Morphological and Agronomic Characterization of Local Vegetable Cowpea Accessions in Coastal Kenya

M.J. Hutchinson[1], F.K. Muniu[2], J. Ambuko[1], M. Mwakangalu[2], A.W. Mwang'ombe[1], J.J. Okello[3] F. Olubayo[1] and J. Kirimi[4]

[1]University of Nairobi, College of Agriculture and Veterinary Sciences, P.O. Box 29053-00625, Nairobi, Kenya
[2]Kenya Agricultural Research Institute, Mtwapa, P.O. Kilifi, Kenya
[3]International Potato Center, P.O. Box 22274, Kampala, Uganda
[4]Chuka University, P.O. Box 109-60400, Chuka, Kenya
***Correspondence:** hutchjesang@yahoo.com

Abstract

Although local cowpea (*Vigna unguiculata* L. Walp) accessions are among the most popular and nutrient-rich indigenous leafy vegetables grown by resource-poor small-scale farmers in Kenya, breeding work and research has been hampered by unavailability of such basic data as morphological and genetic characterization. The objective of this study was to select local accessions with desirable agronomic and morphological characters for commercialization as vegetable cowpeas. Twenty eight cowpeas accessions, collected in April 2012 from Kilifi and Mombasa counties within two major agro-ecological zones (AEZs), were used in this study. The accessions were planted at KALRO Mtwapa in a randomized complete block design with three replications. Morphological characterization and principle component analysis (PCA) was conducted on both the qualitative and quantitative characters. Cluster analysis was performed on the accessions according to their variability using agglomerative hierarchical clustering. All the 28 accessions had sub-globose terminal leaf shape, had coriaceous (leatherly) leaf texture, were glabrescent and had indeterminate growth pattern. The majority of the accessions were green in colour and had V-marks on leaflets. Over 70% of the cowpea accessions evaluated had intermediate to acutely erect growth habit with no or slight twining tendency. The mean number of nodes on the main branch was between 8-10 nodes per plant. The accessions flowered between 36 and 45 days after planting with one accession flowering after 52 days. Ward's Method and Euclidian Distance produced three clusters, showing a perfect correspondence with the morphological classification of the evaluated accessions. The first five principal components (PCs) accounted for about 81.10% of the total genetic variation. However, the PC1 and PC2 could discriminate the evaluated cowpea accessions, suggesting that the accessions may wide adaptability and stability for most of traits evaluated, for instance drought resistant.

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is among the most popular indigenous vegetables consumed in Kenya as grain and leaf especially among resource-poor communities in coastal region of Kenya (Muli and Saha, 2000). Cowpea has been produced mainly for its protein-rich grains and is popularly consumed with cereal foods. Its ability to withstand drought, short growing period and multi-purpose use make it a very attractive alternative crop for farmers in marginal,

drought-prone areas with low and erratic rainfall (Hellensleben et al., 2009). However, despite the numerous benefits, cowpea just like other ALVs, is still viewed as a 'woman's' crop and has therefore not received adequate attention from researchers and policy makers (Abukutsa-Onyango, 2010). To compound this challenge, farmers have maintained a strong preference for local accessions that are said to be more palatable despite their low yields compared to the improved varieties such as KVU and K-80 (Hutchinson et al., 2016, KARI, 2010). Availability of quality seed and breeding of these local accessions is limited by unavailability of basic data of characterization and evaluation (Muli and Saha, 2000).

Although cowpea is a single crop species, there are several variations in plant type, seed color, time taken to maturity among others (Singh et al., 1997). Traditionally, genetic diversity evaluated in crop species are based on differences in morphological characters and qualitative traits (Schut et al., 1997), probably due to the fact that the assay of qualitative traits do not need any sophisticated equipment or complex experiments, they are generally simple, rapid and inexpensive to score. Morphological traits continue to be the first step in the studies of genetic relationships in most breeding programmes (Cox and Murphy, 1990; Van Beuningen and Busch, 1997). Morphological attributes are often employed in establishing phylogenetic relationships among accessions or genotypes between and within species and for various other purposes including correlation of characteristics of agronomic importance. Characterization of genetic diversity among cowpea accessions is important in developing superior cultivars worldwide. Diversity is usually estimated by measuring variation in phenotypic and qualitative traits such as flower colour, growth habit, or quantitative traits such as yield and stress tolerance (Kameswara, 2004).

