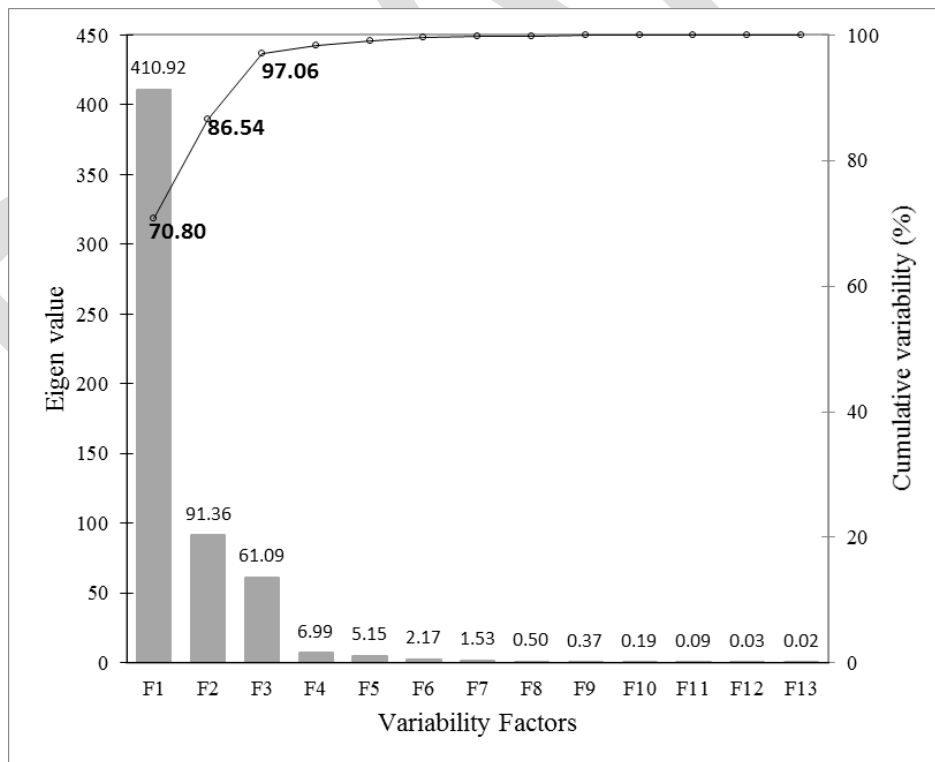


**Fig 1.** Cluster dendrogram depicting morphological diversity between horned melon accessions.

**Table 2.** Variation in agronomic traits of horned melon accessions.

Accessions	Percent Germination	Days to Emergence	Days to Male Flowering	Days to Female Flowering	Days to Maturity
Kathwana	100.00 <sup>a</sup>	11.00 <sup>a</sup>	63.67 <sup>bcd</sup>	66.17 <sup>cdef</sup>	103.00 <sup>de</sup>
Meru	90.17 <sup>abc</sup>	8.83 <sup>e</sup>	59.83 <sup>ef</sup>	63.50 <sup>fg</sup>	105.00 <sup>de</sup>
Siakago	100.00 <sup>a</sup>	9.17 <sup>de</sup>	59.83 <sup>ef</sup>	63.00 <sup>ef</sup>	104.83 <sup>de</sup>
Maragua	91.67 <sup>abc</sup>	9.00 <sup>e</sup>	62.33 <sup>cdef</sup>	65.33 <sup>fg</sup>	106.50 <sup>cde</sup>
Chuka	95.83 <sup>ab</sup>	9.67 <sup>cde</sup>	61.17 <sup>def</sup>	63.33 <sup>fg</sup>	107.17 <sup>cde</sup>
Kehancha	54.33 <sup>d</sup>	9.67 <sup>cde</sup>	58.50 <sup>f</sup>	61.83 <sup>g</sup>	101.67 <sup>e</sup>
Narok	72.33 <sup>bcd</sup>	9.67 <sup>cde</sup>	59.17 <sup>f</sup>	62.33 <sup>fg</sup>	105.00 <sup>de</sup>
Kianjokoma	91.67 <sup>abc</sup>	9.00 <sup>e</sup>	65.17 <sup>bcd</sup>	67.83 <sup>bcd</sup>	108.50 <sup>cd</sup>
Kangundo	100.00 <sup>a</sup>	8.83 <sup>e</sup>	59.50 <sup>ef</sup>	61.83 <sup>g</sup>	102.83 <sup>de</sup>
