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Morphological Diversity among *Corchorus olitorius* Accessions Based on Single Linkage Cluster Analysis and Principal Component Analysis

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Abstract

Corchorus olitorius L. is a leaf vegetable grown in Africa as well in the Middle East. Fifteen accessions of leaf *C. olitorius* were collected from two National Research Institutions and Biotechnological Center and were evaluated for genetic variability using Single linkage cluster analysis (SLCA) and Principal Component Analysis (PCA). The experiment was carried out at Babcock University Teaching and Research Farm during 2009. The experiment was laid out using a Randomized Complete Block Design (RCBD) with three replications. The contribution of Number of leaves per plant, plant height at maturity, Fresh leaf weight, total plant weight and harvest index in the PCA leads to the conclusion that these traits contributes more to the total variation observed in the fifteen accessions of *C. olitorius* and therefore can be used in discriminating among the accessions of *C.olitorius.* BUCor 24 and BUCor 31, with high potential for number of leaves per plant, plant height at maturity, fresh leaf weight and total plant weight would make good parental stock material when breeding for *C. olitorius* leafy vegetative yield, while BUCor 31, and BUCor 05 are more distinct and diverse of all the accessions and can serve as sources for variability in character for *C. olitorius* improvement. The accessions were grouped in four major cluster by the SLCA.

Keywords: Accessions, *Corchorus*, *malukhiyah*, Nijeria and variability

1. Introduction

Corchorus species are known in Arabic as *malukhiyah* and used as green leafy vegetables. *Malukhiyah* is eaten widely in North Africa and the Middle East, such as Lebanon, Palestine, Israel, Syria, Jordan and Tunisia. In Turkey and Cyprus, the plant is known as *molohiya* or *molocha* and is usually cooked into a kind of chicken stew. (Schippers, 2000). *Corchorus olitorius* L. is a leaf vegetable with Africa as the primary center of origin due to wide diverse plant types found in the continent (Benor *et.al*., 2011, Kundu, 1951) and it is widely cultivated for the sliminess of the leaves in local dishes. It is one of the leading leaf vegetables in West Africa (Grubben, 1977). The Egyptian traditional food *Malachia* is made with *C. olitorius* and the slimy property has made it popular beyond the North African origin. Large morphological and physiological variation exist among the leaf *C. olitorius* found on farmers plots in Nigeria (Nath and Denton,1980) and distinct types based on variation in leaf shapes were separated from local cultivars at NIHORT, Nigeria (Denton,1997). Apart from the variation in the leaves, considerable variation in other morphological traits still exists within the various local morphotypes (Akoroda, 1985). Similarly cultivated *Corchorus* with distinctly different leaf shapes are known by different names in Cameroon and Benin (Schippers, 2000; Westphal Stevels, 1986). Soliman *et al.* (2010) found variation in a number of vegetative characters among three Egyptian cultivars of *C. olitorious.* Several studies conducted used molecular marker techniques to detect the genetic variation. Ogunkanmi *et al.* (2010) demonstrated the presence of inter and intra genetic variability among 40 accessions each of *C. incisifolus* and *C. olitorius*, respectively base on Random Amplified Polymorphic DNA (RAPD) markers**.** The study of the genetic diversity of the many African indigenous leaf vegetables, including *Corchorus olitorius* (Chweya and Eyzaguirre, 1999) will aid early and adequate exploitation of the desirable nutritional properties of the crops for better nutrition and good health especially among the rural communities in Africa (Oguntona and Akinyele, 1995). Adequate analysis of germplasm diversity is essential for proper understanding and utilization of genetic variability among accessions and their characters.

Using fuzzy cluster analysis, Yu and Li, (1991), recognized six cluster groups containing varying numbers

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of accessions with up to 16 accessions in one cluster of *C. olitorius* based on observations obtained from 12 morphological characters. There are very few reported improved varieties of leaf *C. olitorius* in Africa and as such the cultivars found on farmers plots are mostly traditionally inherited mixed populations and locally sourced types. This has aided the protection of the genetic variation and the immediate erosion of the crop genetic resources (Fondio and Grubben, 2004). In addition to farmers' conservation of the genetic variability of C. *olitorius*, various other steps had been taken to collect and conserve the germplasm of *Corchorus* sp. in national and institutional gene banks in Nigeria, Ethiopia, Kenya, Sudan and Zambia (Attere,1997). This experiment was set up to assess the genetic variability among fifteen accessions of *Corchorus olitorius*, using Single linkage Cluster and Principal Component analyses based on six morphological characters

2. Materials and Methods

Fifteen accessions of leaf *C. olitorius* were collected from two National Research Institutions and Biotechnological Center namely, National Horticultural Research Institutes (NIHORT), Institute of Agricultural Research and Training (IART) and National Center for Genetic Resources and Biotechnology (NACGRAB), in Nigeria. The accessions represent part of the institutional germplasm which are locally grown by farmers in Nigeria. The experiment was carried out at the research and teaching experimental plot of the Horticultural Unit of Babcock University which is located in the southern rainforest belt of Nigeria with an annual average rainfall of 1500mm and mean daily temperature of 25-27^{o}C between May and December 2009.

