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Genetic Diversity of Pumpkin Accessions in Kenya Revealed Using Morphological Characters, Diversity Index, CATPCA and Factor Analysis

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Abstract

Pumpkin is one of the most morphologically variable genera in the entire plant kingdom. In Kenya, its genetic diversity is undocumented and distribution is haphazard. An expedition was done in Kakamega and Nyeri regions in 2012 using purposive sampling and IPGRI descriptors that led to collection of 155 accessions planted and replicated three times in the Chuka University experimentation farm. The character ranges were green to orange for mature fruit rind, speckled to striped secondary fruit rind, smooth to warty fruit surface, and white to yellow internal flesh, and yellow to pink-red inner flesh and outer flesh. Sex type was monoecious, with most flowers being male and flowering early; only 9 accessions had female flowers appearing early. Most accessions had globular fruits and second fruit cycle. All the accessions had fruit vein tracks and peduncles that abscised when overripe. Deep fruit ribbing was in 40, while small blossom scars were in 69 accessions. Shannon diversity index based on qualitative traits ranged 0.49 to 1.79, with average of 0.97. Fruit shape and seed coat surface displayed high and low indices, respectively. Nyeri accessions had the highest diversity index. CATPCA, factor and cluster analysis determined relationships of the accessions based on the dissimilarity of qualitative characters. CATPCA and factor analysis reduced the dimensionality of the characters to 13 PCs and factors, respectively. CATPCA captured 78.3% and factor analysis 72.1% of the total variation. The two methods jointly identified second fruiting cycle, central leaf lobes, leaf pubescence type, leaf glossiness, and plant growth habit, leaf and flower colour contributing most to divergence of the accessions. The communalities were mostly high except for few characters exhibiting high specificity. Configuration by scatter Bi-plot along the first two PC axes grouped 124 accessions into variegated and green-leafed. Cluster analysis identified four groups with 59, 40, 24 and 1 accessions in clusters one, two, three and four, respectively. The green-leafed accessions were grouped in cluster three and four, and the variegated into cluster one and two. The characters with high discrimination can be useful in identifying variation that can be used for direct selection and in assisting breeders in the identification of pumpkin germplasm with desirable traits for inclusion in breeding and improvement programmes.

Key words: Accessions, Morphological qualitative characters, Categorical Principal Component Analysis, Kakamega, Nyeri

INTRODUCTION

Pumpkin belongs to the family Cucurbitaceae (Jeffrey, 1990). It is one of the most

morphologically variable genera in the entire plant kingdom (Aruah *et al*., 2010). Its genetic diversity and distribution are essential for rational utilization in crop improvement (Padmini *et al*., 2013). Distinction of pumpkin cultivars is easiest by observing fruit shape, size, stalk, stems and leaves (Paris, 2000). Fruits are variable in size, colour and shape (Robinson and Decker-Walters, 1997). Morphological qualitative traits provide information on genetic variability, identification and classification (Lima *et al*., 2012), and determine divergence of pumpkins to a greater degree (Borges, *et al*., 2011). The traits are also used to assess variation between and within accessions (Balkaya *et al*., 2010). These diagnostic features are useful in assessing relationships (Radford 1986), by measuring, counting, differentiating and documenting various morphological characteristics (Xolisa, 2002), using the minimum list of descriptors (Kristkova *et al*., 2003) among pumpkin accessions. The process makes available information collected to prospective breeders and end users that lead to development of new cultivars as well as strengthening the existing ones (Xolisa, 2002).

Principal component analysis (PCA) reveals patterns of variation, eliminates redundancy and identifies unknown trends in a multi-dimensional data set (Maji and Shaibu, 2012). Categorical Principal Component Analysis (CATPCA) is performed when variables are measured on a nominal or ordinal scale. These variables show a fixed a priori order and nonlinear relationships. This method is the nonlinear equivalent of standard PCA, and reduces the observed variables to a number of uncorrelated principal components (Linting *et al.,* 2007). The use of linear or standard PCA is not appropriate, only after linearity in nominal or ordinal variables has been verified (Vilela *et al*., 2015). The most important advantages of nonlinear over linear PCA are that it incorporates nominal and ordinal variables, and that it can handle and discover nonlinear relationships between variables. Also, non-linear PCA can deal with variables at their appropriate measurement level. Every observed value of a variable is referred to as a category. CATPCA converts every category to a numeric value, in accordance with the variable's analysis level, using optimal quantification (Linting *et al.,* 2007). Categorical quantifications of categorical variables by optimal scaling ensures the overall variance accounted for in the transformed variables, given the number of components is maximized. This ensures information in the original categorical data is retained, depending upon the optimal scaling level chosen for each variable separately (Vilela *et al*., 2015). Factor analysis identifies the underlying relationships that exist within a set of variables. Large datasets are reduced into groups of factors that underlie the quality of characteristics of the original variables (Yong and Pearce, 2013). The dimensionality of measurable and observable variables is reduced by regrouping the variables into a limited set of descriptive categories and clusters, and to fewer latent variables that share a common variance and are unobservable. These make it easier to focus on key factors rather than having to consider too many variables that may be trivial. It also summarizes the data into relationships and patterns that can be easily interpreted and understood (Yong and Pearce, 2013).

Cluster analysis (CA) is used in assessing genetic diversity (Lima *et al*., 2012; Maji and Shaibu, 2012). CA group's accessions showing dissimilarity in several traits by displaying similarity or differences between pairs of subjects (Goda *et al*., 2007). CA also defines homogeneous subgroups of a given measure of dissimilarity or similarity from heterogeneous sets of items (Downs and Barnard, 2002). The ward's clustering method is useful in producing desirable compact clusters (Zewdie and Zeven, 1997). The unweighted pair group method of arithmetic averages (UPGMA) minimizes within cluster variance (Hintze, 2001). The Euclidean distance measures dissimilarity and partitions genotypes into exclusive groups according to genetic distance (Lima *et al*., 2012). Kenya being a secondary centre of genetic diversity has a wide array of pumpkin genotypes that

require detailed characterization (Karuri *et al*., 2010). They are referred as “orphaned”, which connotes that they receive very little research and development attention (Naluwairo, 2011). Presently, no information is available that can be used to delineate and standardize the pumpkin accessions in Kenya (Ahamed *et al.,* 2011; Isutsa and Mallowa, 2013; Mwaura *et al*., 2014; Kiharason et al., 2015). The present study was undertaken to characterize phenotypically pumpkin accessions collected among smallholder farmers in Kakamega and Nyeri regions of Kenya. This was aimed at providing useful information on pumpkin accessions qualitative morphological characteristics based on their phenotypic differences, PCA, Shannon diversity index and their dissimilarity using Phylogenetic analysis.

MATERIALS AND METHODS

Research Site

The collected accessions (155) from Kakamega and Nyeri were planted on 23rd May, 2012, in a Complete Randomized Design (CRD) in three replications at Chuka University (CU) farm. The farm lies at 0^o 19` S, $37^o$ 38` E and 1535 m above sea level. Rainfall is about 1,200 mm annually and bimodally distributed. Annual mean temperature is about 20^oC. Soils are mainly humic nitisols, deep, well weathered with moderate to high inherent fertility (Jaetzold & Schmidt, 1983). Land was ploughed and pulverized to fine tilth. Planting holes measuring 2 ft squared on the top and a depth of 2 ft were dug. During digging, top soil was separated from the subsoil. Top soil was then mixed thoroughly with 24 kg of well decomposed farm yard manure (FYM) and returned back to the hole without the subsoil. A six inch unfilled portion of the hole was left (Muyekho *et al*., 2003) to act as a basin for water holding during irrigation to avoid wastage. These holes referred as “*Tumbukiza*”, were spaced at 2m x 2m. Five plants/ accession were planted in each hole using rainfall. Twenty litres of water was applied in two splits in each hole using a calibrated watering jar when there were no rains. Chemical sprays to control insects and diseases were applied. Moles destroying the accessions were trapped manually. Weeding was done during all stages of the accessions growth.

