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PHENOTYPIC INTRASPECIFIC VARIABILITY AMONG SOME ACCESSIONS OF DRUMSTICK (*MORINGA OLEIFERA* LAM.)

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ABSTRACT

Phenotypic characterization of plant species is the basis for selection, conservation as well as improvement. *Moringa oleifera* (Lam.) is commonly grown as multi-purpose medicinal and leafy vegetable crop in Nigeria. This study evaluated phenotypic intraspecific variations among 40 accessions of *M. oleifera* collected from different agro-ecological zones. A total of 30 morphometric traits involving qualitative and quantitative vegetative, floral, fruit/pod and seed traits, seed set and germination percentages were combined for the analyses. Descriptive statistics, variance analysis, correlation coefficient, principal component analysis (PCA) and cluster analysis (CA) were used to evaluate the intraspecific variability. The accessions showed marked differences in floral, fruit pod and seed characters analyzed. Accessions edN037 (71 days), osN024 (73 days), ogN028 (74 days), oyN003 (75 days) and abN059 (76 days) were identified as early maturing accessions. Correlation coefficients $r \geq 0.70$ were high and significant for reproductive characters. The PCA and CA generated similar results. The first five principal component axes explained 61.40% of the total variation with PC1 (23.92%) and PC2 (14.19%) contributing 38.11% of the total variation. The CA showed that the degree of intraspecific similarity was high (66.82%) based on Euclidean similarity index. Nevertheless, four clusters were formed indicating intraspecific phenotypic dissimilarity among the 40 accessions especially with the separation of accessions that were collected from similar environments. The phenotypic variations could be explored for utilization, conservation and for future genetic improvement by selection of accessions with promising agronomic characters.

Keywords: *Moringa oleifera*, phenotypic variation, agro-morphometric, cluster analysis.

INTRODUCTION

Moringa oleifera is grown throughout Africa, usually as a multipurpose medicinal plant, and is cultivated widely for its immature fruit as well as leafy vegetable, stems, roots, pods and seeds (Fahey, 2005; Adejumo *et al*., 2012; Popoola and Obembe, 2013). All parts of *Moringa oleifera* are economically viable as food and nutrition, animal fodder, natural coagulants, forestry products and fertilizer (Fahey, 2005; Afolabi *et al*., 2013; Ahmad *et al*., 2014). In addition, the leaves and the seeds are nutritionally rich containing high concentrations of crude protein, calcium, iron, potassium, manganese, essential vitamins (thiamine, riboflavin, niacin, ascorbic acid), anti-oxidants and anti-inflammatory compounds (Anwar *et al*., 2007; Adisakwattana and Chanathong, 2011; Adejumo *et al*., 2012). The medicinal uses, safety and efficacy of *M. oleifera* have been widely reported by several authors (Anwar *et al*., 2007; Popoola and Obembe, 2013; Hussain *et al*., 2014; Stohs and Hartman, 2015). The seed oil as raw material for production of biodiesel is gaining attention globally as possible replacement for petro diesel fuel in unmodified engines (Fernandes *et al*., 2015). The use as supplementary food has been adjudged to have high biological value, with 20-40% inclusion in groundnut hay based diet recommended for ruminant animals (Akinbamijo *et al*., 2004; ITC, 2004).

However, *M. oleifera*, as a vegetable, is considered neglected and underutilized species (NUS) in some parts of the world (Padulosi *et al*., 2013a; Rudebjer *et al*., 2013). The neglect may be probably due to little or no research attention from agronomic researchers and policy makers, loss of local knowledge and lack of established varieties (Padulosi *et al*., 2011; Padulosi, 2013; Padulosi *et al*., 2013b). Usually, NUS are not traded as commodities; however, the economic importance of *M. oleifera* has elicited utilization in different parts of Africa. There is growing interest in improvement and breeding of *M. oleifera* in Sub-Sahara Africa and thus, a standardized set of morphological characters would be helpful for the purpose of designing hybridization procedure for genetic improvement and utilization as leafy vegetable. Presently, in Nigeria, *M. oleifera* can be said to be underutilized, as available germplasms are scarcely represented in *ex situ* collections and *in situ* collections are poorly managed and conserved (Popoola and Obembe, 2013; Popoola *et al*.,

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2014). In addition, most landraces remain uncharacterized and the knowledge of intraspecific relationships is poorly understood and where available, it is limited in scope. According to National Research Council (NRC) (2006), diversity within underutilized species like *M. oleifera* needs genetic attention to widen its gene pool for genetic improvement, it is therefore crucial to characterize the accessions using agro-morphological characters. Although, morphometric traits are highly influenced by environmental factors, they have, nonetheless, provided the basis for characterizing and evaluating intraspecific relationships among several plant species especially underutilized and economically important species (Amoatey *et al*., 2012; Amponsah *et al*., 2013).

The use of numerical methods as important tools for assessment of phenotypic variations has been variously employed as they are transparent and repeatable in analyzing variable data (Pedersen, 2004; Pinheiro and de Barros, 2007). Multivariate analysis frequently makes it possible to identify important morphological discontinuities and to enhance the selection of diagnostic traits (Palestina and Sosa, 2002). According to Azeez and Morakinyo (2011), analysis of the association between various plant characters would help in identifying traits that are more important than the other. The present study therefore aimed at analyzing phenotypic intraspecific variability among 40 accessions of *M. oleifera* by means of multivariate analysis using minimal descriptors for drumstick (Santhoshkumar *et al*., 2013) and descriptors for legumes (IPGRI, 2006; Adewale and Dumet, 2011).