Kwale	95.83 <sup>ab</sup>	9.67 <sup>cde</sup>	63.50 <sup>bcd</sup>	66.00 <sup>cdef</sup>	104.17 <sup>de</sup>
Rongo	66.67 <sup>cd</sup>	10.00 <sup>bcd</sup>	73.50 <sup>a</sup>	74.67 <sup>a</sup>	114.83 <sup>b</sup>
Machakos	93.00 <sup>ab</sup>	10.33 <sup>abc</sup>	63.33 <sup>bcd</sup>	65.67 <sup>cdefg</sup>	106.00 <sup>cde</sup>
Migori	82.00 <sup>abc</sup>	10.83 <sup>ab</sup>	67.00 <sup>b</sup>	69.00 <sup>bc</sup>	120.50 <sup>a</sup>
Mitunguu	97.17 <sup>ab</sup>	8.67 <sup>e</sup>	60.67 <sup>ef</sup>	63.33 <sup>fg</sup>	107.17 <sup>cde</sup>
Wote	82.00 <sup>abc</sup>	9.66 <sup>cde</sup>	61.17 <sup>def</sup>	64.33 <sup>efg</sup>	105.50 <sup>de</sup>
Kiambere	93.00 <sup>ab</sup>	10.00 <sup>bcd</sup>	65.00 <sup>bcd</sup>	68.00 <sup>bcd</sup>	111.50 <sup>bc</sup>
Embu	100.00 <sup>a</sup>	10.16 <sup>abc</sup>	72.17 <sup>a</sup>	73.50 <sup>a</sup>	121.67 <sup>a</sup>
Nyakoe	95.83 <sup>ab</sup>	10.33 <sup>abc</sup>	61.83 <sup>def</sup>	64.83 <sup>defg</sup>	106.67 <sup>cde</sup>
Oyugis	75.00 <sup>abc</sup>	9.50 <sup>cde</sup>	66.00 <sup>bc</sup>	69.50 <sup>b</sup>	122.16 <sup>a</sup>
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SE	5.381	0.213	0.896	0.825	1.339

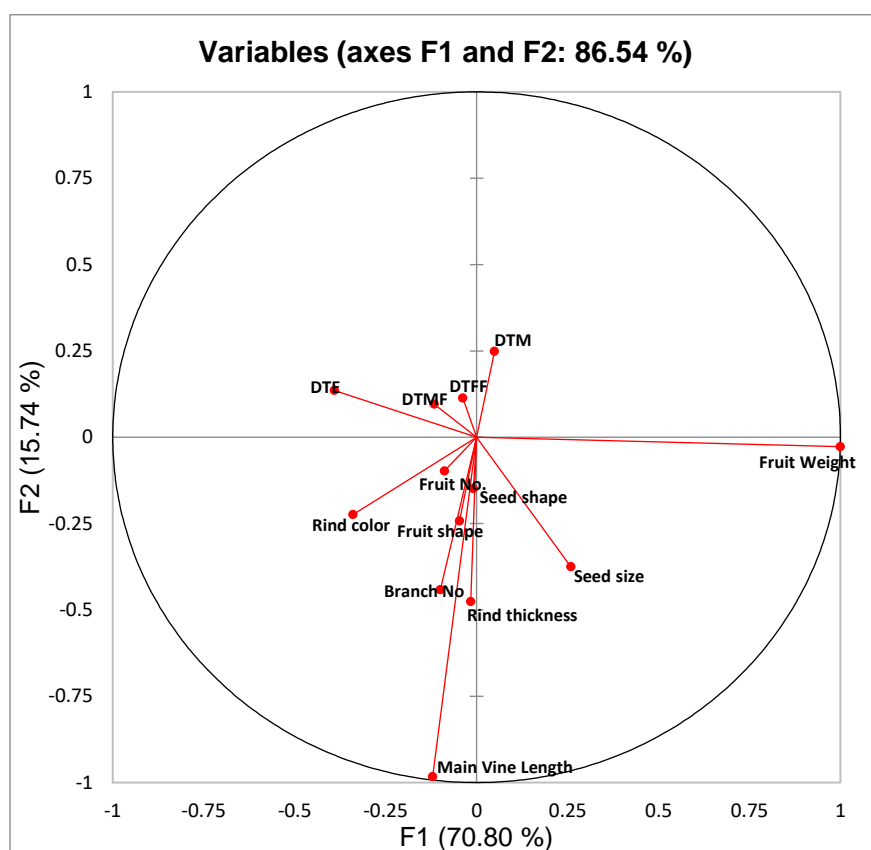
Means followed by the same letter are not significantly different based on SNK test at  $p \leq 0.05$ . SE = Standard Error.



