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Genetic Diversity in *Corchorus Olitorius* L. Grown in South-West Nigeria Inferred from RAPD Data

***Oyenike Adeyemo*[1]✉ *and Abraham Abati*[1]**

ABSTRACT

In this study, we investigated the genetic diversity and relationships in some *Corchorus olitorius* L. grown in South-West Nigeria. We used random amplified polymorphic DNA (RAPD) markers in a total of 17 *Corchorus olitorius* L. cultivars. Twenty-two RAPD markers detected 96 polymorphic alleles (average, 4.36) and a high number of unique alleles (29%) in eight lines. Genetic distance based on RAPD data (Euclidean distance) ranged from 0.05 to 0.53 with a mean of 0.23, indicating low detected genetic diversity among the cultivars. Cluster analysis of RAPD data based on unweighted pair group method with arithmetic mean (UPGMA) grouped *Corchorus olitorius* L. cultivars into two main groups. Majority of the lines (15) were assigned into Group I which included lines collected from the same or different places. The second group consisted of two cultivars (EW05 and EW06) collected from the same place. Principal Component Analysis (PCA) based on genetic distance also separated the cultivars into two groups. Genetic diversity has been narrowed in these cultivars suggesting possible effects of domestication and selection processes.

Keywords: *Corchorus olitorius* L., Cluster analysis, Genetic diversity, Principal component analysis, RAPD markers.

INTRODUCTION

Corchorus olitorius L. is one of the important commercial dark green leafy vegetable crops, grown commonly in the South-West Nigeria and in tropical and subtropical regions of West African countries. It is cultivated mainly for fresh leaves which are used as sources of food for cooking delicious mucilaginous soup (Akoroda, 1988). Studies on the phytochemical composition of *Corchorus olitorius* L. leaves have shown that they contain vitamin C, beta-carotene (provitamin A), vitamin B and other nutrients that are necessary for health (Awobajo *et al.,* 2010). In addition, to the nutritive value, *Corchorus olitorius* L. leaves are known to be good sources of fibre and medicinal values.

Morphological traits have been used to study the genetic diversity of 15 accessions of cultivated *Corchorus olitorius* L. species based on single linkage cluster analysis and principal component analysis (Denton and Nwangburuka, 2012). Information about the genetic diversity and relationship among crop species is useful for genetic improvement and for other goals such as germplasm conservation and cultivar identification. (Senior *et al.,* 1998). The varieties of *Corchorus olitorius* L. are few in number because the cultivated varieties which are edible and preferred are presently available. Efforts to conserve the germplasm of *Corchorus olitorius* L. needs to be put in place to prevent genetic loss of commercially cultivated varieties

[1] Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos, Nigeria
✉ adeyemonik@yahoo.co.uk
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(Adebooye and Opabode, 2004). Estimate of possible loss of genetic diversity during domestication process can be estimated when genetic diversity is assessed among cultivated varieties. In the past, pedigree data, morphological traits or isoenzymes have been used for the assessment of genetic relationship in crop species.

Molecular markers are effective tools to study genetic diversity in species where pedigree data are missing or inaccurate, incomplete and insufficient. Currently, many kinds of PCR-based markers are available for assessment of genetic diversity among genotypes (Adeyemo *et al.,* 2011). Randomly amplified polymorphic DNA (RAPD) marker has been used successfully to assess genetic diversity among crop species and considered to be a useful molecular marker since previous knowledge of the genome is not necessary (Welsh and McClelland, 1990; Roy *et al.,* 2006). Some studies using molecular markers to estimate genetic diversity among *Corchorus* species have been reported using RAPD markers (Ogunkanmi *et al.,* 2010) and SSR markers (Huq *et al.,* 2009). Genetic diversity analysis has been carried out in18 jute genotypes of two cultivated *Corchorus capsularis L.* and *C. olitorius L.* species grown in Bangladesh using RAPD analysis (Haque *et al.,* 2007). Recently, a study conducted genetic diversity and genetic relationship analysis in *C. olitorius* populations from different distribution areas based on molecular and morphological data (Benor *et al.,* 2011).