A study on genetic diversity of Kenyan cowpea accessions collected from different agro-ecological zones showed a relatively low level of genetic diversity among cowpea accessions in the country (Kuruma et al., 2010). Diversity has been used as a powerful tool in the classification of cultivars and also to study taxonomic status (Gbaguidi et al., 2013). To our knowledge, no characterization has been conducted for local cowpea accessions in Kilifi and Mombasa counties of Kenya. The objective of the study was therefore to collect, characterize the morphological traits and evaluate performance of popular local cowpea accessions collected from 2 agro-ecological zones in Kilifi and Mombasa counties of Coastal Kenya.

Materials and Methods

Site Description: The research was conducted at Kenya Agricultural and Livestock Research Organization (KALRO) Mtwapa in Kilifi County in coastal lowland in Kenya. The area has an altitude of 30 m above sea level., a longitude of 39^{o} 45'East, a latitude of 4^{o} South and a high relative humidity of more than 80%. Coastal lowland 3 is characterized by semi-humid conditions with an annual rainfall of 800-1400 mm and temperature range of 24-30^{o}C. Lowland 4 is characterized by semi-humid to semi-arid conditions and a rainfall of 600-1100 mm/year and average temperatures of about 24-32^{o}C. The rainfall in Mtwapa is bimodal with the long rains starting in April/May up to August. Short rains start in October and extend to December. The soils are sandy with pH in the range of 5.3 to 6.9.

Collection and Characterization of Cowpea Accession: Thirty two local cowpeas accessions were collected from farmers in Kilifi and Mombasa counties within two major agro-ecological zones, Coastal Lowland 3 and Coastal Lowland 4 in April 2012. Out of the

32 accessions planted, 28 were selected for accession evaluation in this study. The four discarded were found to be duplicates. The collected accessions were planted at KALRO Mtwapa Research station. Morphological characterization was carried out using International Board of Plant Genetic Resources descriptors (1983) for cowpeas.

Table 1: Number of local cowpea accessions collected per location and agro-ecological zone in coastal Kenya.

County	**Sub-county**	**Ward**	**Agro-ecological zone**	**Number of Accessions studied**
Kilifi	Malindi	Kanyangwa	CL4	2
	Magarini	Fundisa	CL4	3
	Magarini	Kambi waya	CL4	1
	Malindi	Nguruni	CL3	1
	Malindi	Lango Baya	CL4	14
	Ganze	Bamba	CL4	2
		Ganze	CL4	3
		Vitengeni	CL4	2
	Bahari	Roka	CL3	1
	Chonyi	Chasimba	CL3	6
	Kikambala	Junju	CL3	6
	Kikambala	Gongoni	CL3	1
Mombasa	Likoni	Mtongwe	CL3	2

Experimental design: The experiment was laid out in a randomized complete block design with three replications. The field was ploughed and harrowed so as to achieve a moderate tilth. The 28 accessions were planted on 4 m x 3 m plots with a spacing of 60 cm x 30 cm, giving a plant population of 67 plants per plot. Ten plants were randomly selected to constitute experimental plants.

Morphological Characterization: Data were collected on 18 traits according to the International Board of Plant Genetic Resources (IBPGR, 1983) cowpea descriptors at different stages of growth. The following morphological characteristics were recorded: growth habit, growth pattern, twinning tendency, terminal leaflet shape, terminal leaf length, leaf colour, leaf marking, hairiness on leaves, leaf texture, plant vigour, number of branches, number of nodes, plant height, canopy width, root length and number of days to flowering.

Determination of Fresh and Dry Weights: Fresh and dry weights were determined for single and multiple harvests. For single harvest, nine plants were uprooted six weeks after planting. For multiple harvest leaves from nine plants were harvested weekly from the 6^{th} to the 12^{th} week. The total was weights were computed for the six harvests. The fresh leaves were oven dried for 72 hours at 57^{o}C and weighed. Seed yield was determined from plants whose leaves were not harvested.