Data Analysis

Data recording on vegetative characteristics began 20 days after emergence up to fruit maturity. Five plants per accession and a total 775 plants were selected and tagged, for morphological data recording using IPGRI descriptors (IPGRI, 2003). Qualitative characteristics were recorded of leaf, stem, root and inflorescence on 146 accessions, and on 126 and 124 accessions on fruit and seed traits, respectively. Each accession represented a research plot, and at fruit maturity each of the accessions were harvested separately. The colour of fruits, leaves, stems and flowers of the accessions were determined using a colour chart. Qualitative data values were numerically coded and expressed as modes and frequencies. Frequency of mode indicated variation within accessions. Statistical Analysis System generated modes and frequencies.
Shannon diversity index (HS) was used to determine accessions richness and abundance (Equation 1) (Shannon, 1983). Evenness was calculated using the ratio of Shannon diversity index and the natural logarithm of species richness (Equation 2) (Aruah *et al*., 2010).

Shannon Diversity Index $(HS) = \sum_{i=1}^{S} - [(p_i)\ (\ln\ _i)]$ ……….…………………………………………………1

Where: Pi = relative abundance of species, S = number of species in sample and ln = natural logarthm:

Evenness **(J)** = **HS/ln S**……..…….……..…….2

Where: HS = Shannon diversity index and ln S = natural logarithm of the Species richness.

Qualitative characters that contributed significantly to the total variation of the accessions were identified using CATPCA. The CATPCA estimated correlations between the qualitative characters, and generated Eigen values, percentage of variation accumulated by the principal components (PCs), the corresponding load coefficients and the scatter bi-plots. CATPCA was used to extract maximum variance from qualitative data set with each component reducing the large number of variables into smaller number of components. Factor analysis (FA) was used to reduce the measured and observed variables to fewer latent variables that shared a common variance, in order to reduce the dimensionality of variables to the most discriminating qualitative characters. This enabled regrouping of the variables into a limited set of clusters. Factor analysis also helped summarize qualitative data into relationships and patterns that could be easily interpreted and understood (Yong and Pearce, 2013).

The number of PCs and factors that were retained was based on the Eigen value greater than one criterion. The Eigen values were used to estimate the relative contribution of each PC to the total variance of all the variables. The load coefficients were used to generate the correlations of each variable with its relevant PC. The internal consistency of qualitative data was tested using Cronbach's alpha. The data with Cronbach's alpha greater than 0.7, indicated an acceptable internal consistency (Sartipi *et al.,* 2016). Qualitative characters with load coefficients and factor load rotations ≥ 0.20 in the correlation matrix were considered significant contributors to the variability of the accessions and were considered to be highly relevant to that PC or factor. The significant level for retaining discriminative qualitative character was $\alpha = 0.05$. CATPCA and factor analysis were performed using SPSS version 16. Cluster analysis generated dendrograms to infer relationships among accessions based on Euclidean distance matrices, ward and UPGMA methods. Cluster analysis was performed using XLstat.

RESULTS

Morphological Qualitative Characters

All the accessions had leaf veins, large sized leaves, dense and intermediate leaf pubescence adaxial and abaxial, and roots at the internodes. The leaves were pentalobate with cordate base and shallow lobes. Leaf outline was broadly ovate and very broadly ovate in 108 and 38 accessions, respectively. The accession leaves were variegated in 102 and plain green leafed in 44 accessions, leaf senescence when fruits matured moderate in 88, and conspicuous and concurrent in 44 accessions. The flowers of all accessions were monoecious, with most being male and appearing early, than female flowers. Female flowers appeared early in only 9 accessions. The colour of flowers was orange, and the predominant fruit shape globular in most accessions (Table 1).

The second fruiting cycle was observed in 99 accessions, with 27 accessions showing no sign of second fruit cycle. Predominant fruit skin ranged from green to orange and secondary skin pattern from speckled to stripe. The fruit surfaces at maturity were either smooth or wart. Internal fruit flesh colour ranged from white to yellow, and main and outer layer flesh from yellow to salmon (Table 1). Fruit vein tracks and peduncles abscising when overripe, unwinged seeds and no seed coat pattern was observed in all accessions. Deep fruit ribbing, small blossom scars and ease of seed and placenta separation from

the fruit, and elliptic seeds, intermediate in glossiness with tubercular surfaces was observed in most accessions. Predominant seed coat colour ranged from white to cream yellow. The seeds were either sharply or bluntly pointed at the hilum end.

Table 1: Qualitative morphological traits displaying the highest distribution frequency of accessions

Characters	Score code	Descriptor state	Frequency
Flower colour	2	Yellow-cream	1
	3	Yellow	4
	4	Dark-yellow	40
	5	Orange	101
Fruit shape	1	Globular	38
	2	Flattened	13
	4	Elliptical	22
	5	Pyriform (pear-like))	12
	6	Ovate	24
	7	Acorn	12
	8	Elongate	5
Predominant fruit skin colour	3	Cream	7
	4	Pale green	11
	5	Green	45
	6	Dark green	43
	7	Blackish-green	10
	10	Grey	10
Secondary fruit skin colour	2	Light-yellow	58
	3	Cream	11
	4	Pale green	10
	5	Green	2
	8	Orange	41
	10	Grey,	4
Design produced by secondary fruit skin colour	1	Speckled (spots <0.5 cm)	15
	2	Spotted, blotchy (spots >0.5 cm)	61
	3	Striped (bands run from peduncle to blossom scar)	8
	4		27
	5	Short streaked (elongated marks and <4 cm)	15
		Long streaked (as 4 but >4 cm),	
Fruit surface	1	Smooth	39
	2	Grainy	9
	3	Finely wrinkled	11
	4	Deeply wrinkled	2
	5	Shallowly wavy	50
	6	Rare warts	7
	7	Numerous warts	8
Blossom end shape	1	Depressed	33
	2	Flattened	23
	3	Rounded	60

	4	Pointed	10
Stem end shape	1	Depressed	31
	2	Flattened	56
	3	Rounded	11
	4	Pointed	28
Main color of flesh	2	Yellow	54
	4	Pale green	1
	7	Orange	61
	8	Salmon	10
Predominant seed coat colour	1	1 white	2
	2	2 yellow-white	28
	3	3 cream yellow	70
	5	5 light brown or tan	12
	6	6 brown	14

NB - Only qualitative characteristics with more than three descriptor states are displayed in Table 1

Shannon Diversity Index

The genetic diversity of the accessions based on morphological qualitative traits was 0.91 and 1.05, in Kakamega and Nyeri accessions, respectively, with a mean of 0.97 in both regions. The diversity ranged from 0.49 to 1.79, with fruit shape and seed coat surface displaying the highest and lowest indices, respectively. Highest diversity (HS) and evenness (J) were observed in fruit and seed characters. The diversity was positively correlated to evenness. Fruit shape and surface, and predominant fruit skin colour indicated high diversity as attributed to their high evenness (Table 2).