MATERIALS AND METHODS

Study site

The study was carried out at the research farm of Covenant University, Ota, Ogun State, Nigeria. The farm is located within the coordinates 6.6699° N, 3.1574 ° E with an elevation of 55 m above the sea level of guinea and derived savanna. The mean annual rainfall varies from 1000 mm to 2000 mm with average temperature of 27°C. The soils are rich in clay, sand, humus and minerals which support savanna vegetation.

Acquisition of Seeds and Seedlings

Seeds and seedlings of seventy-eight accessions of *Moringa oleifera* were collected from major agro-ecological areas in Nigeria. Forty (40) most geographically and disparate accessions were selected for this study. Mode of collections, total number of accessions collected, and period of collection, geographical distribution and local knowledge use pattern are as described by Popoola and Obembe (2013). Figure 1 shows the study areas and accession codes highlight the collection sites.

Cultivation and Morphological characterization

The seeds of each accession were raised in a nursery grown in planting bags filled with top soil. The bags were arranged in lines of 10 bags per accession in block design. After germination, bamboo leaves were used to provide shade for the seedlings for a period of four weeks. The seedlings were exposed to direct sunlight for two weeks in preparation for transplanting. After seedling establishment, ten healthy seedlings of each accession at eight (8) weeks old were randomly selected and subsequently transplanted to a designed plots of single lines of inter and intra row (3 m x 3 m) of three replicates. Data were collected from five plants per replicate. Base weeding was done manually at interval of four weeks. NPK fertilizer was applied in ring form twice at three months old.

Data Collection

Thirty (30) morphometric traits comprising vegetative, floral, fruit pod and seed characters were recorded and data combined for analysis. Of these characters, 7 were qualitative while 23 were quantitative. Description was as outlined by Santhoshkumar *et al*. (2013), IPGRI (2006) and Adewale and Dumet (2011). Qualitative characters were scored based on visual evaluation while quantitative characters were recorded as SI units. Colors were determined using the Methuen Handbook of Colors (Kornerup and Wanscher, 1978).Vegetative, floral, pod and seed characters were recorded on five middle plants from each accession.

Qualitative characters

The seven qualitative characters recorded include; flower colour (white or creamy, with or without purple strips), stem bark color (grey or whitish), pod shape (straight or curved), fresh pod colour (green or pale green), dried pod colour (golden brown or brownish), pod size (short, medium and long) and branching habit (horizontal branching or vertical branching). All qualitative data were standardized on a numerical scale system of coding to allow for statistical analysis.

Quantitative characters

Data recorded on quantitative vegetative characters include; days to seedling emergence (DSE), stem circumference girth (SCG), mean canopy radius at six month (MCR) and mean plant height at six month (MPH). Floral/reproductive traits recorded are; days to 50% flower bud initiation (DFB), days to first flowering (DFF), number of days to 50% flowering (NDF), number of days to first fruiting (DFR), number of days to 50% fruiting (DR), frequency of flowering per year (FFY) and frequency of fruiting per year (FRY).

The fruit/pod and seed characters include: fruit pod formation period (PFP), days to 95% ripe pod (DRP), mean number of pods per peduncle / panicle (PPD), mean number of pods per plant (NPP), mean pod length (PLT), mean pod width (PW), mean number of seeds per pod (NSP), mean number of locules per pod (NLP), percentage seed set (SS), mean 10 pod weight per plant

(PWT), mean 100-seed weight (SWT) and percent germination after harvesting. Seed set percentage was estimated as follows:

$$\text{Seed set percentage} = \frac{\text{number of seeds per pod}}{\text{number of locules per pod}} \times 100$$

All harvested pods were dried under similar temperature and humidity conditions to reach constant weight. Germination percentage (GP) was also evaluated as the portion of number of germinated seeds to that of plated seeds in germinating plastics and expressed in percentage.