Data Analysis: The agronomic data were analysed using SAS (SAS Version 9) and means separated using Least Significant Difference (LSD) at 5% level of significance. The data was also subjected to cluster analysis using the Number Cruncher Statistical System, NCSS, 2000 (Hintze, 1998). In the process of hierarchical clustering, the un-weighted pair

group method of arithmetic average (UPGMA) was employed.

Results and Discussion

Morphological Characterization of Cowpea Accessions: The growth habit of cowpeas is an important attribute in agronomic practices to be adopted by farmers (Bennet-Lartey and Ofori, 1999). Of all the local accessions tested in the current study, 39%, 39%, 11%, and 7% had acute erect, intermediate, semi-erect and erect growth habits, respectively. The erect accessions have been reported to have potential for good returns in high intercrop adaptability and high reproductive efficiency (Cobbinah et a., 2011). All the 28 local accessions had sub-globose terminal leaf shape, coriaceous (leathery) leaf texture and were glabrescent. While only 1 accession had pale green leaves, the rest 27 had intermediate to dark green ones, suggesting good capacity for photosynthetic activity and resultant high yields and good quality.

Most of the local accessions (17; 61%) had no twining tendency, nine (32%) had slight twining tendency and only two (7%) had intermediate tendency indicating that staking in their management would not be necessary. Similar results were obtained from 134 accessions tested from 8 regions of Ghana (Cobbinah et al., 2011) Of these 28 local cowpea accessions, the majority (22) were very vigorous in growth habit while the other 4 and 2 were rated vigorous and intermediate an indication that it has capacity to be developed for high leaf yield.

The mean number of nodes was between 8-10 nodes per plant. Three accessions had 8 nodes, 21 had 9 while 4 had 10 nodes on the main stem. The number of days from emergence to 50% flowering varied with accessions. Four accessions flowered 36 days after sowing. Eleven flowered 41 days after sowing while 3 flowered after 44 days. One flowered 45 days after sowing, 6 flowered after 47 days while the last accession flowered after 52 days. The mean value of around 42 days to 50% flowering recorded in the present study suggests an element of early flowering by the 28 local accessions studied. Similar results were reported on local Ghanaian cowpea accessions that flowered after 39.5 days after planting (Cobbinah et al., 2011). Both Erskine and Khan (1978) reported heterosis for earliness with early maturity being dominant over late maturity (Mak and Yap, 1980).

There were significant differences in plant height, canopy width, root length, number of branches, number of leaves number of nodes and number of days to 50% flowering among the 28 local cowpea accessions evaluated. The cowpea accession 'Mrahai' had the shortest plants at 39.8cm, while Mnyenze had the tallest at 231 cm (Table 2). 'Usimpe mtu' had the smallest plant canopy of 69.3 cm and the shortest roots (13.2 cm) while 'Mnyenze' had the widest plant canopy of up to 490 cm, the longest roots of 87.5 cm and the largest number of branches (32) and number of leaves (314). Depending on availability of resources such as water and fertilizers, some accessions could respond better than others while some could be potential crops for drought tolerance studies. These results indicate that there may be little genetic variations between the accessions in these attributes although environmental factors may be a factor when say the number of branches are compared to those of accessions studied in Ghana (Cobbinah et al., 2011) where accessions had less than 5 branches.

Table 2: Cowpea morphological characteristics: Plant height (cm), Canopy width, root length, number of branches, number of leaves, number of nodes 6 weeks after sowing and Days to flowering of 28 local cowpea accessions in Coastal Kenya