In this study RAPD markers were used to analyse genetic diversity in cultivated lines of *Corchorus olitorius* L.**.** RAPD markers have not been used to assess this set of lines. The objective of this study was to use RAPD markers to assess the genetic diversity and genetic relationship which exist among a set of *Corchorus olitorius* L. lines that provide information for germplasm conservation.

MATERIALS AND METHODS

Plant materials

A total of 17 cultivars of *Corchorus olitorius* L. were included in this study (Table 1). The materials consisted of cultivars collected from South-West Nigeria representing lines that are predominantly grown and edible. Seeds of each line were planted in a greenhouse for four weeks.

Table 1. The origins of the cultivated *Corchorus olitorius* L. used to evaluate their genetic diversity by RAPD markers

LINES	LOCAL NAMES	ORIGIN (Place, States in Nigeria)
EW01	AGBA-ADU KWARA	NIIHORT, OYO
EW02	YAYA ABEOKUTA	NIIHORT, OYO
EW03	SHAAKI	IFE, OSUN
EW04	ONIYAYA	MILE 12 market, LAGOS
EW05	EWEDU ABEOKUTA	IYANA OBA, LAGOS
EW06	YAYA	IYANA OBA, LAGOS
EW07	UNKNOWN	OSHODI, LAGOS
EW08	UNKNOWN	OSHODI, LAGOS
EW09	ELETI EKU	ILORIN, KWARA
EW10	UNKNOWN	IFE, OSUN
EW11	AGBA-ADU	ODI-ORI FARM, OSUN
EW12	AWOYAYA	EWUPE, OGUN
EW13	ELETI EKU	EWUPE, OGUN
EW14	UNKNOWN	OWO, ONDO
EW15	YAYA	ABEOKUTA, OGUN
EW16	UNKNOWN	IFE, OSUN
EW17	AGBA-ADU ABEOKUTA	OTA FARM,OGUN

DNA extraction and RAPD analysis

From a bulk of 4-8 plants per cultivar, 2-3 g of fresh leaf tissues were grounded in liquid nitrogen and total genomic DNA was extracted following the modified Cetyl trimethylammonium bromide (CTAB) procedure (Saghai-Maroof *et al.,* 1984). A total of 22 random amplified polymorphic DNA (RAPD) primers of Operon Technologies were used. These RAPD primers and their sequences are listed in (Table 2). Reaction mixtures contained 10X buffer, 50 mM $MgCl_2$, 0.1 % , dimethyl sulfoxide (DMSO), 2.5 mM each of dATP, dTTP, dCTP, and dGTP; 5 µM primer; 10 ng of genomic DNA template, ultrapure water and 0.5U of T*aq* polymerase in a reaction mixture of 10 µl. RAPD amplifications were carried out using Applied Biosystems Veriti 96 well PCR thermal cycler (AB, USA) under the following conditions: 94 °C for 3 min, 94 °C for 20 secs, 40 secs at 38 °C, 1 min at 72 °C, repeated 44 times, followed by a final extension period of 5 min at 72 °C and a hold at 4 °C. Amplification products were separated by electrophoresis in 1.5 % (w/v) agarose gels in 1X TBE, and visualized with UV transilluminator after staining with ethidium bromide. Distinct and polymorphic bands were scored manually as present (1) or absent (0) using 50 bp DNA ladder.

Data analysis

Number of alleles per polymorphic locus, the total number of alleles in all polymorphic loci and average number of alleles were estimated. RAPD data was used for calculation of genetic distance (GD) between cultivars using Euclidean distances (Sneath and Sokal, 1973). Estimates of GD was used for construction of dendrogram based on unweighted pair group method using arithmetic averages (UPGMA) clustering and principal component analysis (PCA). The UPGMA and PCA analyses were performed with version 2.01 of the NTSYS-pc package (Rohlf, 1997).

RESULTS

RAPD analysis

Genetic diversity was determined in 17 *C. olitorius* using 22 RAPD markers. The 22 RAPD primers detected a total number of 96 polymorphic alleles (Table 2). The number of alleles per primer ranged from 2 to 10 with an average of 4.36. Fifteen (15) RAPD primers produced 28 unique alleles ranging from 1 to 7 unique alleles in eight *C. olitorius* (EW03, EW05, EW06, EW08, EW09, EW12, EW14, and EW17) (Table 3).