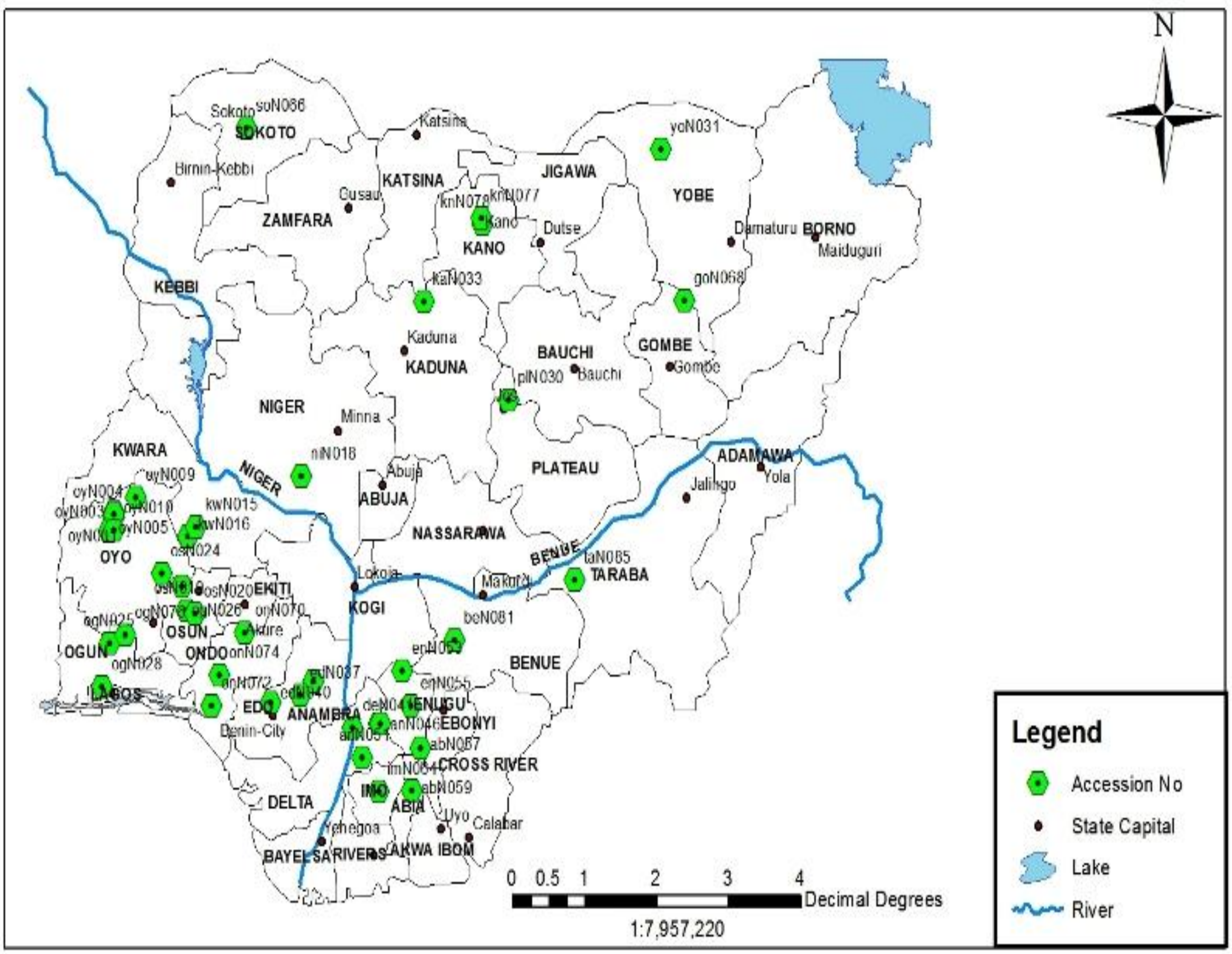

Fig. 1. Study area: Accessions codes highlights collection sites.

Data Analysis

Mean values of all characters were evaluated using descriptive statistics. Analysis of variance, correlation coefficient, principal component and cluster analyses were used in the assessment of the intraspecific variation. A hierarchical cluster analysis and coefficient of variance on the unweighted pair group method (Sneath and Sokal, 1973) was carried out using the PAST software (2013) package. Squared Euclidean distance was used as a measure of distance for cluster formation after standardization of all the morpho-metric data. The percentage variation for the first two principal component analysis (PCA) was used to plot a two dimensional scatter diagram of the dispersal among the 40 accessions.

RESULTS

Qualitative characters

The leaves were bipinnate and tripinnate compound in all the accessions. The flowers were papilionoid with five stamens and described as monosymmetric. Amongst all the accessions studied, the flower colour was generally white with purple pigmentation or creamy white without pigmentation. Only four accessions were observed to possess white with purple pigmentation (enN053, goN068, knN078 and knN077). Stem bark color was grey in all the accessions except two accessions knN078 and oyN003 observed to exhibit whitish grey stem bark color. The fresh pod color was pale green in 25 accessions while it was green in 15 accessions. At maturity, all pods were brown in colour except few that were scored to be golden brown. All accessions showed moderate branching. Fruit shape was straight in all the accessions except in accessions abN057, anN049, anN051 and niN018 that were scored to be curved shaped.

Quantitative characters

The mean, standard deviation, range and variance showed high degree of variability among the 40 accessions of *Moringa oleifera* analyzed for 23 quantitative morphological characters (Table 1). The accessions showed different days to seedling emergence/period of germination. Accession goN068 recorded a longer period of seedling emergence (13 days) while accession onN074 had shorter days (6 days). The mean plant height (MPH) at six months after transplanting ranged from 5.17 m in yoN031 to 10.27 m in oyN003. The accessions showed different flowering and fruiting regime/period. The mean days to 50% flower bud initiation and first flowering were 161 and 167 days, respectively. The days to 50% flowering ranged from 139 days in accession oyN003 to 210 days in accession anN049, with a general mean value of 178.30 days. The days to fruiting ranged from 204 days in accession oyN003 to 321 days in accession anN049.

There was marked differences among the accessions in pod and seed characters analyzed. Pod formation period, which is the number of days from 50% first flowering until 95% of the pods are ripe, ranged from 71 days in edN037 (earlier to flower) to 146 days in yoN031 days (late to flower). Days to 95% ripe pods was higher in anN049 with 331 days and lower in abN059 with 203 days. Number of pods per peduncle/panicle ranged from 2 in enN053 and edN040 to 6 in oyN003, which also produced higher number of pods per plant in a fruiting period. The number of pods produced per plant ranged from 10 in accession onN070 (10 pods) to 62 in accession oyN003, with a generally lower number of pods per peduncle recorded for accession edN035. The mean fruit pod length ranged from 25.45 cm in imN064 to 43.87 cm in oyN004. Estimate of seed set which is a direct function of seeds to locules ratio was generally high in all the accessions. The number of seeds per pod, the number of locules per pod, and thus the total seed set percentage per accession was higher in ogN026, edN035 and imN064 than other accessions.

The weight of 10 pods per accession ranged from 59.37 g in accession enN053 to 91.34 g in accession beN081, with a general mean value of 76.33 g. The 100 seeds weight per accession was higher in accession osN020 (37.32 g) and lower in accession osN019 with 25.45 g. The percentage germination was generally high as it ranged from 60% in accession niN018 to 100% in accession ogN026.