Accession	Plant height	Canopy width	Root length	Number of Branches	Number of leaves	Number of Nodes	Days to 50% Flowering
Usimpe Mtu Mkubwa	65.2	114.8	21	7	79	9	41
Mwandatu	107.3	217.8	40.4	15	181	9	45
Mwakipipi	48	89.3	18	8	76	9	47
MM-01	63.3	109	18.3	6	77	8	36
Mrahai	39.3	89.6	14.9	6	59	9	47
MM-03	132.4	210.6	44.2	21	255	10	47
Kiringongo Mawe	59.4	108.4	18	7	84	9	36
Kunde Kubwa	63.5	120.2	20.2	8	88	9	36
VT-01	52.1	83.8	15.9	7	79	9	41
MM-05A	69.6	117.6	20	10	97	9	41
Usimpe Mtu	39.8	69.3	13.2	5	53	9	41
Mnyenze Madamada	67.2	114.3	20.9	9	101	10	44
VT-02	59.5	106.6	19.2	8	90	9	36
Katsetse	57.3	102.1	18.8	8	98	10	41
MM-05B	61.9	111.6	20.1	9	86	9	41
Kunde za Kigiriama	63	114.1	18.7	8	87	9	41
Charika	40	70.5	17	7	70	9	47
MtsemerI	108.3	157.4	45.2	21	141	8	44
Katatariko	60.3	111.9	18.5	7	76	9	41
Mnyenze	231	489.9	87.5	32	314	9	45
Mesonje	53	101.1	19.3	7	73	9	47
MLB-07	154.8	352.8	63.5	21	214	9	44
Sura Mbaya	60	119.9	17.5	8	89	10	52
MG-01	157.5	324.5	51.1	19	221	9	41
Usimpe Mtu Mdogo	139.3	290.9	47.3	23	197	9	47
MLK-02	58.1	135.8	19.1	7	93	9	41
MLB-06	56.8	113.2	21.3	7	79	9	41
KVU	129.6	186.7	37.7	17	160	8	52
LSD	**16.5**	**46.1**	**33.2**	**1.6**	**22.1**	**0.71**	**0.18**

The accessions had between 8 and 10 nodes per stem with the majority (21) having 9 on the main stem. Earlier studies on cowpeas using morphological traits such as plant pigmentation, plant habit, root traits, leaf traits, pod traits, seed traits, grain quality, and yield have been carried out by many researchers. Marechal et al. (1978) used morphological diversity to study taxonomic relationships between genotypes belonging to the genera

Phaseolus and *Vigna*. Obute (2001) used morphological traits (plant height, number of leaves, leaf length, the number of pods per plant, pod length and number of seeds per pod) to characterize an aneuploid *Vigna unguiculata* from the other cytotypes. These traits were all found to be of great importance to distinguish genetic variability, and have led to a better classification of cowpea genotypes (Magloire, 2005). Qualitative traits, such as yield performance and quality characters are of major importance in breeding and consequently, these traits are usually focused on during the evaluation of accessions. However, these traits express strong environmental effects, and often also genotype with environment interaction. The variety 'Mnyenze madamada' had the highest fresh weight yield of 8.1 tonnes/ha from single harvests while Mesonje had the highest fresh weight of 23.3 tonne/ha from multiple harvests (Table 3). The highest dry matter yield was recorded in variety Katsetse (1tonne/ha) from single harvest while Sura Mbaya accumulated 2.7tonnes /ha from multiple harvests. Other varieties which performed well in fresh and dry leaf yield are MLK 02, 'Mnyenze' and 'Sura Mbaya'. Some of the coded accessions that performed very well and yet could not be verified by the farmers include MM-01, MM-03, MM-05B, MLB06, MLB-07, MLK-02, MG-01, VT-01 and VT-02 and could be compared with the KALRO-Genebank accessions collected from Kilifi and Kwale namely GBK032384, GBK032386, GBK032394 GBK032396, GBK040520, GBK032331, GBK032338, GBK032344, GBK032362 (Kuruma et al., 2010).

Principal Component Analysis: Principal component analysis (PCA) reduced the original set of twenty one variables to five (Table 4). Proportions of variations were based on the first five PC axes, which had Eigen values of 1 or greater than 1. Principal components (PCs), which indicated about 81.10% of the total genetic variation were in five PCs. The first three PCs, explained 64.84% of total variation, whereby the first PC accounted for 31.44%, the second 22.70% and the third 10.69%. The principal component one (PC 1) had the largest Eigen-value (5.345) and accounted for the greatest amount of variance in the original data, while PC 2 accounted for the greatest amount of variation in the residual variation, which was unaccounted for by the first principal axis. Principal component 3 accounted for the greatest amount of variation in the residual variation unaccounted for by PC2. The same process unfolded for principal axes 4 and 5.