The seed dormancy for each accession was first removed with hot water treatment for ten seconds before planting. *C. olitorius* is a small seeded crop and in order to ensure that the seeds are evenly spread, the seeds of each accessions were mixed separately with fine river sand (1gm seed :10kg sand) and then drilled in rows on raised beds. Each row was 5.0 meters long with spacing of 50cm between two rows and within rows, the seedlings were thinned to a spacing of 2.0cm between plants. The trial was set up in a Randomized Complete Block Design (RCBD). There were four rows of plants per plot for each accession and each plot was replicated three times.

Table 1. Accession codes and their sources

Serial Number	Accession Name	Source
1	BUCor 02	IAR&T
2	BUCor 04	NIHORT
3	BUCor 05	NIHORT
4	BUCor 08	NIHORT
5	BUCor 10	IAR&T
6	BUCor 12	NIHORT
7	BUCor 13	NIHORT
8	BUCor 14	IAR&T
9	BUCor 15	NACGRAB
10	BUCor 18	NIHORT
11	BUCor 19	NIHORT
12	BUCor 23	NIHORT
13	BUCor 24	NIHORT
14	BUCor 31	IAR&T
15	BUCor 40	NIHORT

NIHORT: National Horticultural Research Institute, Ibadan, BU: Babcock University Ilishan-Remo, NACGRAB: National Centre for Genetic Resources and Biotechnology, IAR&T: Institute of Agricultural Research and Training, Ibadan.

Before drilling the seeds, a pretreatment of cured poultry manure was applied on all the plots at the rate of 20t/ha. The plants were raised under rain fed conditions and manual weeding was carried out to maintain weed free plots. Regular insecticidal control measure was maintained during the investigation. After eight-weeks of sowing when the accessions were fully established and revealed distinct variations in plant morphological characteristics, ten competitive plants from the two middle rows in each plot were harvested and observations were taken on following morphological characteristics and yield on each plant

1. Plant height at maturity(cm),
2. Number of leaves per plant
3. Fresh leaf weight per plant(gm),
4. Stem weight(gm),
5. Total plant weight (gm).
6. Harvest index (%) :determined by dividing the fresh leaf weight by total plant weight and expressing the value in percentage

The mean value for each character was calculated as the average for the ten harvested plants and was used for the statistical analysis.

2.1. Data analysis

Data were analyzed using SAS Microsoft windows 8.0 (SAS, 1999) adopting the method of Steel and Torrie (1980). The PCA and SLCA were used to determine the extent of genetic variation and percentage similarity within accessions. Eigen-values and factor scores were obtained from PCA, which were used to determine the relative discriminative power of the axes and their associated characters. FASTCLUS procedure was used to group the fifteen accessions based on their genetic relationship. A dendrogram is generated from SCLA to display position of accessions and their percentage similarities.

3. Result

The result of the PCA showed that two of the six Principal Component Axes (PCA) had Eigen-values greater than two and all together accounted for over 80% of the total variability (Table 2). The first Principal Component Axes (PCA accounted for 56.80% of the total variation while the second Principal Component Axes (PCA 2) accounted for 23.60% of the total variation. The PCA 1 and PCA 2 are loaded with characters such as number of leaves per plant (0.46), plant height at maturity (0.40), Fresh leaf weight (0.52), total plant weight (0.53) and harvest index (0.74). The relative discriminating capacity of the PCA is shown by their Eigen-values. The PCA 1 had the highest discriminating power as revealed by its highest Eigen -value of 3.40 followed by PCA 2 with Eigen value of 1.42. The accessions were classified

into four distinct cluster groups using the FASTCLUS procedure (Table 3).