Pearson's Correlation Coefficient (r) of morphological characters

The Pearson correlation matrix among 23 quantitative characters of *Moringa oleifera* is presented in table 3. Number of days to flowering (NDF) was significantly correlated with days to flower bud initiation r = +0.965. Days to first flowering (DFF) was significantly correlated with days to flower bud initiation (DFB) r = +0.964 while days to 50% flowering (DF) correlated with days to flower bud initiation (DFB) r = +0.891 and days to first flowering r = +0.94. Period of flowering and frequency of flowering were associated with fruiting characters as frequency of fruiting per year (FRY) was moderately correlated with frequency of flowering (FFY) r= +0.59. Among reproductive characters, pod formation period (PFP) was significantly correlated with days to first fruiting (DFR) r = +0.73 and days to 50% fruiting (DF) r = +0.83 while it was negatively correlated with frequency of flowering in a year (FFY) r = -0.35. Days to 95% ripe pod (DRP) was strongly correlated with days to 50% flowering (DF) r = +0.71, days to first fruiting (DFR) r = +0.83, pod formation period (PFP) r = +0.83 and moderately correlated with days to flower bud initiation and days to first flowering at r = +0.56 and +0.58, respectively. Number of seeds per pod was correlated with pod length (PLT) and pod width (PW) at r = +0.69 and +0.54, respectively while number of locules per pod was also strongly correlated with pod length r = +0.69, pod weight r = +0.61 and number of seeds per pod r = +0.98.

Principal Component Analysis

In the PCA, the first five axes were considered informative with 61.40 % of the total variation. The first and second principal components (PC1 and PC2) of the 40 accessions of *Moringa oleifera* generated 23.92% with eigen value 7.179 and 14.19% with eigen value 4.27 of the total variation, respectively. PC3 accounted for variation of 9.36% with eigen value of 2.81, PC4 contributed 7.75% while PC5 contributed 6.11%. The eigen values ranged from 7.179 (PC1) to 1.832 (PC5). Eigen values $\geq$ 0.20 were identified as the logical cut-off points where each selected character made an important contribution to the PC axes. The respective morphological characters which largely controlled each PC axes were presented in Table 2. The two dimensional scattered plot of PC1 and PC2 based on normalized variance-covariance matrix was shown in Figure 2. The analysis clustered the 40 accessions into four clusters: Cluster A comprised 7 accessions, cluster B had 3 accessions, cluster C comprised 25 accessions while cluster D had 5 accessions. The PCA analysis was similar and comparable to the cluster analysis (Fig. 3).

Table 1. Mean, Standard Deviation (SD), Range, Mean Square and Variance for 23 quantitative characters.

S/N	Character	Mean	SD	Range	Mean Square	Variance	Sig.
1	Days to 50% Seedling emergence(DSE)	9.88	1.73	6.33 (onN074) – 13.33 (goN068)	4.33	2.99	0.0229
2	Mean Plant Height at 6 Months (m)	7.75	1.23	5.17 (yoN031) – 10.27 (oyN003)	1.48	1.52*	0.5375
3	Stem Circumference Girth (cm)	7.99	2.26	4.00 (oyN003) – 13.00 (ogN028)	7.02	5.13	0.0535
4	Mean Canopy Radius (m)	3.09	0.59	1.80 (oyN009) – 4.07 (edN040)	1.15	0.35*	0.3773
5	Days to 50% Flower Bud initiation (DFB)	161.55	16.07	129.00 (oyN003) – 198.00 (anN049)	378.91	258.36	0.0186
6	Days to first flowering (DFF)	167.40	16.59	134.00 (oyN003) – 200.00 (anN049)	416.04	275.37	0.0105
7	Days to 50% flowering (DF)	178.30	18.06	139.00 (oyN003) – 210.00 (anN049)	484.79	326.01	0.0144
8	Days to first fruiting (DFR)	266.18	27.14	204.00 (oyN003) - 321.00 (anN049)	1086.04	736.40	0.0168
9	Days to 50% Fruiting (DR)	269.38	29.99	203.00 (abN059) – 331.00 (anN049)	1297.48	899.16	0.0245
10	Frequency of flowering per year (FFY)	1.73	0.45	1.00 (abN057) – 2.00 (oyN010)	0.34	0.20	0.0007
11	Frequency of Fruiting per year (FRY)	1.48	0.51	1.00 (abN057) – 2.00 (deN041)	0.40	0.26	0.0055
12	Pod Formation Period (PFP)	102.85	18.88	71.00 (edN037) – 146.00 (yoN031)	506.83	356.54	0.0312
13	Days to 95% ripe pod (DRP)	269.38	29.99	203.00 (abN059) – 331.00 (anN049)	1297.48	899.16	0.0245
14	Pods per Peduncle (PPD)	3.53	1.06	2.00 (edN035) – 6.00 (oyN003)	1.04	1.13*	0.6245
15	Number of Pods per Plant (NPP)	26.15	10.91	10.00 (onN070) – 62.00 (oyN003)	103.74	119.11*	0.7081
16	Pod Length (PLT) cm	33.35	3.85	26.83 (imN064) – 43.87 (oyN004)	19.48	14.84*	0.0874
17	Pod Width (PW) cm	2.06	0.21	1.63 (imN064) – 3.00 (osN020)	0.05	0.04*	0.2796
18	Number of Seeds per Pod (NSP)	10.63	3.00	5.00 (kaN033) – 16.21 (osN020)	8.96	8.99*	0.2196
19	Number of Locules per Pod (NLP)	11.52	2.94	7.00 (kaN033) – 18.43 (osN020)	10.61*	8.67*	0.4417
20	Seed set Percentage SS%	91.70	7.22	71.43 (kaN033) – 100.00 (ogN026)	71.24	52.14	0.0053
21	10 pod weight (PWT)	76.33	8.81	59.37 (enN053) – 91.34 (beN081)	79.12*	77.68	0.4669
22	100 seed weight (SWT)	30.59	2.65	25.45 (osN019) – 37.32 (osN020)	5.71*	7.00	0.7845
23	Germination Percentage (GP)	85.50	11.44	60.00 (abN057) – 100.00 (goN068)	136.69*	130.97	0.4249