The variables with high scores (>0.20) on PC 1 were quantitative traits: plant height, canopy width, root length, number of branches and number of leaves. . The variables with highest scores on PC 2 were: single harvest fresh weight, single harvest dry weight, multiple harvest fresh weight and multiple harvest dry weight. Hence, the variables with high coefficient in the first and second PCs were considered the most relevant as they explained over half of the total variation. Plant vigor had Eigen vector of >0.60 on PC 5. Plant vigour had the highest eigen vector of 0.609 in PC 5 as shown with bold in Table above. The characters with Eigen vectors of 0.60 and above are considered very important for their large effect contribution to variation.

Table 3: Fresh and Dry Leaf Yield for Single and Multiple Harvests of 28 Local Cowpea Accessions at KALRO Mtwapa

Accession Name	Single harvest Fresh weight (tons/ha)	Single Harvest (Dry Weight (tons /ha)	Cumulative Multiple Harvest-Fresh Weight (tons/ha)	Cumulative Multiple Harvest-Dry Weight (tons/ha)
Usimpe Mtu Kubwa	5.2	0.7	18	2.4
Mwandatu	2.8	0.3	11.4	1.4
Mwakipipi	5.9	0.7	20.8	2.5
MM-01	5.4	0.7	18.1	2.1
MRAHAI	3.7	0.4	9.5	1.3
MM-03	5.7	0.7	17.7	2.2
Kiringongo Mawe	6	0.8	16.5	2.4
Kunde Kubwa	6.3	0.8	18.7	2.3
VT-01	5.3	0.7	15.1	2.1
MM-05A	3.6	0.4	9.3	1.1
Usimpe Mtu	4	0.6	13.2	1.8
Mnyenze Madamada	8.1	0.9	19.5	2.4
VT-02	5.4	0.6	17.6	2.3
Katsetse	7.9	1	22.5	2.7
MM-05B	5.6	0.6	18.7	2.5
Kunde Za Kigiriama	5.7	0.8	17.1	2.3
Charika	3.5	0.4	14.8	1.9
Mtsemeri	3.9	0.5	18.1	2.1
Katatariko	5.7	0.7	18.6	2.3
Mnyenze	6.5	0.7	17.7	2.2
Mesonje	6	0.7	23.3	2.7
MLB-07	6	0.7	21.5	2.6
Sura Mbaya	6.5	0.7	22.7	2.7
MG-01	6.4	0.7	17.7	2.4
Usimpe Mtu Mdogo	6.2	0.7	20	2.6
MLK-02	7.3	0.6	20.6	2.5
MLB-06	4.9	0.6	17.5	2.3
KVU	4.7	0.5	17.1	2.2
LSD	**1.63**	**0.22**	**5.7**	**0.72**

Table 4: Principal Component Analysis (PCA) of 28 vegetable cowpea accessions evaluated in coastal Kenya

PCA analysis	PC 1	PC 2	PC 3	PC 4	PC 5
Eigen values	5.345	3.860	1.818	1.527	1.237
Explained proportion of variation (%)	31.443	22.704	10.693	8.984	7.279
Cumulative proportion of variation (%)	31.443	54.147	64.839	73.823	81.102
TRAITS	**EIGEN VECTORS**				
Leaf marking	-0.039	-0.016	0.434	-0.066	0.353
Leaf Colour	0.131	-0.037	0.491	0.220	0.039
Growth habit	-0.040	-0.162	0.018	0.432	0.364
Plant Vigour	0.200	0.085	0.137	-0.102	**0.609**
Twinning tendency	0.094	-0.198	-0.041	-0.551	-0.125
No. of nodes	0.000	0.255	-0.483	0.136	0.062
Number of days to flowering	0.118	-0.065	-0.060	0.575	-0.323
Terminal leaf length	0.006	0.099	0.504	-0.080	-0.487
Plant height	0.422	-0.092	-0.018	-0.037	-0.022
Canopy width	0.414	-0.075	-0.031	-0.087	-0.017
Root length	0.416	-0.105	-0.003	-0.004	-0.037
Number of branches	0.411	-0.111	-0.014	0.056	-0.047
Number of leaves	0.413	-0.089	-0.147	0.025	0.020
Single harvest Fresh weight	0.136	0.457	-0.056	-0.085	0.044
Single Harvest Dry Weight	0.061	0.463	-0.048	-0.192	-0.004
Multiple Harvest-Fresh Weight	0.133	0.432	0.127	0.142	-0.044
Multiple Harvest-Dry Weight	0.124	0.438	0.119	0.117	-0.020