Table 2: Shannon diversity index and evenness of pumpkin accessions based on qualitative traits

Character	Kakamega accessions		Nyeri accessions		Kakamega and Nyeri accessions	
	HS	J	HS	J	HS	J
Fruit shape	1.78	0.43	1.72	0.42	1.79	0.37
Predominant fruit skin colour	1.35	0.33	1.60	0.39	1.51	0.31
Secondary fruit skin colour	1.20	0.29	1.39	0.34	1.31	0.27
Primary colour of immature fruit	0.90	0.22	1.03	0.34	0.99	0.21
Secondary colour of immature fruit	0.86	0.21	0.90	0.22	0.90	0.19
Fruit skin glossiness	1.04	0.25	1.09	0.26	1.07	0.22
Fruit surface	1.13	0.27	1.59	0.39	1.53	0.32
Fruit ribbing	1.15	0.28	1.07	0.26	1.09	0.23
Blossom scar appearance	0.90	0.23	0.96	0.23	0.93	0.19
Blossom scar size	0.80	0.19	0.93	0.2	0.89	0.18

				3		
Blossom end shape	1.11	0.27	1.24	0.3		
				0	1.22	0.25
Stem end shape	1.26	0.30	1.24	0.3		
				0	1.25	0.26
Fruit stem/peduncle colour	1.01	0.24	0.99	0.2		
				4	1.0	0.21
Main colour of flesh	0.95	0.23	0.85	0.2	0.95	0.20
				1		
Flesh colour of outer layer	0.79	0.19	1.03	0.2	096	0.20
				5		
Seed shape	0.90	0.22	1.06	0.2	1.0	0.21
				6		
Predominant seed coat colour	1.14	0.28	1.16	0.2		
				8	1.19	0.25
Seed glossiness	0.96	0.23	1.08	0.2		
				6	1.03	0.21

*** Only morphological qualitative characters with diversity index values above 0.9 are displayed in Table 2*

Categorical Principal Component Analysis (CATPCA)

Total Cronbach's alpha was greater than 0.7 in PC 1 and 2 (Table 3). The characters with Eigen values greater than one were reduced to 13 PCs that explained 78.2% of the total variation. The first four PCs with eigen-values greater than 2.0 explained more than half of total variation (Table 3). The first PC had the highest Eigen-value and accounted for the greatest amount of total variation (35.5%) in the original data (Table 3). It was highly and positively loaded with second fruiting cycle, central leaf lobes, leaf glossiness, leaf pubescence type, leaf senescence and predominant seed coat colour. It was highly and negatively loaded with plant growth habit, leaf and flower colour (Table 4). The second PC 2 accounted for 6.4% of the residual variation unaccounted for by PC 1, and was highly and positively loaded with predominant and secondary fruit skin colour, and primary colour of immature fruit. It was highly and negatively loaded with seedling vigour, plant size, blossom scar appearance and size. The third PC accounted for 5.0% of the residual variation unaccounted for by PC 2, and was highly and positively loaded with fruit shape. It was highly and negatively loaded with blossom scar appearance and size. The fourth PC accounted for 4.4% of the residual variation unaccounted for by PC 3, and was positively loaded with seedling vigour and earliness of male flowers. It was highly and negatively loaded with earliness female flowers (Table 3 and 4). The same process unfolded for PC 5 up to 13 (Balkaya *et al*., 2010). The first PC was defined by plant, leaf, flower, fruit and seed characters, PC 2 and PC 3 were mainly delineated by fruit characters, while PC 4 was mainly outlined by flower characters (Table 4).

Table 3: Categorical principal components, Cronbach's alpha, Eigen values and percent variation

Principal components (PCs)	Cronbach's Alpha	Total (Eigen value)	% Proportion of Variance	Cumulative variance %
PC 1	1.0	16.7	35.5	35.5
PC 2	0.7	3.0	6.4	42.0
PC 3	0.6	2.3	5.0	46.9
PC 4	0.5	2.1	4.4	51.3

PC 5	0.5	1.9	4.1	55.4
PC 6	0.4	1.7	3.7	59.1
PC 7	0.4	1.6	3.3	62.5
PC 8	0.3	1.5	3.1	65.6
PC 9	0.2	1.3	2.8	68.3
PC 10	0.2	1.3	2.1	71.1
PC 11	0.2	1.2	2.2	73.6
PC 12	0.1	1.2	2.4	76.0
PC 13	0.0	1.0	2.1	78.2
Total		36.7	78.2	

Table 4: Categorical principal component loadings (Eigen vectors) for PCs with Eigen values ≥ 2

Characters	PC 1	PC 2	PC 3	PC 4
Seedling vigour	0.08	**-0.41**	-0.05	**0.51**
Plant growth rate before flowering	-0.13	**-0.34**	-0.08	**0.44**
Plant growth rate after flowering	**-0.61**	-0.06	**-0.28**	0.07
Plant growth habit	**-0.96**	-0.02	0.01	-0.02
Plant size	0.07	**-0.47**	**-0.20**	**0.44**
Number of nodes	**-0.25**	0.01	0.10	-0.07
Internode length	-0.03	**-0.23**	-0.11	-0.03
Stem colour	**-0.61**	**0.35**	-0.11	0.04
Leaf outline	**0.86**	0.13	0.00	0.03
Central leaf lobe shape	**0.99**	0.05	-0.03	0.00
Leaf pubescence type	**0.99**	0.04	-0.04	0.00
Leaf colour	**-0.98**	-0.05	0.01	-0.02
Leaf glossiness	**0.97**	0.04	-0.05	-0.03
Leaf senescence	**0.96**	0.02	-0.01	0.01
Earliness of male flowers	-0.08	0.19	**-0.29**	**0.68**
Earliness of female flower	0.15	-0.14	**0.34**	**-0.67**
Flower colour	**-0.94**	-0.01	0.03	0.00
Fruit shape	**0.29**	0.01	**0.48**	**0.38**
Fruit size	**-0.33**	-0.12	-0.15	-0.13
Fruit size variability	**-0.51**	-0.17	-0.03	**0.22**
Second fruit cycle	**0.99**	0.04	-0.03	0.00
Predominant fruit skin colour	-0.14	**0.71**	**-0.35**	-0.02
Secondary fruit skin colour	**-0.32**	**0.57**	**-0.22**	0.14
Primary colour of immature fruit	-0.17	**0.66**	**-0.32**	-0.02
Secondary colour of immature fruit	**-0.40**	-0.18	-0.01	-0.12
Fruit skin glossiness	**-0.32**	-0.09	0.19	0.07
Secondary skin colour	**-0.71**	0.02	0.18	0.05
Fruit surface	**0.88**	0.11	0.07	0.06
Fruit ribbing	**-0.53**	**-0.30**	-0.14	**-0.28**
Shape of fruit ribs	**-0.20**	0.06	**0.26**	-0.07
Vein track colour	0.06	0.04	**-0.27**	-0.11
Blossom scar appearance	**0.32**	**-0.40**	**-0.57**	-0.18
Blossom scar size	**0.21**	**-0.42**	**-0.68**	-0.16
Blossom end shape	**0.43**	-0.12	**0.29**	0.13
Stem end shape	**-0.21**	**0.26**	**0.31**	**0.21**