*Significance at P > 0.05

Cluster Analysis

The degree of intraspecific similarity was high (66.82%) based on Euclidean similarity index and segregated the 40 accessions into four clusters: cluster A had single accession abN059, cluster B comprised of 14 accessions and formed three sub-clusters; BI with 5 accessions (enN055, enN040, osN024, edN037, ogN028), BII clustered 6 accessions (beN081, osN020, ogN076, ogN026, edN035, plN030) while BIII clustered 3 accessions (deN041, osN019 and oyN003). Cluster C comprised 17 accessions and formed two sub clusters: CI clustered 7 accessions (goN068, enN053, kwN016, kwN015, kaN033, ogN025 and oyN005) while CII clustered 10 accessions (abN057, knN078, imN064, knN077, soN066, onN072, oyN001, onN070, oyN009 and oyN004). Cluster D comprised 8 accessions (anN049, anN046, anN051, onN074, taN085, niN018, oyN010 and yoN031) Figure 3. At higher Euclidean distance, four clusters were formed indicating intraspecific morphological dissimilarity among the 40 accessions especially with the separation of accessions that were collected from similar environments.

Table 2. Principal Component Analysis of Morphological characters of *Moringa oleifera*.

Characters	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Flower color	-	0.328	-	-0.219	0.431
Stem Bark Colour	-	0.280	-0.358	0.403	-
Branching Habit	-	-	0.326	0.343	0.553
Pod Shape	0.310	0.502	-	0.481	0.229
Fresh Pod Colour	0.264	-	0.345	-	0.648
Dried Pod Colour	-0.215	-0.361	-	-	-0.362
Pod Shattering	0.346	-	-	0.563	-0.306
Days to Seedling Emergence	-	-	0.641	0.328	-
Mean Plant Height (cm)	-0.517	0.479	-	-	0.235
Stem Circumference Girth (cm)	-	-	0.774	0.229	-
Mean Canopy Radius (cm)	-0.220	-	-0.504	-0.282	-
Days to 50% Flower Bud Initiation	0.746	0.282	-	-0.358	-
Days to First Flowering	0.790	0.316	-	-0.311	-
Days to 50% Flowering	0.869	0.273	-	-	-
Days to First Fruiting	0.903	0.292	-	-	--
Days 50% Fruiting	0.837	0.329	-	0.279	-
Frequency of Flowering per Year	-0.607	-	-	-	-
Frequency of Fruiting per Year	-0.394	0.297	-0.268	-	-
Pod Formation Period	0.656	-	-	0.516	-
Days to 95% ripe Pod	0.837	0.329	-	0.279	-
Pods Per Peduncle	-0.204	-	-0.555	0.352	0.292
Number of Pods per Plant	-0.435	-	-0.544	0.335	0.398
Pod Length (cm)	-0.380	0.682	0.365	-	-
Pod Width (cm)	-0.564	0.561	-	-	-
Number of Seeds per Pod	-0.234	0.834	0.290	-	-
Number of Locules per Pod	-0.310	0.798	0.269	-	-
Seed Set Percentage	-	0.486	0.201	-0.314	-
10 Pod Weight	-	0.221	-0.243	-	-
100 Seed Weight	-0.555	0.427	-	0.202	-
Germination Percentage	-0.390	0.219	-	-	-
Eigenvalue	**7.179**	**4.272**	**2.809**	**2.326**	**1.832**
% variance	**23.92**	**14.19**	**9.36**	**7.75**	**6.11**

PC = Principal Component.
Only eigenvectors with values ≥ 0.20 which largely influenced each PC axes are presented.

Table 3. Correlation Matrix of quantitative morphometric characters of *Moringa oleifera* accessions studied.