The characters on PC 3 which had high coefficient (>0.20) scores were: leaf marking (0.43), terminal leaf length (0.5) and leaf colour (0.49) The fourth axis (PC 4) had the highest coefficient for growth habit (0.43), and leaf colour (0.22). The fifth axis (PC 5) had the high scores leaf marking (0.35) growth habit (0.36) and plant vigour (0.6).

Terminal leaflet shape, leaf texture, growth pattern and hairiness on leaves had no variation. The variables in the table above are the ones which contributed to the variations seen in the cowpeas accessions. Accessions with high PC1 scores could be good genitors for diversity. The rich diversity within the accessions provides more selection chances in breeding.

Clustering based on Qualitative and Quantitative Characters of cowpeas accessions: The associations of qualitative and quantitative characters with the PC axis were obtained and given as their factor scores or Eigen-vectors. These factor scores are used to construct scatter plot for the (Figure 2). A combination of both quantitative and qualitative characters contributed a variation of 81.70% in five PCs. The first two PCs explained 54.02% of total variation, whereby the first PC accounted for 40.34%, the second 13.67%.

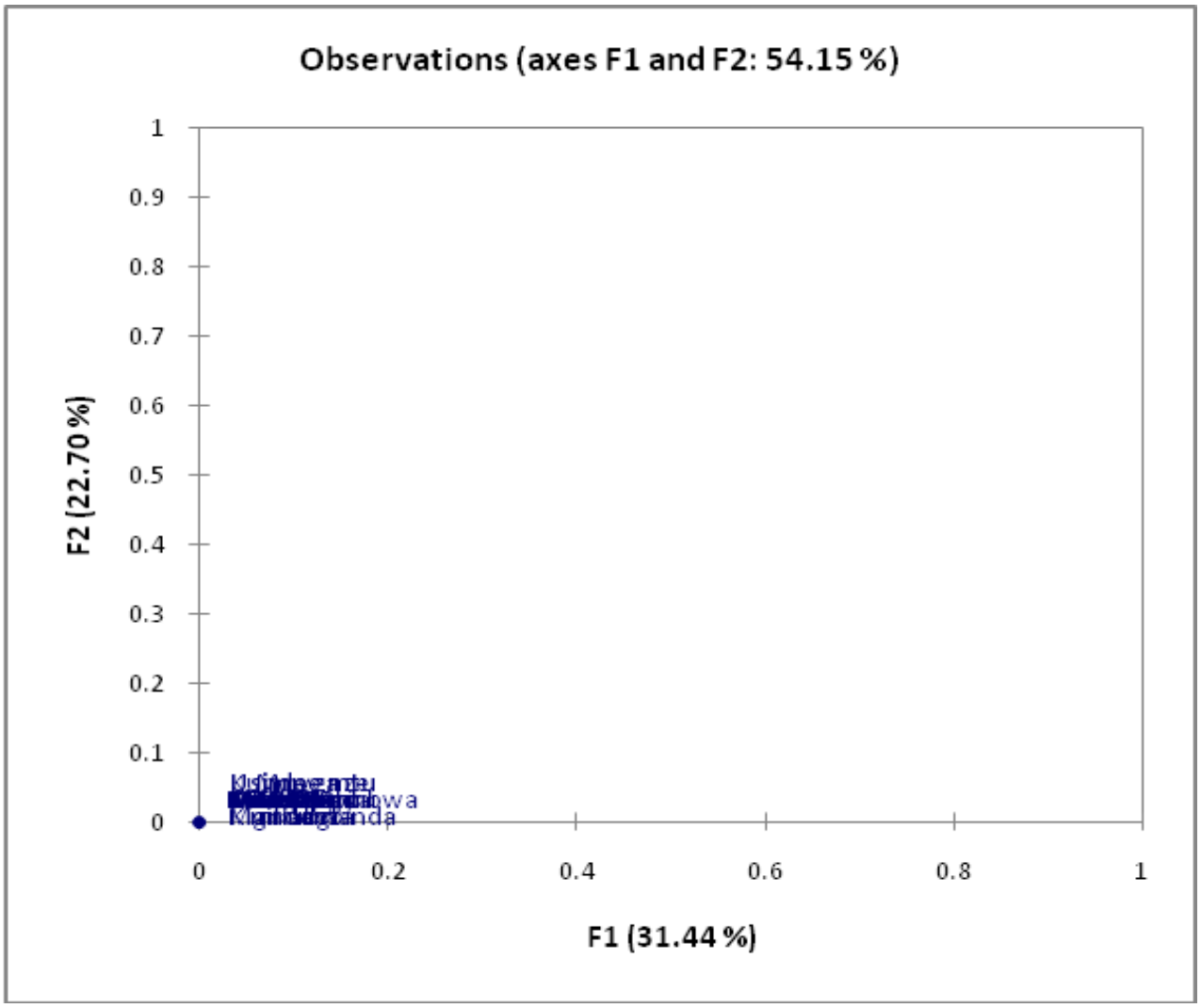

Figure 2: Factor scores for the first two principal components (PCs), showing their distribution in PC 1 and PC 2. (Both qualitative and quantitative characters; F1 and F2, 54.02%).

Cluster analysis further helps to group the genotypes on the basis of morpho-genetic traits. The distribution of the accessions on the PC 1 and PC 2 plot, show that morphological variation among the cowpeas does not exist. The characters of those clustered close together are not different. They are densely clustered on the negative end of the F1 axis. Dense clustering on the bi plot shows close relationships with little or no variation. Principal component analysis showed that morphological variation among the cowpea accessions evaluated does not exist, which means the good traits for instance drought resistant are still retained within the accessions.