**Fig 2.** Scree plot of eigen values corresponding to variability factors.

**Table 3.** Contribution of the variables to observed variation.

Agro-morphological Variables	Variability Factors		
	F1	F2	F3
Main Vine Length	0.322	96.152	2.728
Branch Number	0.002	0.134	0.112
Rind Colour	0.000	0.051	0.260
Fruit Shape	0.022	0.043	0.247
Seed Shape	0.001	0.134	0.003
Fruit Number	0.011	0.058	0.099
Days to Seed Emergence	0.016	0.008	0.085
Days to Male Flowering	0.054	0.162	22.369
Days to Female Flowering	0.004	0.180	18.092
Days to Maturity	0.023	2.650	55.946
Fruit Weight	99.544	0.334	0.013
Seed Size	0.001	0.013	0.016
Rind Thickness	0.000	0.081	0.030
Eigenvalue	410.922	91.356	61.095
Variability (%)	70.797	15.740	10.526
Cumulative %	70.797	86.537	97.063



**Fig 3.** Pictorial illustration of the principal component analysis.

**Table 4.** List of horned melon accessions evaluated in the study.

S/No.	Accession Code	County	Place of Collection	AEZ	GPS Coordinates
1.	ACC1	Tharaka-Nithi	Kathwana	UM	0.327201S, 37.866059E
2.	ACC2	Meru	Meru	UH	0.051541N, 37.645855E
3.	ACC3	Embu	Siakago	LM	0.577330S, 37.640544E
4.	ACC4	Murang'a	Maragua	UM	0.799959S, 37.133016E
5.	ACC5	Tharaka-Nithi	Chuka	UH	0.331004S, 37.647958E
6.	ACC6	Migori	Kehancha	UM	1.193604S, 34.615860E
7.	ACC7	Narok	Narok	LH	1.085049S, 35.877356E
8.	ACC8	Embu	Kianjokoma	UM	0.395741S, 37.503827E
9.	ACC9	Machakos	Kangundo	LM	1.299215S, 37.350726E
10.	ACC10	Kwale	Kwale	LH	4.181473S, 39.460144E
11.	ACC11	Migori	Rongo	LM	0.746204S, 34.595284E
12.	ACC12	Machakos	Machakos	LM	1.528227S, 37.263005E
13.	ACC13	Migori	Migori	UM	1.070804S, 34.472522E
14.	ACC14	Meru	Mitunguu	UH	0.108490S, 37.784815E
15.	ACC15	Makueni	Wote	LM	1.788249S, 37.633152E
16.	ACC16	Embu	Kiambere	LM	0.684613S, 37.792102E
17.	ACC17	Embu	Embu	UM	0.487726S, 37.458003E
18.	ACC18	Kisii	Nyakoe	LM	0.627900S, 34.762945E
19.	ACC19	Homabay	Oyugis	LM	0.507982S, 34.737482E

(122 days) to mature. Seasonal variations were significant for days to emergence ( $p < 0.001$ ), days to male flowering ( $p < 0.0001$ ) and days to female flowering ( $p < 0.0001$ ). These variables therefore responded differently to different seasons.