	DSE	MPH	SCG	MCR	DFB	NDF	DFF	DF	DFR	DR	FFY	FRY	PFP	DRP	PPD	NPP	PLT	PW	NSP	NLP	SS	PWT	SWT	GP
DSE	1																							
MPH	-0.001	1																						
SCG	.473**	-0.248	1																					
MCR	-.371*	0.179	-.486**	1																				
DFB	-0.126	-0.199	0.032	-0.02	1																			
NDF	-0.118	-0.226	0.001	-0.041	.965**	1																		
DFF	-0.107	-0.218	0.006	-0.047	.964**	.961**	1																	
DF	-0.075	-0.262	-0.009	-0.12	.891**	.916**	.940**	1																
DFR	-0.117	-0.312	-0.039	-0.188	.749**	.785**	.799**	.896**	1															
DR	-0.135	-.326*	0.044	-0.093	.559**	.580**	.635**	.711**	.835**	1														
FFY	0.078	.356*	0.006	-0.096	-.374*	-.406**	-.416**	-.505**	-.441**	-.469**	1													
FRY	-0.13	.378*	-0.28	0.184	-0.282	-0.299	-0.252	-.322*	-0.193	-0.097	.586**	1												
PFP	-0.103	-0.287	-0.015	-0.165	0.183	0.227	0.244	.432**	.726**	.834**	-.350*	-0.049	1											
DRP	-0.135	-.326*	0.044	-0.093	.559**	.580**	.635**	.711**	.835**	1.000**	-.469**	-0.097	.834**	1										
PPD	-0.138	0.108	-0.297	0.15	-0.139	-0.073	-0.085	-0.066	-0.082	-0.019	0.095	0.192	-0.01	-0.019	1									
NPP	-0.186	0.204	-.360*	0.242	-.368*	-.404**	-.389*	-.374*	-.323*	-0.271	0.232	0.187	-0.106	-0.271	.741**	1								
PLT	0.116	.526**	0.117	0.056	-0.11	-0.121	-0.112	-0.189	-0.138	-0.112	.380*	0.288	-0.108	-0.112	-0.029	-0.07	1							
PW	-0.105	.405**	-0.112	0.196	-0.223	-0.241	-0.271	-0.311	-0.299	-0.269	.352*	0.26	-0.205	-0.269	0.156	0.255	.586**	1						
NSP	-0.053	.447**	0.129	0.063	0.058	0.011	0.09	0.01	-0.04	0.01	0.127	0.232	-0.091	0.01	0.033	0.013	.694**	.539**	1					
NLP	-0.002	.444**	0.137	0.062	-0.006	-0.042	0.028	-0.047	-0.101	-0.05	0.192	0.259	-0.138	-0.05	0.083	0.055	.685**	.609**	.977**	1				
SS	-0.263	0.247	-0.009	0.062	0.215	0.16	0.228	0.174	0.165	0.175	-0.208	0.007	0.111	0.175	-0.211	-0.151	.321*	-0.006	.534**	.349*	1			
PWT	-0.052	-0.092	-0.11	0.104	-0.102	-0.103	-0.091	-0.071	-0.111	0.008	-0.026	0.002	-0.023	0.008	0.168	0.218	0.035	.367*	0.256	0.302	-0.142	1		
SWT	0.143	.500**	-0.049	0.166	-.318*	-.333*	-.343*	-.362*	-.355*	-0.23	0.308	0.179	-0.157	-0.23	0.225	0.235	.548**	.707**	.355*	.415**	-0.074	0.172	1	
GP	-0.088	0.279	0.076	0.139	-0.266	-0.257	-0.245	-0.262	-.315*	-0.26	-0.032	-0.011	-0.25	-0.26	0.126	0.114	.322*	0.281	0.242	0.227	0.239	-0.049	.370*	1

**. Correlation is significant at the 0.01 level

*. Correlation is significant at the 0.05 level

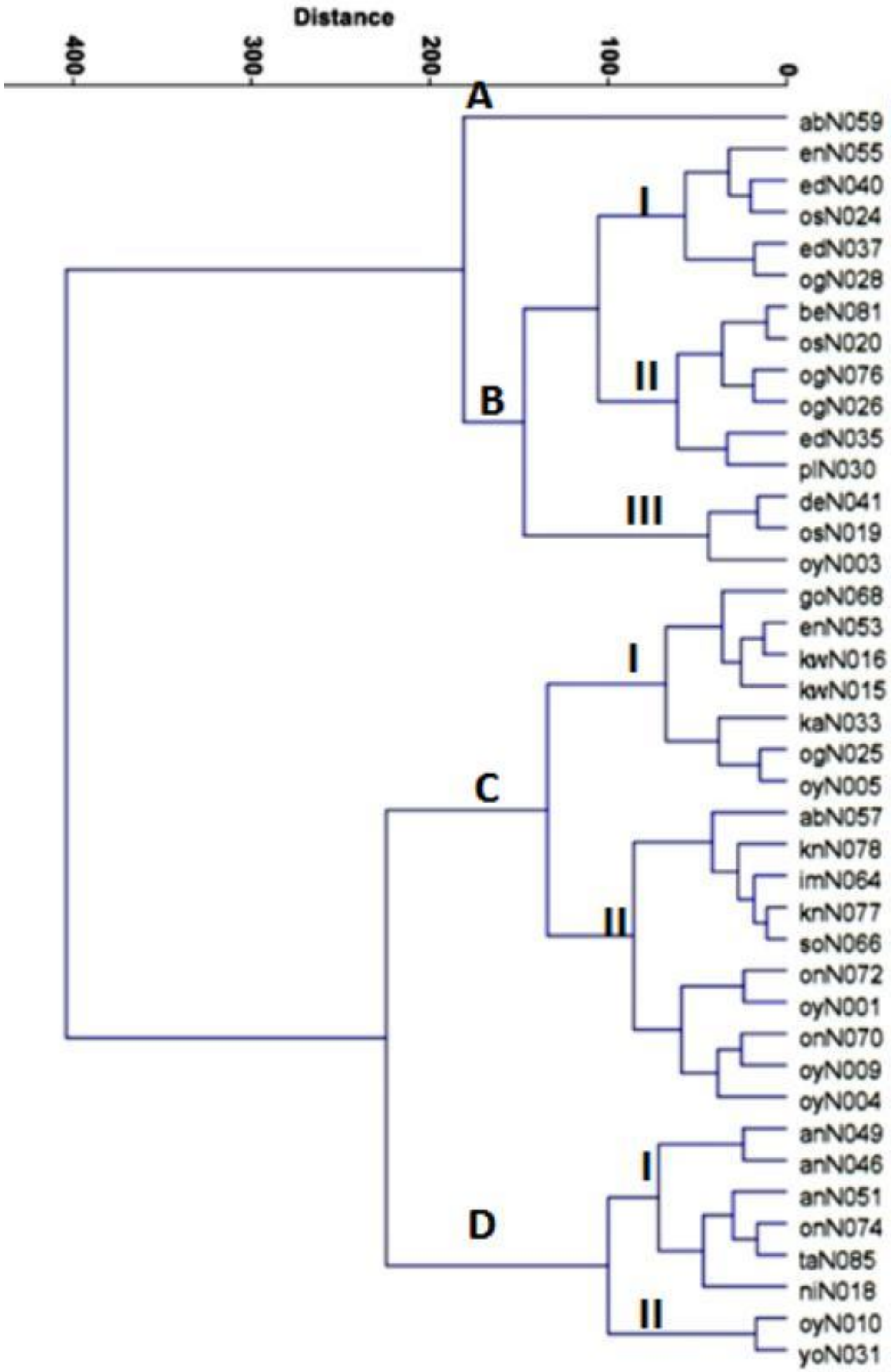

Fig. 3. Cluster analysis based on morphological characters.