Table 2. RAPD primer sequences and number of alleles in 17 cultivated *Corchorus olitorius* L.

S/N	Primer names	Sequences (5′ - 3′)	No. of alleles
1	OPB01	5′ GTTTCGCTCC 3′	6
2	OPB02	5′ TGATCCCTGG 3′	8
3	OPB03	5′ CATCCCCCTG 3′	10
4	OPB04	5′ GGACTGGAGT 3′	2
5	OPB05	5′ TGCGCCCTTC 3′	6
6	OPB06	5′ TGCTCTGCCC 3′	4
7	OPB07	5′ GGTGACACGG 3′	3

S/N	Primer names	Sequences (5′ - 3′)	No. of alleles
8	OPB08	5′ GTGCACACGG 3′	4
9	OPB10	5′ CTGCTGGGAC 3′	2
10	OPH01	5′ GGTGCGACAA 3′	7
11	OPH02	5′ TCGGACGTGA 3′	3
12	OPH03	5′ AGACGTCCAC 3′	4
13	OPH04	5′GGAAGTCGCC 3′	5
14	OPH05	5′ AGTCGTCCCC 3′	4
15	OPH09	5′ TGTAGCTGGG 3′	4
16	OPH10	5′ CCTACGTCAG 3′	2
17	OPT01	5′ GGGCCACTCA 3′	7
18	OPT02	5′ GGAGAGACTC 3′	3
19	OPT05	5′ GGGTTTGGCA 3′	2
20	OPT07	5′ GGCAGGCTGT 3′	2
21	OPT08	5′ AACGGCGACA 3′	5
22	OPT10	5′ CCTTCGGAAG 3′	3
	TOTAL		96

Table 3. Cultivated *Corchorus olitorius* L. with unique alleles for RAPD markers

S/N	Lines	Number of alleles	Name of primers
1	EW03	1	OPB03
2	EW05	6	OPB01, OPB05*,OPH01, OPH04, OPH07
3	EW06	5	OPB05*, OPB06, OPH01, OPH04,
4	EW08	7	OPB02, OPB06, OPB08,OPH01,OPH03,OPH05, OPT07
5	EW09	2	OPT08*
6	EW12	1	OPB08
7	EW14	1	OPT01
8	EW17	5	OPH03, OPH10,OPT01,OPT08*
	Total	28	
		*RAPD primer produced two unique allele in a species	

Genetic distance, UPGMA and PCA analyses

Genetic distance based on the RAPD markers (Euclidean distance) ranged from 0.05 (EW01 and EW02; EW01 and EW15; EW02 and EW15) to 0.53 (EW06 and EW16) with a GD mean of 0.23. The EW02 and EW15 had similar local names and very lowest genetic distance but were collected from different locations. The *C. olitorius* cultivars with lowest genetic distances do not possess unique alleles. A dendrogram was constructed using the Euclidean distance. RAPD markers separated the cultivated *C. olitorius*. The 17 *C. olitorius* were grouped into two main groups. The majority of the lines (15) were assigned into Group I which included lines collected from the same or different places. The second group consisted of two cultivars (EW05 and EW06) which were collected from the same place (Figure 1). Furthermore, because of the very small genetic relationships between two or three cultivars, the 9 cultivars in group I were further divided into four different subgroups (EW01, EW15 and EW02; EW04 and EW13; EW07 and EW14; EW11 and EW12). Principal Component Analysis (PCA) based on genetic distance separated the *C. olitorius* into a major group. Three lines (EW05, EW06 and EW09) distantly separated from each other and from the major group, formed the second group comparable to the dendrogram (Figure 2).