#### Correlation analysis

Relationship between the quantitative and agronomic traits was assessed using a Pearson's correlation matrix. There was positive significant ( $p < 0.05$ ) correlation between days to male flowering and days to female flowering ( $r = 0.992$ ), days to male flowering and days to maturity ( $r = 0.785$ ), as well as days to female flowering and days to maturity ( $r = 0.809$ ). All the other variable combinations (data not presented) had no significant relationship.

#### Cluster analysis

A cluster dendrogram constructed using the quantitative and qualitative traits was used to estimate the genetic diversity among the nineteen horned melon accessions. Results of the cluster analysis using the combined season morphological data are illustrated in Figure 1. The accessions separated into seven (7) clusters with a between classes diversity of 79.20% and within classes diversity of 20.80%. Accessions from Kathwana, Kehancha, Kangundo, Mitunguu, Kiambere, Kianjokoma, Kwale and Nyakoe clustered together indicating higher level of similarity between them. The second largest cluster had four accessions from Oyugis, Maragua, Meru and Siakago while Machakos, Chuka and Wote accessions clustered together. Migori and Rongo accessions clustered together in their own cluster while accessions from Embu and Narok each separated into their own singleton cluster.

#### Principal component analysis

Principal component analysis (PCA) was used to analyse the contribution of the variables to the total variation observed between the nineteen horned melon accessions. The results for the 13 variables used indicated that the first three variability factors (F1, F2 and F3) explained 97.06% of the total variation with F1 explaining variability of 70.78%, F2 with 15.74% and F3 with 10.53% of the total variation. All the other ten (10) variability factors explained only 2.94% of the total variation (Figure 2). The variables contributed differently to the observed variation with fruit weight, main vine length and days to maturity contributing the highest to F1 (99.54%), F2 (96.15%) and F3 (55.95%) respectively (Table 3; Figure 3). Days to male and female flowering also contributed significantly to F3 with 22.37% and 18.09% contribution respectively. The rest of the variables contributed minimally to the three principal factors (Table 3; Figure 3).

#### Discussion

##### Variation in qualitative traits of horned melon

Significant genetic diversity was observed between the horned melon accessions based on morphological and agronomic variation. However, most of the qualitative traits were similar in all accessions except fruit shape, seed shape and predominant rind colour. Similar observation was made by Bisognin (2002) and Gichimu et al. (2009a) who reported that cucurbits are similar in above ground development but have high genetic diversity in fruit characteristics such as fruit shape, flesh colour, seed shape and seed colour. Most of the accessions (74%) had elliptically shaped fruits while the rest (26%) had cylindrically shaped fruits. The seeds for most of the

accessions (63.16%) were also elliptically shaped while rest (36.84%) had pinonette shaped seeds. This observation concurred with earlier report by Bester and Condy (2013) that the fruits of *C. metuliferus* were ellipsoid-cylindrically shaped. The rind colour varied from light green to dark green with majority of the accessions being dark green. However, the fruit colour in all accessions changed to bright orange at ripening. Similar observation was made by Dembitsky et al. (2011) and Usman et al. (2015).