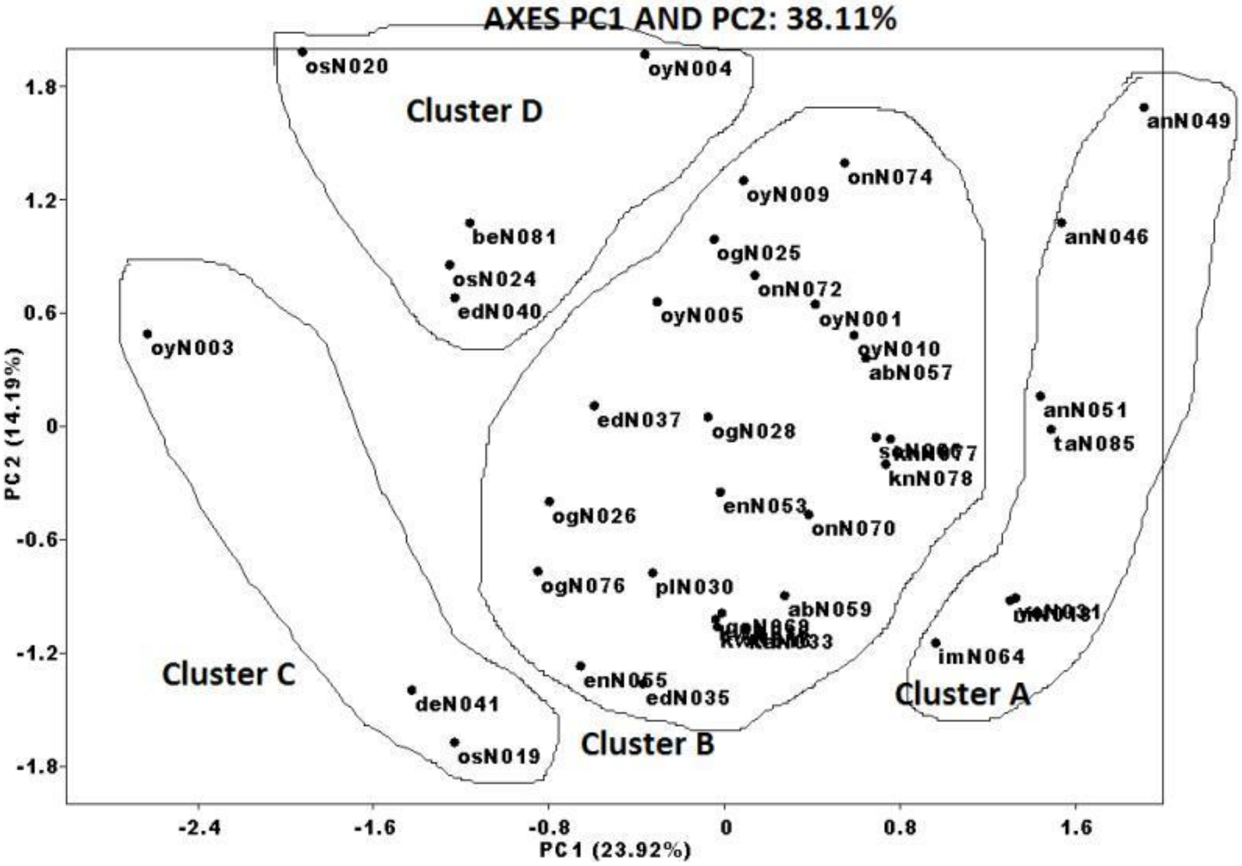

Fig. 2. A two dimensional plot of Principal Components Analysis of the 40 accessions of *Moringa oleifera* based on normalized variance-covariance matrix using PAST software 2013.

DISCUSSION

The use of heritable phenotypic and agronomic characters is a standard way of assessing genetic variation for many species because the inheritance of characters/markers can be monitored visually without specialized biochemical or molecular techniques (Karaca, 2013). It will continue to be relevant particularly in the characterization of underutilized and neglected species to identify and select good and promising characters/accessions for possible breeding trials. In this study, phenotypic variabilities were assessed among 40 accessions of *Moringa oleifera*. All the accessions showed considerable similarities and differences in growth, flowering and reproductive attributes. Accessions with better and promising agronomic traits were identified which could invariably be useful in conservation, utilization and breeding efforts.