Fruit stem peduncle colour	0.02	**0.24**	-0.03	-0.03
Fruit stem peduncle length	**-0.70**	0.03	-0.10	0.01
Internal colour of skin	-0.07	**0.46**	**-0.26**	-0.01
Main colour of flesh	**-0.55**	0.10	-0.01	-0.04
Flesh colour of outer layer	**-0.61**	0.06	-0.14	-0.04
Seed size	**0.67**	0.01	-0.19	0.08
Seed shape	**0.68**	0.18	-0.08	0.03
Seed shape at hilum end	0.08	0.18	**0.22**	0.08
Predominant seed coat colour	**0.98**	0.04	-0.03	0.00
Seed coat surface	**-0.88**	0.07	0.13	0.06
Seed surface glossiness	**0.83**	0.06	0.00	-0.08
Number of seeds per fruit	**-0.43**	-0.18	-0.17	-0.05

Configuration of Accessions

The configuration along the first two PCs (1 and 2) grouped the accessions into two distinct groups (Figure 1). On the positive end of PC 1 axis after the truncation line are the green leafed accessions, and on the negative end of PC 1 are the local variegated accessions. The local variegated accessions KK-63, KK-51, NY-94, NY-95 and NY-117 on the positive end of PC 2 axis, and KK-60, KK-46 and NY-144 on the negative end of PC 2 axis, and green leafed KK-19 on the positive end of PC 1 of the configuration were placed a distant away from the other accessions (Figure 1). The accessions were mainly discriminated on the basis of plant growth habit, second fruiting cycle, leaf outline, central leaf lobes, leaf glossiness, leaf pubescence type, leaf senescence, leaf and flower colour, fruit surface, predominant and secondary fruit skin colour, fruit stem peduncle length, predominant seed coat colour, seed coat surface and seed surface glossiness.

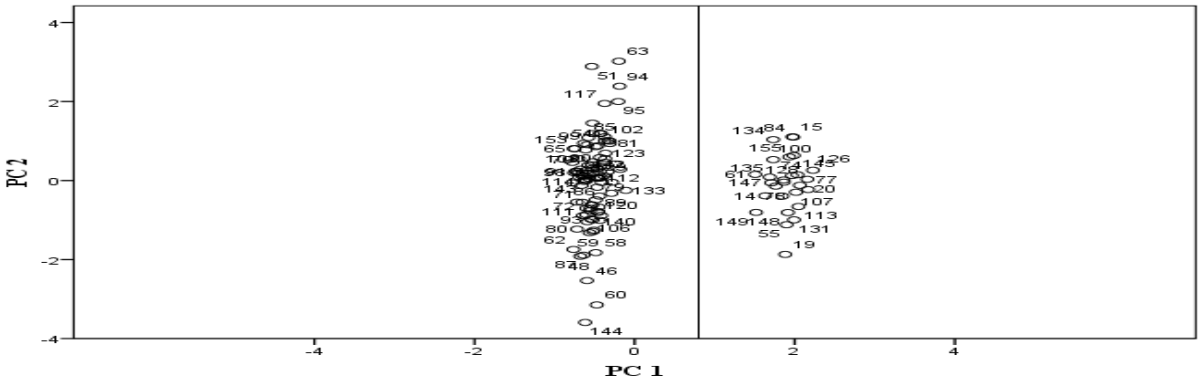

Figure 1: Scatter bi-plot showing the distribution of 124 accessions under PC 1 and 2 axis, 1-70 – Kakamega (KK) and 71-155- Nyeri (NY) - 1 - 155 accessions codes

Factor Analysis

Factor analysis based on principal components reduced qualitative traits into 13 factors, which explained 72.1% of total variation. Three factors with eigen-values ≥ 2.0 explaining more than half of total variation were retained (Norman *et al*., 2011). The degree of association of qualitative characters within the first three factors was given as factor scores. This information was used to construct three dimensional ordinations for morphological qualitative traits that explained 38.7 % of the total variation (Figure 2).

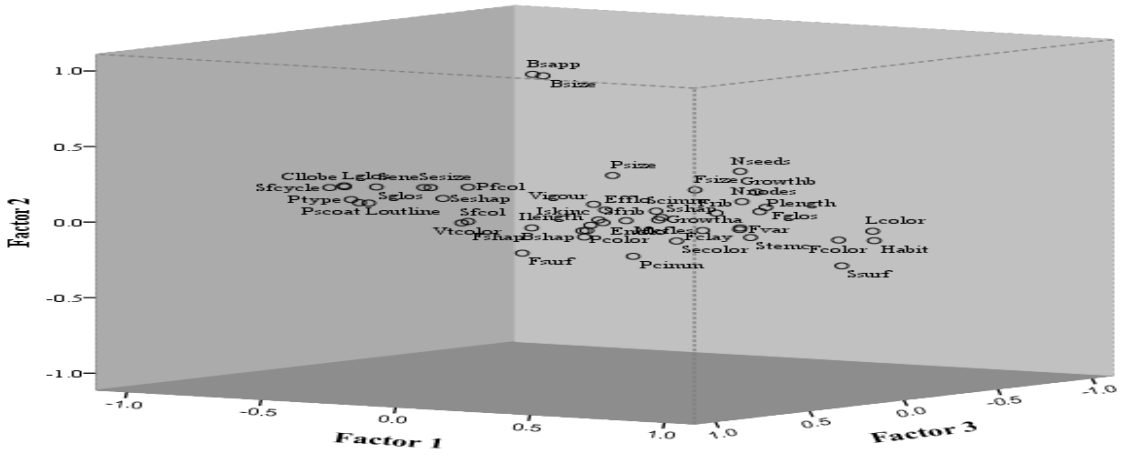

Figure 2*:* Three dimensional ordination for morphological qualitative traits contributing 38.7 % of the total variation. Growthb-plant growth rate before flowering; Growtha- plant growth rate after flowering; Vigour-seedling vigour; Psize- plant size; Habit-plant growth habit; Stemc-stem colour; Loutline-leaf outline; Cllobes=central leaf lobes; Ptype-leaf pubescence type; Nnodes-number of nodes; Ilength-internode length; Lcolor-leaf colour; Lglos-leaf glossiness; Sene-leaf senescence; Emflo-earliness of male flowers; Efflo-earliness of female flowers; Fcolor-flower colour; Fsize-fruit size; Fshap-fruit shape; Sfcycle-second fruit cycle; Pfcol-predominant fruit skin colour; Sfcol-secondary fruit skin colour; Pcimm-primary colour of immature fruit; Scimm- secondary colour of immature fruit; Fglos-fruit skin glossiness; Sfcol-secondary skin colour; Fsurf-fruit surface; Frib-fruit ribbing; Sribs-shape of fruit ribs; Vtcolor-vein track colour; Bsapp-blossom scar appearance; Bsize- blossom scar size; Bshap-blossom end shape: Sshap-stem end shape; Pcolor-fruit stem peduncle colour; Plength-fruit stem peduncle length; Iskinc-internal colour of skin; Mcfles-main colour of flesh; Fvar-fruit size variability; Fclay-flesh colour of outer layer; Seshap-seed shape; Shilum-seed shape at the hilum end; Secolor-predominant seed coat colour; Ssurf-seed coat surface; Sglos-seed surface glossiness; Nseeds-number of seeds per fruit

The first factor accounted for the greatest amount (29.3%) of total variation in the original data. It was highly and positively loaded with plant growth habit, leaf and flower colour, and seed coat surface, and was highly and negatively loaded with second fruit cycle, central leaf lobes, leaf glossiness, leaf pubescence type, leaf outline, leaf senescence and predominant seed coat colour. The second factor accounted for 4.8%, and was highly and positively loaded with blossom scar size and appearance. The third factor accounted for 4.6% of the total variation, was moderately and positively loaded with secondary fruit skin colour, fruit vein track colour and fruit surface, and moderately and negatively loaded with fruit skin glossiness (Table 5). The communalities were all high except for number of nodes, internode length and fruit skin glossiness which exhibited low communality and high specificity. The first factor was mainly associated with plant, leaf, flower, fruit and seed characters. Factor 2 and 3 were mainly delineated by fruit characters (Table 5).