#### **Variation in quantitative traits of horned melon**

Quantitative characters that were evaluated include main vine length, number of branches on the main vine, fruit number, fruit weight, rind thickness and seed number. The former four are considered to be yield components while the latter two are quality components (Gichimu et al., 2009b). Among the yield components, only fruit number recorded significant differences among accessions; ranging from an average of 17 fruits produced by the Rongo accession to 7 fruits produced by Kangundo accession. Unfortunately, the prolific nature of the Rongo accession may have compromised the fruit weight which averaged 204 g, a far cry from 259 g recorded by Oyugis and Meru accessions. However, this could be possibly improved by enhancing the soil fertility since high yielding accessions are expected to have a relatively higher demand of essential nutrients. Apparently, *C. metuliferus* is highly variable in terms of fruit yield as different studies have reported divergent data on fruit number and weight. April et al. (2018) reported that each vine is capable of producing up to 100 fruits. In a study evaluating 26 *C. metuliferus* plant introductions in Missouri, United States, Marsh (1993) obtained fruit numbers ranging from 14 to 101 fruits per plant but with relatively lower fruit weights ranging from 12 to 31 grams.

For successful yield selection, all the yield components should be considered. Although there were no significant variations among accessions in vine length and branch number, these variables are expected to have a significant influence on yield as reported by April et al. (2018). Similar observation was also made by Deepa et al. (2018) in cucumber who also reported that the yields were strongly correlated to branch number and vine length. In the contrary and against expectations, this study observed no significant correlation between the yield components. This observation was attributed to the fact that some accessions managed to produce many fruits despite not being well endowed with long vines and many branches. Similarly, some accessions with long vines and many branches produced relatively fewer fruits. This was an indication that there is high breeding potential to improve the yields of horned melon through selective hybridization of accessions possessing desirable yield components. For example, the potential of the Rongo accession can be further expanded by increasing the vine length and branch number through hybridization with the Wote accession which was found to combine long vine length with high branch number and fruit number.

Significant differences among accessions were also recorded in seed size and rind thickness. This evaluation was important because both the seeds and the rind of horned melon are edible. The seed size ranged from 3.17 mm recorded in

Kiambere accession to 4.10 mm recorded in Maragua accession. This observation was divergent from what was reported by Wilkins-Ellert (2004) that the seeds of *C. metuliferus* are 5 – 8 mm long. All accessions had more than 100 seeds per fruit. Rind thickness also varied from 5.37 mm recorded in the Machakos accession to 3.01 mm recorded in the Nyakoe accession. There was no available data in literature comparing the rind thickness among different accessions of horned melon. There were no significant seasonal variations between accessions for all the quantitative variables indicating that the accessions were genetically stable and may not be significantly affected by genotype by environment interactions. This is a positive agronomic trait that would ensure constant productivity of the horned melon across variable weather conditions.

#### **Contribution of variables to the observed diversity**

Although there were no significant variations among accessions in fruit weight and main vine length, the PCA indicated that the two had the highest contribution to the total variation observed between the nineteen accessions studied. This was an indication that there was a high potential of agronomic selection among the accessions based on these variables. The average length of the main vine ranged from 236.75 cm recorded in the Narok accession to 277.08 cm recorded in Wote accession. This big gap between the shortest and the longest vine is an indication of the improvement potential among the accessions studied. However, the vine length obtained in this study fell short of the 5 m length reported by April et al. (2018). Similarly, high improvement potential was observed in fruit weight whose variation among accessions varied from 195.0 grams produced by Machakos accession to 259.33 grams produced by Meru and Oyugis accessions. This was slightly high than the average of 200 grams reported by Wilkins-Ellert (2004) in Israel. However, unlike vine length whose potential is a combined effect of the genetics and agronomic nourishment, fruit weight may be affected by fruit number unless the plant is adequately and timely supplied with all essential growth resources.

#### **Variation in agronomic traits of horned melon**

Unlike the growth variables, other agronomic variables namely, percent germination, days to seed emergence, days to male and female flowering and days to maturity recorded highly significant variations between accessions. This was expected as all these variables are liable to be affected by the weather especially the temperature and the accessions were all collected from different geographical locations where they may have adopted to different weather patterns. Variation in flowering time on *C. metuliferus* was also reported by Weng (2010). Most of the accessions recorded high viability of between 80 and 100% except five accessions whose germination percentage was lower than 80%. These were accessions from Kehancha, Rongo, Narok and Oyugis. This observation was consistent over the two seasons indicating that its cause could be genetically linked and acquired from the original habitat. Aliero and Gumi (2012) also observed high germination percentage averaging 96.66% with untreated seeds of horned melon. Significant positive correlation that

was observed between days to male and female flowering and days to maturity was an indication that these variables are genetically linked thus selection for one would automatically improve the others. Principal component analysis also showed that these three variables contributed significantly to the total variation observed between the accessions and therefore they can be easy targets for selective improvement of the accessions.