Accessions with shorter seedling emergence and flowering days resulted in earlier maturity days and showed higher potentials for fruit pod production and consequently higher seed potentials. This might suggest that days to seedling emergence can be selected among the accessions to facilitate early flowering, early maturity and in effect higher fruit pod and seed production. Accessions (oyN003, osN019, abN059, ogN026, ogN076, deN041, osN024 and plN030) were identified as early maturing accessions. These accessions can serve as potential parent materials for breeding and for genetic improvement of *M. oleifera*. According to Amoety *et al*. (2012) variations in days to maturity and frequency of flowering in a year could be used to map out breeding strategies towards synchronized flowering to facilitate hybridization among the accessions. In addition, accessions with longer fruit pods resulted in higher number of locules per fruit pod, number of seeds per pod and consequently higher seed set percentage. Accession oyN004 had the longest fruit/pod length (43.87 cm), an average of 15 seeds per pod and seed set percentage of 93.75%. This accession can be a good candidate for high seed yield. Likewise, accessions knN077 (31.32 cm, 99.12%), oyN003 (34.50 cm, 94.12%) and oyN009 (36.91 cm, 94.12%) could also be considered for high seed yield. Accession beN081 recorded higher total weight of pods (91.34 g) while enN053 recorded the least (59.37 g). Previous studies have consistently identified the number of fruit/pods per plant, seeds per fruit pod and weight of fruit pod as very important components of fruit pod and seed yields (Ariyo, 1989; Udensi *et al*., 2012; Ganesan *et al*., 2014). Similarly, days to flowering, numbers of fruit pods per plant and flowers per branch have been reported as some of the most variable quantitative characters of *Moringa oleifera* (Genesan *et al*., 2014), which could be used for breeding purpose.

The results from this work lend credence to the existence of substantial variation in quantitatively inherited traits in natural populations of *M. oleifera* (Ramachandran *et al*., 1980; Jyothi *et al*., 1990; Resmi *et al*., 2005; Popoola *et al*., 2014). The high fruit set and seed set percentages can be attributed to flowering (and thus fruiting) period and pattern, pollination mechanism and ability to produce fertile pollen grains. The studies of Silva *et al*. (2011) support the claim on high pollen viability (88%) with pollen grains reflecting three apertures resembling pollen types in Fabaceae. Likewise, *M. oleifera* is an out-crossing species adapted to a mixed system of selfing (geitonogamy) and out crossing (xenogamy) with ability to produce viable pollens, thus favoring high fruit and seed set (Jyothi *et al*., 1990; Mgendi *et al*., 2010). In many plant species including *M. oleifera*, breeding systems, duration of flowering and fruiting coupled with high pollen viability have been variously connected to high seed set percentages and by extension high yield (Muluvi *et al*., 1999; Matthew and Rajamony, 2004; Popoola *et al*., 2011; Ojuederie *et al*., 2015). These observations also explain the phenotypic variations among the accessions. However, some accessions (onN070, niN018 and kaN033) showed poor performance for some agronomic traits; including lower total number of fruit pod per plant, fruit/pod length, and number of seeds per fruit pod. Hence, such accessions cannot be considered for yield related characteristics for breeding trials.

Mutual association of traits is usually expressed by the phenotypic, genotypic and environmental correlations (Akinyele and Osekita, 2006; Aremu *et al*., 2007). In this study, the correlation coefficients were high and significant for reproductive and yield related characters, which are important for improving the yield of *Moringa oleifera*. For instance, days to 95% ripe pod (DRP) and pod formation period ($r = 0.83$), number of pods per plant (NPP) with number of pods per branchlet/peduncle (PPD) $r = +0.70$ were positively significantly correlated phenotypically. Similarly, number of seeds per fruit pod was significantly correlated with fruit/pod length $r = +0.71$, while 100 seed weight was significantly correlated with fruit/pod length, pod weight, number of seed per locule and number of seed per pod. The significant relationship between days to 50% flowering and days to 50% maturity is indicative that there is a strong relationship between the stage of plant growth at which flowering is initiated and the time taken to complete the crop's life cycle (Akinyele and Osekita, 2006; Aremu, 2011).These suggest that preference can be given to these characters in the selection of parent accessions for hybridization trials. This observation may not be unconnected with the fact that morphological differences of different accessions cultivated in a common environment makes it plausible that at least some of the differences involved are heritable. Though, this study did not delve into heritability estimates, it is a critical component of the production formulae for computing expected response to selection since it is based on the phenotypic variance (Ojo *et al*., 2006; Ukaoma *et al*., 2013). Several authors have reported the relationships between different traits in different crops such as rice (Ukaoma *et al*., 2013), cowpea (Udensi *et al*., 2012) and African Yam bean (Aremu and Ibirinde, 2012; Osuagwu *et al*., 2014).

From the five axes of the Principal Component Analysis, more than 60% of the total variations among the accessions were accounted for by the reproductive traits, suggesting their stability and consistency. The PC1 and PC2 with 38.11% variation were mostly correlated with reproductive quantitative characters that recorded higher PC loading values. The PCA identified such traits like days to 50% flower bud initiation, days to 50% flowering, days to 50% fruiting and pod formation period as characters that significantly contributed to the variation.
Though, the degree of phenotypic similarity among the 40 accessions of *Moringa oleifera* was high, the separation of the clusters showed a considerable degree of phenotypic variation among the accessions. The cluster analysis grouped the 40 accessions of *Moringa oleifera* into four clusters based on similarity for the quantitative and qualitative characters. The cluster groups comprised of accessions from different ecological locations, with cluster A being separated (abN059) from similar accessions (abN057) collected from the same area. These might be attributed to genetic component, breeding system and phenotypic similarities. The accessions were raised in a common environment and subjected to similar treatments which invariably might have reduced the effects of the environment on their phenotypic expression.

Conclusion and Future work

The present study revealed that *Moringa oleifera* exhibit variability in morphometric traits, which can be explored for utilization, conservation and genetic improvement. The inter-character correlation and relationship can serve as a guide in designing hybridization procedure to employ for genotypic selections. The degree of phenotypic variation recorded among the accessions indicates wide differences that may necessitate further evidence, probably genotyping by sequencing (GBS) so as to lay a solid foundation for genetic improvement of the species via breeding and genetic engineering.

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