Table 2. Principle Component Analysis among Fifteen *Cochorus olitorus* accessions

Character	PC Axis 1	PC Axis 2	PC Axis 3
Number of leaves per plant	0.46	0.26	-0.20
Plant Height at Maturity	0.40	-0.52	-0.03
Stem weight	0.25	-0.29	0.87
Fresh leaf weight	0.52	0.17	-0.15
Total plant weight	0.53	-0.01	-0.14
Harvest index	0.11	0.74	0.41
Eigen-Value	3.40	1.42	0.88
% Variance	56.80	23.65	14.69
Cumm. % Variance	56.80	80.45	95.14

Table 3. Mean, Standard Deviation (sd) in parenthesis of four clusters with major characteristic patterns of 15 *Corchorus* accessions

Character	I	II	III	IV
Accession	10,12,13,14, 18,19,2,4,8	24,31	5	15,23, 40
Number of leaves per plant	11.32 (2.19)	19.072 (4.53)	10.2 (0.0)	9.65 (2.04)
Plant Height at maturity	29.10 (1.5)	31.02 (1.25)	31.27 (0.0)	19.7 (2.02)
Stem weight	2.94 (0.55)	4.34 (0.8)	11.73 (0.0)	1.75 (0.29)
Fresh leaf weight	1.93 (0.55)	3.27 (0.57)	1.7 (0.0)	1.3 (0.17)
Total plant weight	4.87 (1.06)	7.6 (1.32)	4.73 (0.0)	2.92 (0.29)
Harvest index	0.39 (0.04)	0.43 (0.0)	0.42 (0.0)	0.45 (0.02)

The highest number of accessions was located in cluster I with nine accessions, clusters IV and II had three and two accessions respectively whereas cluster III had only one accession. Cluster II contained accessions with the highest number of leaves per plant followed by accessions in cluster I, while accessions in cluster IV had the least number of leaves per plant. Clusters II and III contain plants with the highest plant height at maturity. This was closely followed by accessions in cluster I, whereas, the lowest value was recorded in accessions found in cluster IV.

Stem length was found to be the highest in accessions located in cluster III and the lowest in accessions presented in cluster IV. Meanwhile, fresh leaf weight was highest in accessions found in cluster II and lowest in accessions found in cluster IV. Total plant weight was highest in accessions found in cluster II, but lowest in accessions found in Cluster IV. The highest harvest index was recorded in accessions found in cluster IV, followed by cluster II, whereas the least was recorded in accessions found in cluster I.

The plot of (PCA 1 and 2) is shown in Figure 1. Result shows that accessions BUCor 31(31), BUCor 40(40) BUCor 15(15), BUCor 23(23) and BUCor 05(5) were the most dispersed and diverse of all the accessions considered in this study. BUCor 23, BUCor 15 and BUCor 40 are mostly described by characters in PCA 2, whereas characters in the PCA 1 best described BUCor 05 and BUCor 31. The dendrogram drawn from the SLCA shows the relationship between the 15 accessions (Figure 2). The dendrogram revealed four distinct clusters that joined to form one big cluster at 1% level of similarity. At 100% level of similarity all the accessions were totally

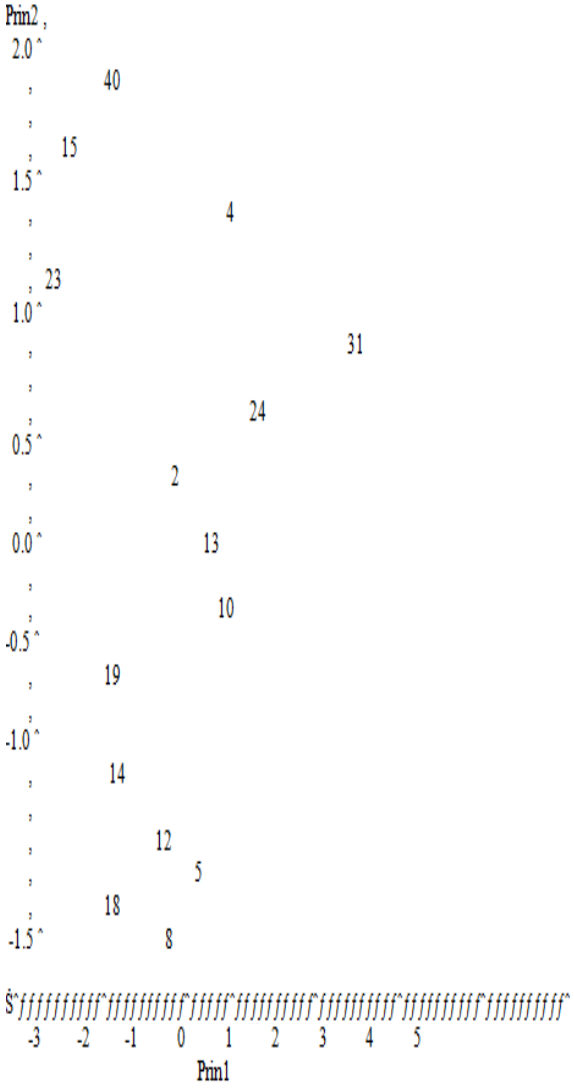

Figure 1. Configuration of fifteen *Corchorus* genotypes under PCA 1and 2.