Table 5: Factor loadings for the first three factors with Eigen values ≥ 2, communality and specificity of qualitative characters

Characters	F 1	F 2	F 3	Communalities	Specificity
Seedling vigour	-0.07	0.06	-0.05	0.78	0.22
Plant growth rate before flowering	0.14	-0.03	-0.08	0.81	0.19
Plant growth rate after flowering	**0.64**	0.23	0.11	0.65	0.35
Plant growth habit	**0.95**	-0.09	-0.08	0.92	0.08

Plant size	0.00	0.25	-0.04	0.69	0.31
Number of nodes	0.24	0.06	-0.38	0.47	**0.53**
Internode length	0.08	-0.03	0.19	0.45	**0.55**
Stem colour	**0.60**	-0.08	0.08	0.65	0.35
Leaf outline	**-0.86**	0.00	0.07	0.81	0.19
Central leaf lobe shape	**-0.92**	0.11	0.07	0.89	0.11
Leaf pubescence type	**-0.91**	0.02	0.05	0.86	0.14
Leaf colour	**0.94**	-0.02	-0.07	0.91	0.09
Leaf glossiness	**-0.92**	0.10	0.06	0.88	0.12
Leaf senescence	**-0.83**	0.11	0.03	0.78	0.22
Earliness of male flowers	0.06	-0.03	0.09	0.71	0.29
Earliness of female flower	-0.03	0.02	-0.04	0.80	0.20
Flower colour	**0.85**	-0.09	-0.03	0.79	0.21
Fruit shape	-0.29	-0.12	-0.02	0.68	0.32
Fruit size	0.29	0.18	-0.07	0.72	0.28
Fruit size variability	**0.53**	-0.04	0.04	0.60	0.40
Second fruit cycle	**-0.98**	0.09	0.07	0.98	0.02
Predominant fruit skin colour	-0.38	0.17	0.18	0.74	0.26
Secondary fruit skin colour	-0.05	0.04	**0.69**	0.64	0.36
Primary colour of immature fruit	0.41	-0.17	0.43	0.73	0.27
Secondary colour of immature fruit	0.28	0.07	0.13	0.83	0.17
Fruit skin glossiness	0.21	-0.01	**-0.56**	**0.47**	**0.53**
Secondary skin colour	0.38	-0.11	0.16	0.56	0.44
Fruit surface	0.08	-0.17	**0.55**	0.52	0.48
Fruit ribbing	0.48	0.07	0.09	0.74	0.26
Shape of fruit ribs	0.20	0.01	0.17	0.63	0.37
Vein track colour	-0.06	0.04	**0.64**	0.51	0.49
Blossom scar appearance	-0.30	**0.89**	-0.05	0.91	0.09
Blossom scar size	-0.23	**0.90**	0.00	0.88	0.12
Blossom end shape	-0.16	-0.18	-0.12	0.78	0.22
Stem end shape	0.21	0.00	0.00	0.71	0.29
Fruit stem peduncle colour	-0.08	-0.11	0.00	0.69	0.31
Fruit stem peduncle length	**0.67**	0.11	0.13	0.62	0.38
Internal colour of skin	0.07	-0.01	0.13	0.73	0.27
Main colour of flesh	0.48	-0.03	0.17	0.70	0.30
Flesh colour of outer layer	**0.56**	-0.02	0.09	0.79	0.21
Seed size	**-0.65**	0.12	0.03	0.62	0.38
Seed shape	**-0.56**	0.06	0.06	0.66	0.34
Seed shape at hilum end	-0.09	-0.12	-0.04	**0.75**	**0.25**
Predominant seed coat colour	**-0.89**	-0.02	-0.02	0.82	0.18
Seed coat surface	**0.84**	-0.26	-0.06	0.79	0.21
Seed surface glossiness	**-0.71**	0.10	-0.09	0.62	0.38
Number of seeds per fruit	**0.49**	0.33	-0.03	0.60	0.40

Phylogenetic analysis

Cluster analysis clustered the accessions into 4 groups. Group I (GI) and GII consisted of 59 and 40 variegated accessions, GIII and GIV consisted of 24 and 1 green leafed accession, respectively (Figure 3).

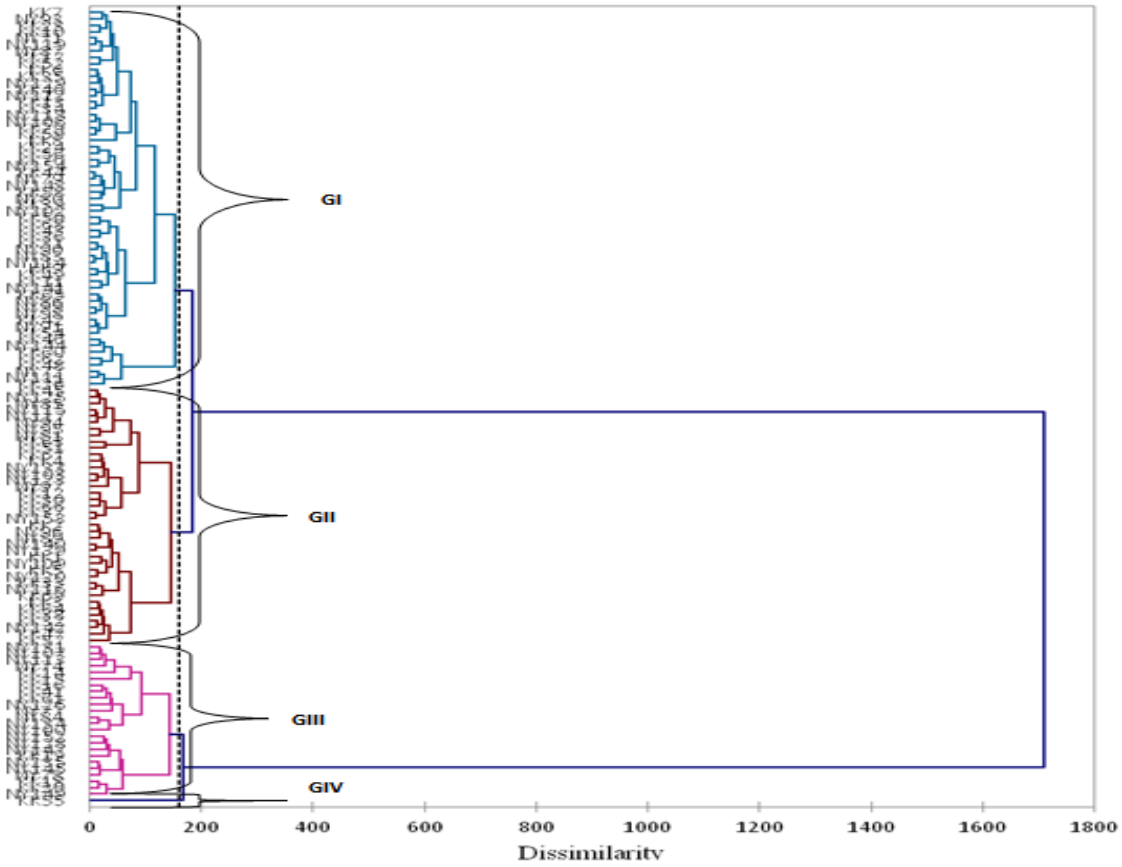

Figure 3: A dendrogram portraying relationships among 124 accessions using qualitative characters. G = Group I–IV.