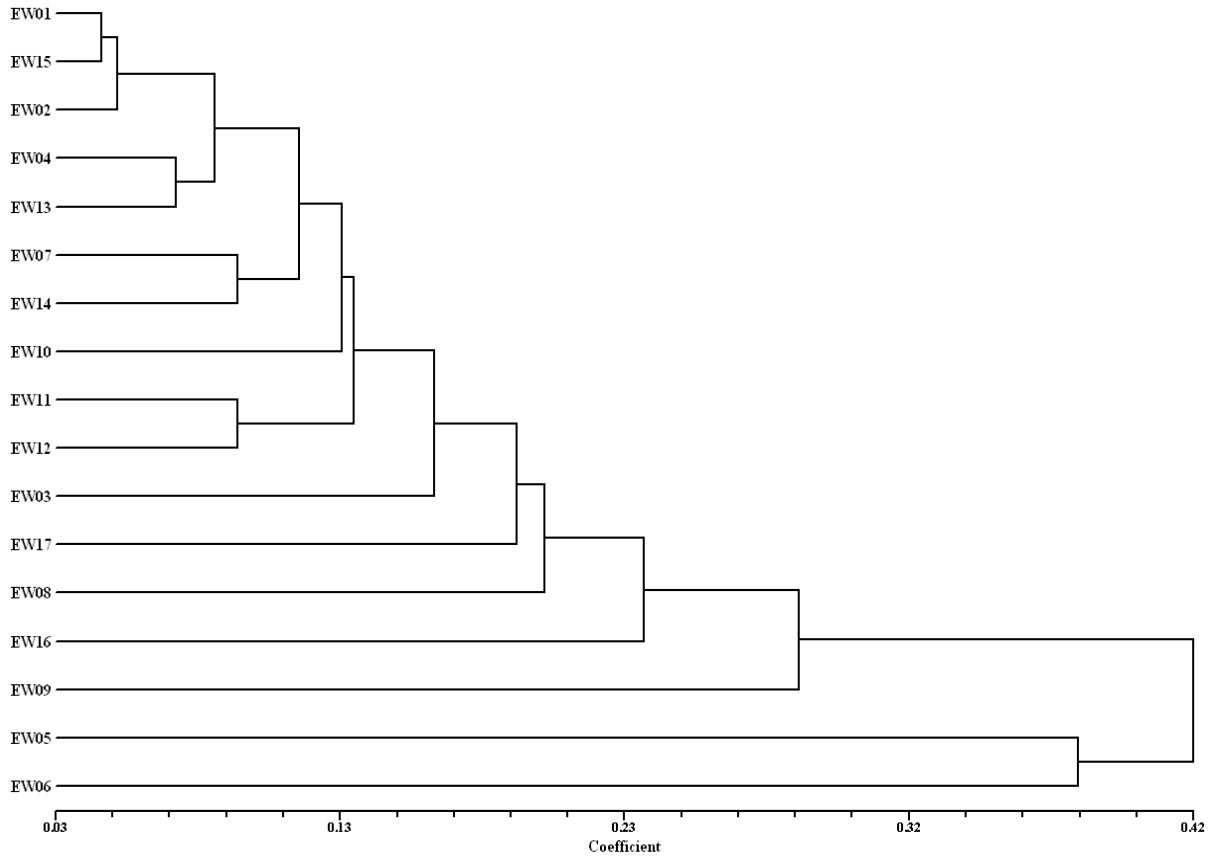

Figure 1: Dendrogram analysis of 17 *Corchorus olitorius* using RAPD-based estimates of Euclidean distance