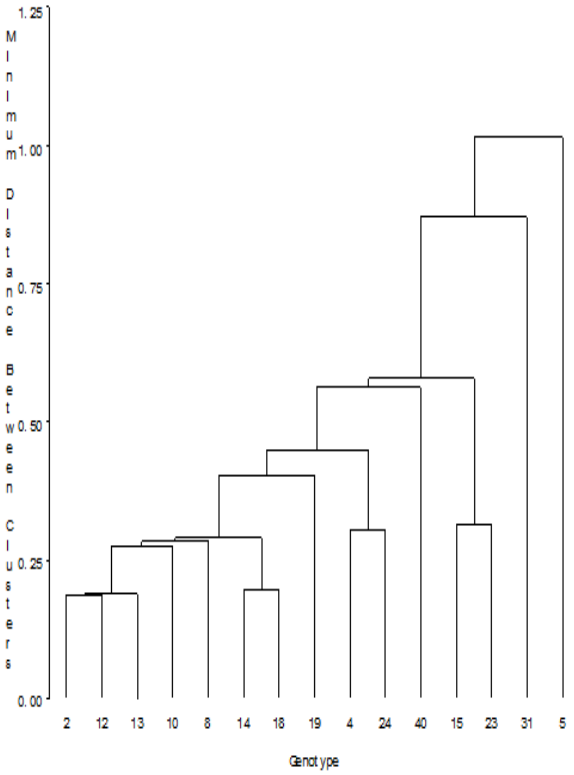

Figure 2. Dendrogram from SLCA of fifteen *Cochorus olitorus* accessions.

distinct from each other and had formed one single cluster at 1% similarity level. At 68% level of similarity eight of the accessions BUCor 2 (2), BUCor 12 (12), BUCor 13 (13), BUCor 10 (10), BUCor 8 (8), BUCor 14 (14), BUCor 18 (18) and BUCor 19 (19) had joined together to form a cluster. These accessions also formed another major cluster joining with three other accession at 52.5%.

At 43.5% similarity level these accessions had join with three others to form one major cluster. At 25% level of similarity all the accessions have joined to form one major cluster except accession BUCor 31 (31) and BUCor 05(5), which were still distinct from all the others. However, these two accessions later joined the other accessions to form one major single cluster at 1% level of similarity.

4. Discussion

The relatively high mean contribution of number of leaves per plant, plant height at maturity, fresh leaf weight, total plant weight and harvest index confirm the individual contributions of these traits to the total variation observed in the fifteen accessions of *C. olitorius*, hence these characters can be used in discriminating between the accessions of *C. olitorius*. This suggests that any selection from any cluster group for leaf vegetable yield must take into consideration these traits. This agrees with the report of Islam *et al*., (2002) in their report on *C. olitorius*

Accessions in cluster II with high potential for number of leaves per plant, plant height at maturity, fresh leaf weight and total plant weight would make good parental stock material when breeding for *C. olitorius* leafy vegetative yield which is the economic yield of *C. olitorius* in Nigeria and some other African countries. The clustering scores among the PCA suggests that there is a strong relationship amongst individuals in a cluster (Nwangburuka *et al*., 2011). The range in the similarity indices (1 – 82%) among the accessions is large enough to suggest sufficient variability among the accessions (Tokpol *et al*., 2006; Morsy, 2007 and Rawashdeh, 2011). BUCor 31, and BUCor 05, which were more distinct and diverse can serve as sources for variability in characters for the improvement of the accessions studied. Meanwhile, crosses between accessions in a cluster may not produce meaningful improvement in the offspring's, since these accessions are expected to have similarities in gene and therefore may not introduce reasonable variation. This agrees with the report of Torkpol *et al*., (2006).

BUCor 12 (2) and BUCor 12(12) were the closest accessions with (82%) similarity, high percentage of similarity could be related to these accessions may have similar ancestral origin with a common gene.

5. Conclusion

The contribution of number of leaves per plant, plant height at maturity, fresh leaf weight, total plant weight and harvest index leads to the confirmation of the individual contributions of these traits to the total variation observed in the fifteen accessions of *C. olitorius* and therefore can be used in discriminating among the accessions of *C. olitorius*.

Furthermore, BUCor 24 and BUCor 31, with high potential for number of leaves per plant, plant height at maturity, fresh leaf weight and total plant weight would make good parental stock material when breeding for *C. olitorius* leafy vegetative yield.

The clustering scores among the PCA suggested that there is a strong relationship amongst individuals in a cluster. The accessions were grouped in four major cluster by the SLCA. The range in the similarity indices (1– 82%) among the accessions as shown in the dendrogram drawn from SLCA is large enough to suggest sufficient variability among the accessions. Finally, BUCor 31, and BUCor 05, are more distinct and diverse of all the accessions and can serve as sources for variability in character for *C. olitorius* improvement.

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