The dissimilarity among the accessions was attributed mainly to stem colour, leaf glossiness, leaf senescence; earliness of male and female flowers, flower colour; fruit shape, predominant and secondary fruit skin colour, primary and secondary colour of immature fruit, fruit skin glossiness, secondary skin colour, fruit surface, fruit ribbing and shape of the ribs, fruit vein track colour; blossom scar appearance and size, blossom and stem end shape, fruit stem peduncle colour and length, internal colour of skin, main and outer layer colour of flesh, separation of seed and placenta from the fruit, seed shape, seed shape at the hilum end, predominant seed coat colour, seed coat surface and glossiness. Group I (GI) accessions were delineated by stem colour, earliness of female flowers, primary and secondary colour of immature fruits, secondary skin colour, fruit ribbing, internal colour of skin, main and outer layer colour of flesh. They were further clustered into three sub groups. Group II (GII) were differentiated by flower colour, fruit skin glossiness, shape of the ribs and seed coat surface, and further clustered into two sub groups. Group III (GIII) were qualified by leaf glossiness, fruit shape, fruit stem peduncle colour, seed shape at the hilum end and predominant seed coat colour, and were further divided into three sub groups. In group IV (GIV), one green leafed accession was clustered in simplifolious. These accession was delineated by leaf senescence; earliness of female flowers, predominant and secondary fruit skin colour, fruit surface, fruit vein track colour; blossom scar appearance and size, blossom and stem end shape, fruit stem peduncle length, separation of seed and placenta from the fruit, seed shape and seed surface glossiness.

DISCUSSION

a) Phenotypic Variation of the Accessions

i. Vegetative, stem and root characters

The growth habit in most of the accessions was bushy and multilateral. Maynard (2007)

reported multilateral branches forming at nodes and following the same general pattern of growth as the main stem. Multilateral branching creates more locations for flowering and fruit development (Gichimu *et al*., 2008). The variations in internode length among accessions was attributed to genetic effects which induce reduced internode length and shorter vines “bush”, or “semi-bush”, or “restricted vine” (Maynard, 2007). The stems in most accessions were dark-green. Ajuru and Okoli (2013) reported light-green, highly pubescent stems. All the accessions had tendrils at the nodes. Saboo *et al.* (2013) reported branched tendrils arising in the axial or opposite to the leaf at the node. All the accessions had roots at the internodes. Internodal roots improve nutrient absorption (Aderi *et al*., 2011).

ii. Leaf characters

Most of the accessions had soft leaf pubescence. Ajuru and Okoli (2013) reported highly pubescent, hairs forming a cushion on the adaxial surface of leaves. Agbagwa *et al*. (2007) reported large pilose leaves, and Nesom (2011) moderately hirsutulous to puberulent and moderately villous to unicellular-based hairs. The leaves were variable with green and silvery variegation in most of the accessions. Xiaohua *et al*. (2011) reported leaf colour ranges from light-green to dark-green. Ajuru and Okoli (2013) reported light-green leaves, and Agbagwa *et al*. (2007) white blotched leaves. The variegation in leaves of pumpkins is controlled by dominant M gene for silver-gray areas in axils of leaf veins, which are dominant to *m* for absence of silver-gray (Paris and Brown, 2005). The silvering is caused by air spaces within the palisade cell layer and between that layer and the epidermis (Brown, 2002). Leaf senescence among accessions was slightly visual, moderately, to conspicuous when fruits matured. Precocious yellowing of leaves, under certain environmental conditions is caused by *B* (*Bicolor*) genes. Dominant selective suppressor of *B (Ses-B)* gene prevents leaf yellowing in the presence of B (*Bicolor*) gene (Brown, 2002). The slight to moderate senescence of local variegated accessions was attributed to segregation of *Ses-B* with age of plants as the fruits matured (Brown, 2002). Conspicuous concurrent senescence, just after flowering was observed in green leafed accessions. Early senescence is a genetically programmed self-attrition program accompanied by recycling of nutrition released during degradation of macromolecules such as proteins (Gan, 2014), and due to the presence of *B* (*Bicolor*) genes (Brown, 2002). The leaves were large, pentalobate and cordate, broadly to very broadly ovate in all the accessions. Saboo *et al.* (2013) reported large leaves shallowly 5 lobed, alternate broad, palmately-veined and reticulate, with long hollow petiole and different shapes. Agbagwa *et al*. (2007) reported large, cordate and shallowly 5-lobed leaves, and Ajuru and Okoli (2013) simple, alternate, broadly ovate, roughly serrate, broadly cordate and palmately lobed leaves. Leaf shape and margin characteristics are useful in distinguishing cultivated *Cucurbitaceae* from others (Agbagwa and Ndukwu, 2004).

iii. Inflorescence characters

The flower colour was orange in most accessions. Ahamed *et al.* (2011) reported yellow flowers in all accessions of *Cucurbita moschata*. The flower colour variations are attributed to genetics (Maynard, 2007), and are important in classification of plants (Nesom, 2011). The accessions were all monoecious. McCormack (2005) reported monoecious plants in the four major domesticated species of cucurbits, with staminate flowers developing, maturing, and shedding their pollen before the female flowers. Most male flowers appeared early compared to female flowers. Maynard (2007) and. Agbagwa *et al.,* (2007) also reported similar results. Earliness of female flowers was recorded in only nine accessions. McCormack (2005) reported earliness of female flowers in summer squash varieties planted early. It is not uncommon for *Cucurbits* to bear female flowers

first (OECD, 2012). Flowering in cucurbits is influenced by climactic and hormones produced within the plant as well as synthetic growth regulators. Gibberellins promote male flowers, while ethylene, natural and synthetic auxins promotes pistillate flowers. High temperatures, and light intensity, and long days favour male flowers, while, low temperatures and light intensity, and short days favour female flowers (McCormack, 2005). The flower ratio in most of the accessions was mostly male. This ratio may be affected by the number of already developing fruits present in a plant (McCormack, 2005).

iv. Fruit characters

Fruit in most accessions were globular in shape. Ahamed *et al*. (2011) reported elliptical to round and pyriform shapes. Labrada *et al*. (1997) reported pyriform, elongate, globular and flat shapes and Xolisa (2002) cylindrical, oblate, flattened, globular or elliptical shapes. Fruit skin colours were predominantly green or dark green, with blotchy secondary pattern in most accessions. Mladenovic *et al*. (2014) reported predominant fruit skin colour ranges from green to orange, and secondary pattern from speckled to stripe. Ahamed *et al*. (2011) reported colour ranges from green, yellow to brown. The colour of fruit skin is controlled by 3 loci (*Gr, Mldg* and *B*). Dominant *Gr* results in green fruits, *Mldg* mottled immature fruit colour and *mldg* non-mottled rind, while B (*Bicolor)* gene confers a precocious yellow colour (Paris and Brown 2005; Lietzow *et al*., 2005; 2006). Warty, grainy, smooth, wrinkled and wavy fruits were observed in some of the accessions. Warts were either rare or numerous. Xolisa (2002) reported smooth, and Mladenovic *et al*. (2014) smooth to warty fruits. Fruit warts are controlled *Wt* gene dominant to non-warty (*wt)*, and complementary to hard rind gene *Hr*. Wartiness is expressed only in the presence of the dominant *Hr* allele (Paris and Brown, 2005), that controls the activity of phenylalanine ammonia lyase (PAL) (Schaffer *et al*., 1986), to biosynthesize lignin in the fruit rinds resulting into warts (Brown, 2002). Main and internal fruit flesh colour was orange and green in most accessions, respectively. Mladenovic *et al*. (2014) and Xiaohua *et al*. (2011) reported flesh colour ranges from white to orange. Brown (2002) reported white through cream and yellow to various shades of orange, including greenish tints, and Ahamed *et al*. (2011) white to green, orange to deep orange fruit flesh. Genes *A/-B/-* result in orange fruit, associated with high carotenoid levels (Brown, 2002), and *A/-b/b* in green fruits (Paris, 1994).