### **Cluster analysis**

A cluster dendrogram developed using the qualitative and quantitative traits as well as the agronomic variables indicated that there was significant genetic variation between the accessions studied. The accessions separated into seven (7) clusters with a between classes diversity of 79.20% and within classes diversity of 20.80%. This was a considerable high level of genetic diversity for accessions of the same species and further underscores the enormous selection potential that exists within horned melon accessions. There are scanty reports on the genetic characterization of *C. metuliferus* accessions in Kenya. A related study conducted by Gichimu et al. (2009a) on watermelon accessions obtained relatively lower genetic diversity of 42-54% between classes and 8-27% within classes. Other previous studies conducted by Henan et al. (2013) on muskmelon in Tunisia and Solmaz et al. (2010) on melon genotypes collected from Eastern and Central Anatolia region of Turkey also reported high morphological variation between cucurbits. In another genetic diversity study conducted by Weng (2010) among *C. metuliferus* accessions using cucumber microsatellites, unexpectedly low genetic diversity was observed between 36 accessions. The 42 microsatellite markers only managed to separate the accessions into six supported groups detecting on average 3.3 alleles across the 36 *C. metuliferus* accessions and 12 of them were monomorphic. The effect of the region of origin of the accessions was evident as most accessions clustered according to the proximity of their places of origin (where they were collected). For example, the Rongo and Migori accessions that were both from Migori County clustered together. Similarly, the Wote, Machakos and Chuka accessions all of which were from the Eastern Kenya region clustered together as it was the case with Kathwana, Kangundo, Mitunguu, Kiambere and Kianjokoma accessions which were also from Eastern Kenya region. Similar observation was reported by Weng (2010) when studying the genetic diversity of *C. metuliferus* populations using cucumber microsatellites where he noted that accessions that were collected from nearby locations were clustered in the same group. However, some accessions that were collected from distinctively diverse geographical regions clustered together which was an indication of high agronomic potential of horned melon over a wide range of environmental conditions. This observation was further supported by the fact that all the nineteen accessions with diverse geographical origin managed to produce appreciably in a common location. Therefore, there is wide agronomic range of horned melon in Kenya and this supports the previous report by Wilkins-Ellert (2004) that horned melon occurs at altitudes from near sea level to 1800 m and tolerate a wide range of soil types throughout their natural distribution area.

## **Materials and Methods**

### **Description of the study site**

The study was carried out at University of Embu research farm. The University is located along Nairobi – Meru highway in Manyatta sub-county in Embu County, Kenya. The research site lies on latitude 0° 31' 52.03" N and longitude 37° 27' 2.20" E at an elevation of 1480 m above sea level (Jaetzold et al., 2006). The area receives mean annual rainfall of about 1200 mm received in two distinct rainy seasons; the long rains (mid-March to August) averaging 650 mm and the short rains (mid-October to February) averaging 450 mm (Muthee et al., 2019). The mean annual temperature is 19.5°C, a mean maximum and minimum of 25°C and 14.1°C, respectively. The mean annual potential evaporation is 1422 mm while mean annual evapotranspiration is 950 mm (Jaetzold et al., 2006). The soils are mainly humic nitisols derived from basic volcanic rocks. They are deep, highly weathered with friable clay texture and moderate to high inherent fertility (FAO, 2011). Most farmers rely on production of crops like maize and horticultural crops such as melons.