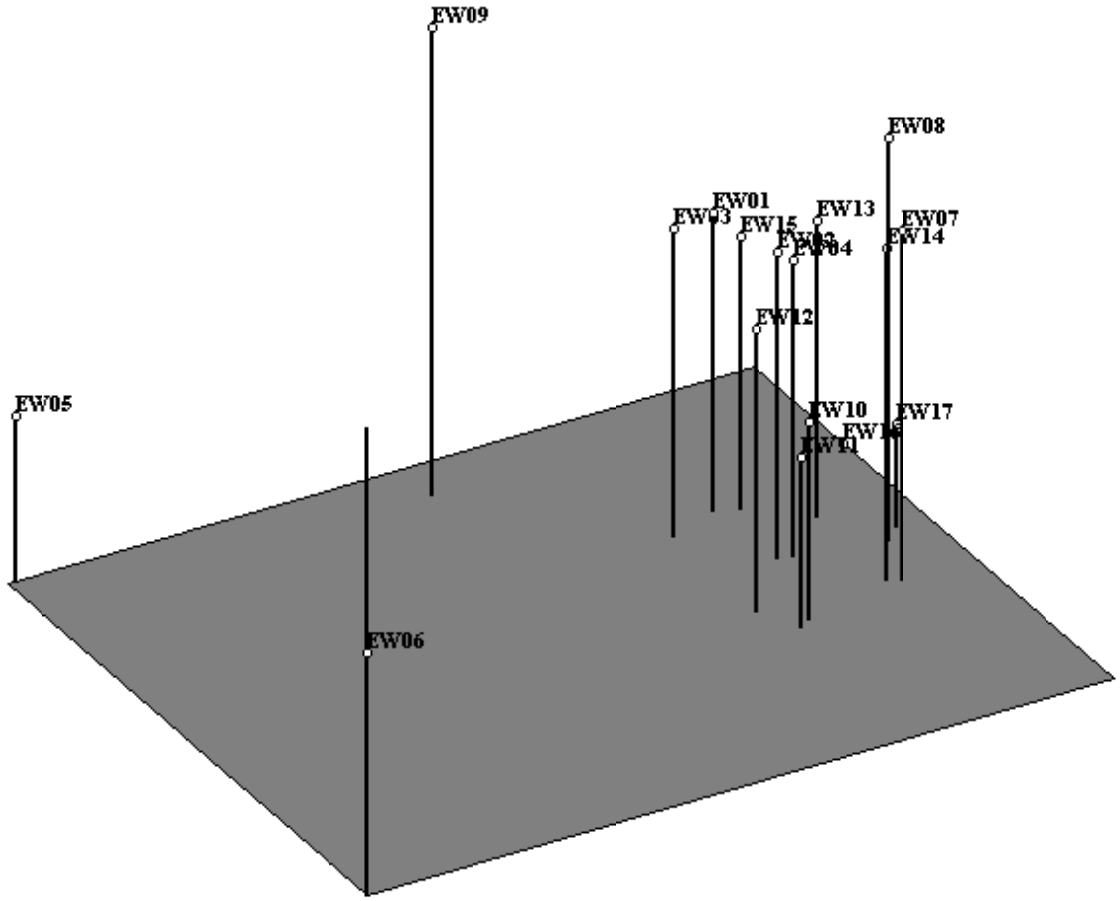

Figure 2: 3D plot of 17 *Corchorus olitorius* determined on the basis of principal component analysis based on RAPD data

DISCUSSION

Genetic diversity analysis can be used to explore whether a set of cultivated species is homogenous or not and to help in germplasm conservation. RAPD analysis uses dominant markers which are inexpensive and simple to use and can detect genetic diversity depending on the types of the primers that are evaluated (Williams *et al.*, 1990). Using RAPD analysis, genetic diversity in 17 cultivated *C. olitorius* was assessed. The average number of alleles (4.36) detected in the present study was moderate. The explanation for this is that the lines used were mainly cultivars within a *C. olitorius* and also due to different RAPD markers used. A study of two *Cochorus* species which are grown in Nigeria indicated that *C. incisifolus* has higher level of allelic diversity than *C. olitorius* (Ogunkanmi *et al.*, 2010).

A high number of unique alleles (29 %) were detected in these lines in spite of great similarity among the cultivars. The presence of unique alleles in some lines might be due differences in origin before

domestication. The unique alleles were detected by15 RAPD markers which can be utilized for cultivar identification in the eight cultivated *C. olitorius*. The diversity study conducted in *C. olitorius* with Amplified Fragment Length Polymorphism (AFLP) by Benor *et al.* (2011) detected high number of private bands (N=35). This study is the first report of identification of RAPD markers that produced unique alleles in cultivated *C. olitorius*. The present analysis also shows the utility and reliability of RAPD markers in molecular analysis for assessment of genetic relationships in *C. olitorius*. Previous studies have shown that RAPD markers are effective for assessing genetic diversity in some crops (Roy *et al.*, 2006).