Fruit peduncle colour ranged from light-green to dark-green. Brown (2002); Paris and Nerson (1986) and Paris, (1996) reported dark-green peduncles at the stem end, gradually lightening to yellow at the fruit end in plants homozygous for $D,^{s}$ and light peduncles and dark stems at the base of heterozygous plants. Fruit peduncles of all accessions abscised when fruits over-ripened. Abscission layer at the peduncle is attributed to ethylene (Pitrat, 2008), and is a good indicator of full ripeness (Beaulieu, 2006). The layer also plays an important role in growth and survival by discarding unnecessary organs, and protecting plants by eliminating pathogen-infected organs (Tsukahara *et al*., 2013). The appearance and size of fruit blossom scars ranged from obscure to conspicuous, and small to large, respectively. Xolisa (2002) reported intermediate to conspicuous appearance, and Loy (2006) reported small sized scars in fruits from pistillate flowers, than in fruits developed from perfect flowers. Blossom and stem end were rounded, depressed, flattened or pointed. Similar findings were reported by Xolisa (2002). Fruit ribbing ranged from superficial to deep, obtuse or intermediate in shape in fruits among the accessions. Xolisa, (2002) and Mladenovic *et al*. (2012a) reported superficial, intermediate or deep-ribbed fruits. Second fruiting cycle was observed mostly in local variegated accessions. This was attributed to genetic diversity and adaptation to agro-ecological conditions (Du *et al.,* 2011). Cucurbits continue producing flowers and fruits for an extended period of time with appropriate conditions,

(OECD, 2012). Lack of second fruiting of green leafed accessions was attributed to a recessive gene *de* for determinate plant habit (Paris and Brown, 2005). Most of the local variegated accessions had second fruiting cycle, unlike green leafed accessions which had no second fruit cycle. OECD (2012) reported some pumpkin species behaving as facultative annuals, dying in their first year. Paris and Brown (2005) linked a recessive gene *de* being responsible for determinate habit which results to no second fruit cycle.

v. Seed characters

The seed coats in most of the accessions seeds were predominantly cream yellow. Balkaya *et al*, (2010) reported cream tones seed coats in most pumpkins, and cream, tawny, dark-cream, light cream and brown in other pumpkin seeds. Aruah *et al*. (2010) reported brown and light-brown seeds. The separation of seed and placenta from fruit in most accessions was easy. McCormack (2005) reported seeds separating easily from the pulp in some varieties than in others. The seed shapes were elliptic, with seed size ranging from large to very large in most of the accessions. Balkaya *et al.* (2010) reported widely elliptic seeds being most common, Ajuru and Okoli (2013) spherical or oval seeds. The tubercular seed coats in most of the local variegated accession seeds were attributed to deficiency of lignification in hypodermis, sclerenchyma and aerenchyma, and cellulose in epidermis. The smooth seed coat surfaces found mostly in seeds of green leafed accessions were attributed to strong lignification of epidermis, hypodermis, sclerenchyma and aerenchyma outer layers (Teppner, 2000).

b) Genetic Diversity among Accessions

The mean genetic diversity among accessions across Kakamega and Nyeri was below 1.0. Aruah *et al*. (2010) reported genetic diversity of 1.559 for *C. moschata*, 1.474 for *C. maxima* and 1.103 for *C. pepo*. Nyeri accessions had high genetic diversity, compared to Kakamega accessions. This was attributed to many exotic green leafed cultivars introduced by farmers in Nyeri. Introduction of genetically dissimilar or closely related species by farmers increase gene flow through exchange of genes. These activities whether intentional or unintentional release genotypes that later on reproduce with the local landraces (Lundqvist *et al*., 2008). Introduction of new species could lead in gene escape and farmers acquiring unique constructs through pollen dispersal that leads to depletion in the quality and performance of local landraces (Groot *et al.,* 2003). Variation in biotic and abiotic resistance, or tolerance or competition due to gene exchange could generate variance in demographic parameters (Bolnick, 2011). Demographic and genetic processes act synergistically through interaction to foster the ‘‘extinction vortex’’ of small populations (Gugerli *et al*., 2008). The low genetic diversity recorded among the Kakamega accessions was attributed to the existence of many local accessions that were recycled for many generations and also to the few exotic green leafed cultivars that were introduced in the region. The highest genetic diversity was recorded in fruit shape among the accessions. Studies by Du *et al*. (2011); Onyishi *et al*. (2013); Gichimu *et al*. (2008) and Aruah *et al*. (2010) reported similar findings. Intraspecific variation among the accessions was high in predominant and secondary fruit skin colour, blossom and stem end shape, and fruit surface among other characters. Intraspecific variation represents a large proportion of total variation, which has important consequences for competition, co-existence, productivity and resistance (Siefert, 2014). The high genetic diversity observed in these characters was attributed to their high evenness. Evenness is an important component in diversity studies, as it expresses even distribution of the individuals among different species (Bibi and Ali, 2013). The value of evenness indicated high genetic diversity among the accessions more in fruit shape and surface and predominant fruit skin colour. Genetic diversity is of prime importance for the long-term preservation of biodiversity in changing environments (Gugerli *et al*., 2008). Characters with high

diversity provide raw material that can be used as a basis for selection (Aruah *et al.,* 2010; Bolnick, 2011). The local variegated accessions were more diverse compared to green-leafed. This was attributed to their adaptation to diverse growing conditions, and due to genetic adaptability to diverse agro-ecological conditions over the years (Xiaohua *et al*., 2011).

c) Genetic Variation among Accessions

The total Cronbach's alpha for the 13 PCs was above 0.7, which indicated an acceptable internal consistency of the collected morphological data. This meant that the variance derived from these data originated from the characters measured, and not from experimental design or method of data collection used (Sartipi et al., 2016). The characters in the first four PCs and three factors with Eigen values equal or greater than 2.0 were selected because they accounted for more than half of the total variation (Mladenovic *et al*., 2012b), and were mainly defined by plant, leaf, flower, fruit and seed characters. These characters were considered important and emboldened, which indicated how they were related to that PC and factor (Balkaya *et al*., 2010b; Mladenovic *et al*., 2012b). The functional relationship assigned to the first PC and factor was growth, leaf, flower, and fruit and seed factors. Fruit factors were assigned to PC and factor 2 and 3, and flower factors to PC 4, respectively. They were summed as growth, quality, yield and maturity factors. These factors could thus, be used as priority indices for selection, screening and breeding of pumpkin germplasm (Xiaohua *et al*., 2011). Studies by Xiaohua *et al*. (2011) reported PC 1 being mainly defined by leaf length and width, and leaf petiole length. PC 2 by fruit width, flesh thickness and fruit weight and PC 3 by taste, texture and flavour of flesh. The three PCs were called leaf, fruit and flesh quality factor, respectively. Odiyi *et al*. (2014) reported marketable leaf yield, vine length, number of branches/plant and number of leaves/plant being important characters of genetic variability in *T. occidentalis* for both factor and principal component analysis. The functional relationships assigned to the traits were thus yield and numeric factors. The PCs and factors with Eigen values below two were considered weak and had no discriminatory power (Maji & Shaibu, 2012).