### **Plant materials and seed preparation**

Nineteen accessions of mature horned melon fruits were collected from the wild and farmers' fields from selected counties in Kenya where the crop is grown. These counties include Meru, Embu, Makueni, Kisii, Homabay, Muranga, Narok, Machakos, Tharaka Nithi, Kwale and Migori. The materials were collected from different agro-ecological zones (AEZ) as shown in Table 4. The ripened fruits were then transported in well labelled bags to the University of Embu for extraction of seeds that were used in the establishment of the crop. The fruits were cut open with a sharp knife and the pulp carefully scooped out using clean spatula. The obtained seeds were separately fermented to remove the jelly-like substance and to soften the seed coat (McCormack, 2005). This was done in plastic containers using pure water for three days after which the seeds were sun-dried and then winnowed to separate the seeds from chaff (Aliero and Gumi, 2012).

### **Experimental design and layout**

The experiment was laid out in a Randomized Complete Block Design with three replications. The nineteen different accessions were used as treatments and were allocated experimental plots measuring 3m by 3m. A 1m wide alley was provided between plots to avoid any interference with the plants during data collection and crop husbandry. Three seeds were planted per hole at a spacing of 1m by 1m and later thinned to one seedling a week after germination. Well decomposed farm yard manure and TSP fertilizer were applied in the planting holes before sowing at the recommended rates of 30 t/ha and 200 Kg/ha, respectively. Each plot comprised of five plants and data were collected from four tagged plants. Two rows of the Embu accession were used as guard rows around the experimental field. Other agronomic practices including irrigation, pests and weeds control were done uniformly in all the plots whenever necessary. The study was



carried out in two seasons from October 2018 to January 2019 and from March to July 2019.

### Data collection

A descriptor list with 21 morphological characters was adopted from International Plant Genetic Resources Institute (IPGRI) (Díez et al., 2005; Jarret and Griffin, 2007) and was modified to suit the characterization of horned melon. Data were collected on qualitative and quantitative morphological characters of horned melon including leaf shape, growth habit, predominant rind colour, flesh colour, fruit shape, seed shape, seed size, seed colour, rind thickness, number of seeds per fruit, main vine length, number of branches per plant, fruit number per plant and average fruit weight. Data were also collected on other agronomic characters such as percent germination, days to seedling emergence, days to female and male flowering and days to maturity. Qualitative traits were scored as described in the descriptor while quantitative data were taken as an average of the measurements made on four tagged plants per replicate. A digital vernier calliper was used to measure the rind thickness of the fruits while average fruit weight was measured using a digital balance model PLT 750DXXL with a readability of 50 grams.

### Statistical analysis

Quantitative and agronomic data for the two seasons was combined and subjected to a two-way analysis of variance (ANOVA) at 5% level of significance using XLSTAT Version 2019 to test for the significant differences between accessions, seasons and interactions between accessions and seasons. Separation of means was done using Students Newman's Keuls (SNK) at 95% level of confidence. Pearson correlation coefficients were carried out between growth and yield components. Qualitative and quantitative data were then organized into a matrix and subjected to cluster analysis. Estimates of similarity among the genotypes were calculated using dissimilarity units and expressed as Euclidean genetic distance (Kaçar et al., 2012). Dissimilarity indices were used to generate a dendrogram using the unweighted pair group method with arithmetic average (UPGMA). Truncation was performed based on the classes' diversity. Principal component analysis (PCA) was also carried out using the quantitative and agronomic variables to analyse their contribution to the total morphological diversity.

### Conclusion

This study established that there exists considerably high genetic variation within horned melon which can be exploited by plant breeders for genetic improvement of this high value crop whose potential is yet to be fully utilized. The study further established that there is great breeding potential to improve the yields of horned melon through selective hybridization of accessions possessing desirable yield components. In addition, the study findings indicated that horned melon has a high agronomic potential over a wide range of environmental conditions as accessions from diverse geographical regions recorded appreciable yields in a common location. Therefore, plant breeders should embark on

improving the agronomic and quality attributes of this crop through targeted selection and hybridization. The best performing cultivars should then be promoted among farmers in all agro-ecological zones in Kenya.

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