The RAPD analysis indicated low genetic diversity among cultivated *C. olitorius* and the genetic distance ranged from 0.05 to 0.53 with a mean of 0.23. Genetic diversity has been narrowed in the cultivars during domestication and selection processes. *C. olitorius* being a self pollinating plant may also account for the low genetic diversity. Low genetic diversity has been reported within populations of 61 *C. olitorius* accessions consisting of 34 wild populations and 27 cultivars from Africa and Asia based on molecular analysis (Benor *et al.,* 2011). In the UPGMA dendrogram analysis, the 17 *C. olitorius* cultivars clearly separated into two main groups. Lines such as EW01, EW02 and EW15 that grouped together are genetically highly similar and may share the same ancestral origin. Study on the molecular study of 40 *C. olitorius* grouped the lines into two main groups (Ogunkanmi *et al.,* 2010). Recently, a set of SSR markers have been developed and used to study polymorphism in jute (*Cochorus*) (Mir *et al.,* 2009; Das *et al.,* 2012). SSR markers are co-dominant markers, has high reproducibility and they can detect heterozygotes. Additional studies involving the transferability of the SSR markers and its possibility for genetic diversity assessment of this set of *C. olitorius* for more genetic data at the molecular level will be necessary.

Conservation of *C. olitorius* germplasm is highly necessary to prevent loss of genetic diversity. Extensive seed collection, evaluation and genetic characterisation of *C. olitorius* distributed in South West Nigeria will contribute for its conservation strategy and genetic improvement. Studies on morphological diversity using phenotypic characters and molecular markers can be used to assess genetic relationships among both the wild and commercial populations of *C. olitorius*. The use of Next generation sequencing (NGS) to enhance conservation studies for *C. olitorius* can be an alternative approach.

Furthermore, germplasm conservation based on gene banks is also important to be put in place. Geographical distribution of *C. olitorius* genetic resources in Nigeria needs to be mapped for *in situ* conservation. More research efforts in the areas of development of new varieties are necessary.

CONCLUSIONS

In summary, this study assessed the genetic diversity among the 17 *C. olitorius* grown in South West Nigeria. Based on RAPD data, extremely low genetic diversity was revealed among cultivated lines. Genetic diversity has been narrowed in these cultivars suggesting possible effects of domestication and selection processes. This information will be useful for germplasm conservation, in cultivar identification and can also assist in genetic crossing studies in *C. olitorius*. Strategies for the conservation of *C. olitorius* germplasm in the South-West Nigeria are necessary.

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التنوع الوراثي في نبات الملوخية (*Corchorus olitorius*) في الجنوب الغربي من نيجيريا بأستخدام طريقة (RAPD)

اويناك اديمو[1] و ابراهام اباطي[1]

ملخص

تم في هذه الدراسة التنوع الوراثي ما بين 17 صنفاً من نبات الملوخية (*Corchorus olitorius*) والذي ينمو في الجنوب الغربي من نيجيريا بأستخدام طريقة ال (RAPD) . عند استخدام 22 نوع من البادئات تم التعرف على 96 من أليلات المتعددة الأشكال (بمتوسط 4.36 لكل بادئ) بالاضافة لعدد كبير من الأليلات الفريدة (بمتوسط 29 ٪) في ثمانية أصناف. استنادا إلى نتائج ال RAPD وجد أن المسافة الجينية تراوحت ما بين 0،05 الى 0،53 بمتوسط 0.23، مما يدل على وجود تباين وراثي منخفض ما بين الأصناف . باستخدام أسلوب المزاوجة الحسابي المتوسط (UPGMA) على نتائج ال RAPD، تم تقسيم الأصناف المدروسة إلى مجموعتين رئيسيتين حيث احتوت المجموعة الاولى 15 صنفاً والتي تم جمعها من نفس المكان أو اماكن مختلفة. في حين تألفت المجموعة الثانية من صنفين فقط (EW05 وEW06) و اللذان تم جمعها من نفس المكان. بالأضافة تم تحليل المكونات الرئيسة باستخدام ال(PCA) على أساس المسافة الوراثية، حيث تم ايضا فصل الأصناف إلى مجموعتين. إن النتائج المعروضة تبين وجود خسارة في التنوع الوراثي في هذه الأصناف مما يدل على وجود تأثيرات لعمليات تدجين واختيار في مناطق الجنوب الغربي من نيجيريا.

الكلمات الدالة: *Corchorus olitorius* L ، تحليل الكتلة، التنوع الوراثي، تحليل المكون الرئيس، وبادئات RAPD.

[1]. قسم بيولوجيا الخلية والجينات، جامعة لاجوز، اكوكا، لاقوز، نيجيريا
adeyemonik@yahoo.co.uk

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