The highest weightings of similar traits using CATPCA and factor analysis ignoring the sign was revealed in second fruiting cycle, central leaf lobes, leaf pubescence type, leaf glossiness, plant growth habit, leaf and flower colour in the first PC and factor. The sign on the weighting indicated the direction of the relationship between the PCs and factors and the characters (Balkaya *et al*., 2010b). Odiyi *et al*. (2014) also reported factor and principal component analysis identifying similar characters in pumpkins, okra, rice and groundnut. The similarity of the two techniques gives a good description of variability and discrimination of the accessions (Odiyi *et al*., 2014) using qualitative characters. The characters with high weighting contributed maximum to the discrimination of the accessions and were under similar genetic control (Odiyi *et al*., 2014). The high weighting in these characters also indicated high correlation, that could be used in assessing and identifying genetic variability in pumpkins (Norman *et al*., 2014; Odiyi *et al.,* 2014), without adversely affecting other characters of economic importance (Odiyi *et al.,* 2014). Second fruit cycle and leaf glossiness were mostly observed in the local variegated accessions. Second fruiting cycle keeps cucurbits producing flowers and fruits for an extended period of time given appropriate conditions (OECD, 2012). Yogranjan *et al*. (2014) reported leaf glossiness lessening the impacts of climate change and optimizing overall yield potential of crops due to utmost reflectivity with least/no impact on photosynthetic yield. Nawalkar *et al*. (2015) reported positive correlation of leaf glossiness with yield/plant. The yield of plants increased with an increase in leaf glossiness. These traits should thus, be considered when selecting pumpkins for crop improvement.

Predominant and secondary fruit skin colour, and primary colour of immature fruit had high weighting in the second PC. Brown (2002), Paris and Nerson (1986), and Paris (1996) linked fruit colour to peduncle stem colour, with light-green and dark-green peduncles producing white and green fruits, respectively. Blossom scar appearance and size had high weighting in the second factor and the third PC, respectively. Loy (2006) reported variability in size of blossom scars, with fruits derived from pistillate flowers having smaller scars than those developed from perfect flowers. Greater fruit size was achieved in plants with smaller blossom scars and monoecious genes. The fourth PC was highly loaded with earliness of female and male flowers. Studies conducted by Organization for Economic Cooperation and Development [OECD], (2012) reported flower development in Cucurbitaceae being regulated by both genetic and environmental mechanisms such as temperature and the duration of days. Temperature and day length influences how long the plant remains in the male phase. High temperatures, high light intensity, and long days favour production of male flowers. This leads to a longer male phase and a larger number of male flowers relative to female flowers. Low temperature, low light intensity and short days favour development of female flowers relative to male flowers (OECD, 2012). Generally, male flowers usually develop and mature (McCormack, 2005) and open before any female flowers (Maynard, 2007), and shed their pollen to ensure cross-pollination occur early in the plant's development (McCormack, 2005). However, it's not uncommon for *C. moschata* to bear female flowers first (McCormack, 2005). Earliness of female flowers in summer squash varieties planted early was reported by McCormack (2005).

Total genetic variation captured by CATPCA was high compared to factor analysis. PCA accounted for over 78% of the total variation in the first four PCs while factor analysis accounted for 72.1% of the variation in the first three factors. Odiyi *et al.* (2014) reported high total variation using PCA compared to factor analysis. From the results of the factor analysis and principal component analysis, it was clear that second fruiting cycle, central leaf lobes, leaf pubescence type, leaf glossiness, plant growth habit, leaf and flower colour were important characters of genetic variability in pumpkin accessions. These characters were considered as the most important for classifying the variation among pumpkin accessions. In addition CATPCA identified leaf senescence and predominant seed coat colour also as important characters. CATPCA captured all forty seven characters as important to discriminate the accessions unlike factor analysis which identified thirty eight characters. The variation in most characters was largely influenced by communality compared to specificity. The communality value ranges from zero to 1 where 1 indicates that the variable can be fully defined by all the factors associated with it and has no uniqueness. In contrast a value of 0 indicates that the variable cannot be predicted at all from any of the factors (Beaumont, 2012). The communality observed in most of the characters measured indicated that the observed variation in each character was as a result of total influence from all the factors associated with it. Three of the characters measured loaded more than 50% as specific factors (specificity) compared to common factors (communality). This indicated how significance each of the characters contributed to the observed genetic variation among the accessions (Beaumont, 2012). Common factors were shared in more than one of the observed variables, whereas specific factors only affected particular variables, unique to a specific variable (Yong and Pearce, 2013).

d) Configuration of Accessions

The configuration along the first two PC axes grouped accessions into two distinct (variegated and green-leafed) groups. The configuration was very significant in visualizing the genetic relationships (Mussane *et al*., 2010), and interrelations (Oliveira and Munita, 2011), among the accessions based on the most discriminating morphological traits. The

accessions placed a distant away from the others in the configuration were considered genetically diverse. Thus, direct selection could be made on these accessions. The accessions that clustered together were considered genetically similar. Cluster analysis grouped the accessions into four clusters on the basis of their dissimilarity of morphological qualitative traits and not according to geographical origin. Accessions clustered in the same cluster were considered to belong in a heterotic group. Clustering of accessions using phylogenetic analysis agreed with PCA scatter bi-plot configuration results. Both methods grouped variegated and green leafed accessions into two distinct groups of variegated and green leafed accessions. The fact that the accessions were separated into two dissimilar groups based on qualitative character differences, it proved that use of morphological characters was an inexpensive means for distinguishing species (Balkaya *et al*., 2010b).

CONCLUSION AND RECOMMENDATIONS

The present study collected 155 pumpkin accessions in Kakamega and Nyeri regions. Morphological characters were used to reveal the great variations existing in pumpkins. Most of the accessions that were assessed exhibited high variability with respect to fruit and seed characters. However, greater variation was mainly centered in fruit characters. High genetic diversity was revealed in accessions collected in Nyeri using diversity index. This was attributed to intentional or unintentional introduction of genetically dissimilar pumpkin species by farmers that later on reproduced with the local pumpkins. Fruit and seed characters also revealed high genetic diversity using the index. CATPCA was important in analyzing qualitative nominal data. Factor analysis reduced the dimensionality of characters into a limited set of descriptive categories and fewer latent characters that shared a common variance. Both CATPCA and factor analysis were useful in investigating genetic diversity of the accessions by comparing the characters capturing the most variation. This helped to give supplementary information on the usefulness of these characters in defining groups of accessions. The traits that showed the most discrimination power among the accessions using both CATPCA and factor analysis **were** second fruiting cycle, central leaf lobes, leaf pubescence type, leaf glossiness, plant growth habit, leaf and flower colour. Thus, these characters can be used to make direct selection of the accessions for pumpkin improvement. Configuration and cluster analysis grouped the accessions into homogeneous groups based on their morphological dissimilarity. The accessions that clustered together were considered genetically similar and shared some biological relationship. Those that were placed a distance away were considered genetically diverse. The high genetic diversity indicated that direct selection of these accessions would be effective in their utilization in breeding to improve specific traits. Therefore, estimating genetic diversity is of paramount importance for future production, conservation and maintenance of pumpkins for use in breeding. Genetic diversity could also be achieved by crossing the accessions showing the most genetic variability to come up with new cultivars. Thus, information on the the genetic diversity of pumpkins can be used to contribute to conservation and utilization of pumpkins.

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