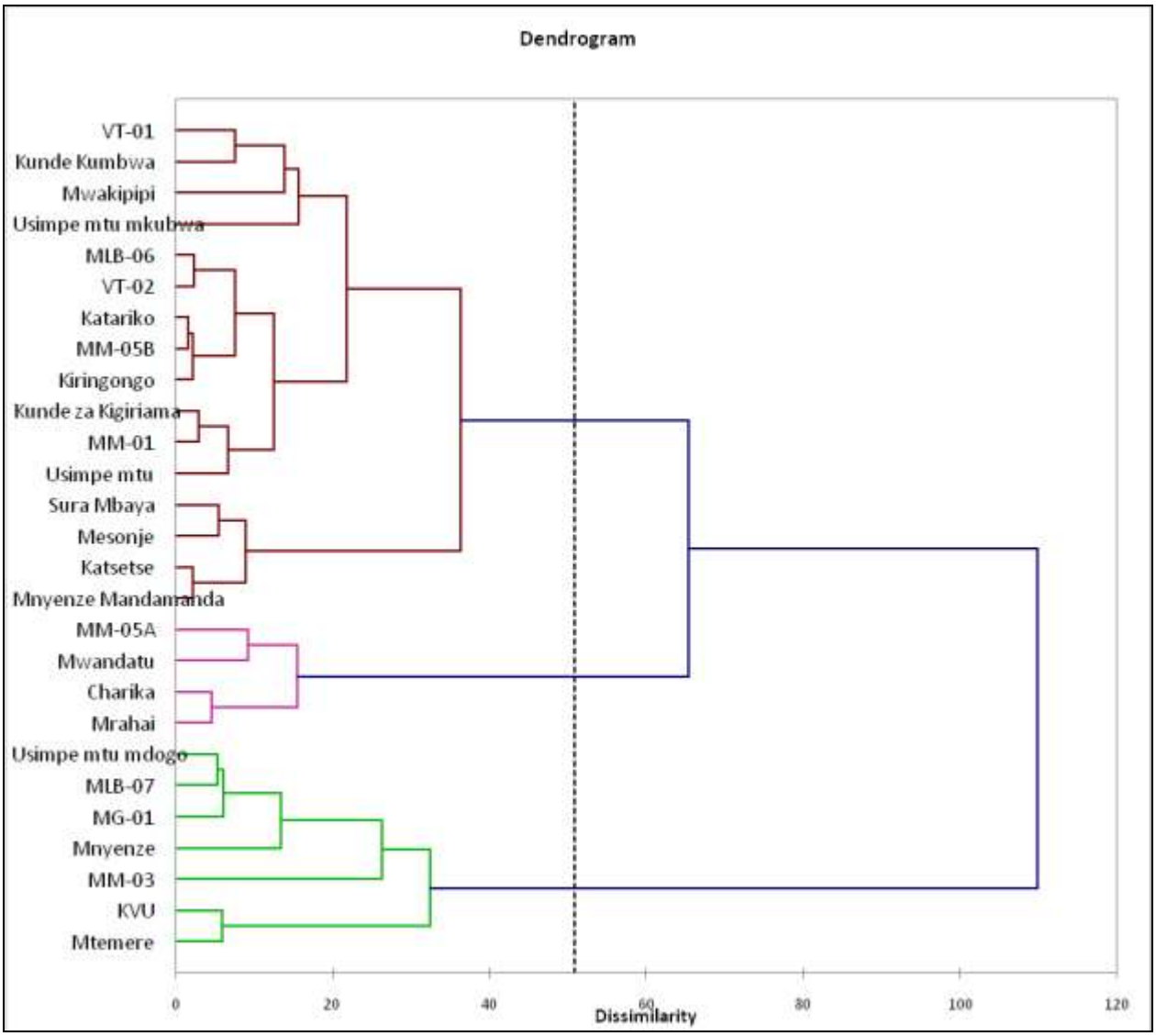

Figure 3: Dendogram based on quantitative and qualitative traits of 28 Kenyan cowpea accessions evaluated at KALRO-Mtwapa

Cluster analysis performed on the accessions according to their variability using agglomerative hierarchical clustering, Ward's Method and Euclidian Distance produced three clusters. An examination of the dendrogram does not show a perfect correspondence with the morphological classification of the accessions analysed, an indication that the accessions are fairly similar genetically. Similarly, there was no distinction among the accessions collected from the 2 Agro-ecological zones.

In conclusion, most of the 28 local cowpea accessions collected from farmers in the 2 agro-ecological zones in coastal part of Coastal Kenya, exhibited vigorous growth with acute/intermediately erect habits with no or slight twining tendency. Although differences were noted in some morphological and agronomic indicators studied, clustering of the accessions indicated no genetic variations among them.

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Supplementary Materials

Table S1: Morphological characteristics: Growth habit, Growth pattern, Twining tendency, Terminal leaflet shape, Leaf colour, Leaf markings, Leaf texture and Hairiness on leaves of 28 local cowpea accessions from Coastal Kenya

Growth habit		**Growth pattern**		**Twining tendency**		**Terminal leaflet shape**		**Leaf marking**		**Leaf texture**		**Hairiness on leaves**		**Leaf Colour**	
Trait	**#**	**Trait**	**#**	**Trait**	**#**	**Trait**	**#**	**Trait**	**#**	**Trait**	**#**	**Trait**	**#**	**Trait**	**#**
Acute erect	11	Indeterminate	28	None	17	Sub-globose	28	Absent		Coriaceous	28	Glabrescent	28	Intermediate green	20
Intermediate	11			Slight	2			Present	26					Dark green	7
Semi-erect	4			Intermediate	9									Pale green	1
Erect	2														