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Assessment of Genetic Relationships Between Two Species of Jute Plants Using Phenotypic and RAPD Markers

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Abstracts: The genus *Corchorus* is one of the most important fibre crops, in order to improve this largely neglected crop, it is essential to understand the pattern of diversity in this important crop species. In this study, inter and intra genetic diversity analysis of 40 genotypes from *C. olitorius* and 40 genotypes from *C. incisifolus* was carried out using sixteen RAPD markers. The molecular study of the two species classified *C. olitorius* into two main clusters with five genotypes unresolved while there are three clusters with all the genotypes clearly separated in *C. incisifolus*. The results of both inter and intra genetic diversities revealed a higher level of allelic diversity in *C. incisifolus* than in *C. olitorius*. In the morphological study, higher mean and higher upper range values for all the five quantitative traits in *C. incisifolus* indicated its superiority over *C. olitorius*. However, both species show divergent attributes in four qualitative traits but similar in two characters. This could be attributed to higher level of natural outcrossing in *C. incisifolus* relative to that of *C. olitorius*. Results of this study therefore show that RAPD marker is a useful tool for assessing inter and intra genetic diversity in the two species of *Corchorus*. Therefore selection could be made from the diverse genotypes as parents for crosses designed for breeding improved jute cultivars and for producing mapping populations for QTL analysis.

Key words: Jute, RAPD, interspecific, intraspecific, *Corchorus*, dendrogram

INTRODUCTION

The Genus *Corchorus* consists of some 40-100 species, of which about 30 are found in Africa (Makinde *et al.*, 2009). It belongs to the family Tiliaceae and the chromosome number is 2n-14. Several species of *Corchorus* are used as a vegetable, of which *Corchorus olitorius* is most frequently cultivated as vegetable in Nigeria.

Corchorus olitorius, also called Bush Okra, Jews mallow or jute mallow in English, Krin Krin in French speaking area of West Africa, Mlenda in Swahili, tege in Cameroon, derere in Zimbabwe and Otigo in Uganda (Roy *et al.*, 2006). Some Nigerian names include ewedu in Yoruba, aluhara in Igbo, where as the Hausa people call it malafiya.

While the Indo-Burma region, including South China, is the center of origin for *C. capsularis*, Africa is the center of origin for *C. olitorius* (Roy *et al.*, 2006). These two species constitute an important crop of the South

East Asian countries and Brazil, providing environment-friendly (biodegradable and renewable) ligno-cellulose fiber. Jute fiber is second in importance only to cotton and was also identified as an alternative to European hemp (*Cannabis sativa* L.) as early as the end of 18th century.

However, the presence in Africa of more wild *Corchorus* species and the larger genetic diversity within *C. olitorius* point to Africa as the first centre of origin of the genus, with a secondary centre of diversity in the Indo-Burmese region. At present, *Corchorus olitorius* is widely spread all over the tropics and it probably occurs in all countries of tropical Africa. In tropical Africa, it is a leading leaf vegetable in Coted'Ivoire, Benin, Nigeria, Cameroon, Sudan, Kenya, Uganda and Zimbabwe (Makinde *et al.*, 2009).

The two most common types in Nigeria are Oniyaya, widely branched with broad, deeply serrated (*Corchorus incisifolus*) leaves and Amugbadu, a plant growing even taller with large, finely serrated (*Corchorus olitorius*) leaves that are oblong in shape Among the Yoruba

speaking group of Southwestern Nigeria, there is a preference for oniyaya because of the belief that it is more mucilaginous. In Cameroon and other West African countries, there are numerous local types varying among others in height, stem color, leaf and fruit shape. This Oniyaya in Cameroon is more deeply lobed than the Oniyaya in Nigeria (although there are overlaps). A large, broad-leaved variety from Cameroon with shorter swallow tails that is commonly cultivated near Yaoundé is called Greant de Bertoua. Its leaf tips are more round than those of Amugbadu, the most common one in Nigeria, which resembles the Cameroonian Ewondo (Pal *et al.*, 2006).

Fresh leaves of *C. olitorius* are rich source of vitamins A and C. The small amount of protein present is rich in methionine. The leaves are used in the treatment of chronic cystitis, gonorrhoea, dysuria, for toothache (Hillocks, 1998). The seeds are used for fever and as a purgative; they possess broad antibacterial properties (Pal *et al.*, 2006).

There are several other minor local morphotypes, for example Eleti ehoru with small ovate leaves like the ear of a hare, oblong and with fine serration and Eti eku' with a leaf shape like the ear of a rat. Another Nigerian variety is Yaga (Makinde *et al.*, 2009).

In Nigeria, little or no molecular study is known on the diversity of *Corchorus*, most researches are expressed in vegetative and physiological characteristics. However, such traits are limited in number and are often influenced by the environment, thus making them unsuitable for correct assessment of the genetic diversity. This limitation can be largely overcome by the use of molecular markers, which are unlimited in number and are not influenced by the environmental factors, growth stages and agronomic practices (Reyazul *et al.*, 2009).

The present study was carried out to determine the genetic diversity between and within the two cultivated species using RAPD markers.

MATERIALS AND METHOD

Plant collection: Two sets of jute genotypes were collected from National Horticultural Research Institute (NIHORT), Ibadan Nigeria in 2009. The first set belongs to *C. olitorius* while the second set belongs to *C. incisifolus*. The seeds were tied in a piece of cotton cloth immersed in warmed water for 20 sec to break dormancy and allowed to dry before planting. The two species were separated apart in the same screen house in the biological garden of University of Lagos, Nigeria. The planting date was noted and plants were monitored till seedling emergence and data recorded at fully grown

period to detect the true picture of the adult leaf for finely or deeply serrated margin as this is the main descriptor that distinguishes the two species.

Phenotypic data were recorded on 10 random plants of each genotype using the following traits: growth habits, leaf margin, leaf color, leaf shape, days to flowering, leaf length, leaf width, number of leaves per plant, petiole length and stem length.

DNA extraction: DNA was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thompson, 1980) and purified by RNase A treatment followed by extraction using phenol and chloroform. The mucilage and polyphenolic compounds were removed by passing DNA through an Au-Prep purification column (Life Technologies, Rockville, MD, USA). The DNA was quantified using 1% agarose gel stained with ethidium bromide in the presence of different concentrations of undigested λ -DNA and a final concentration of 25 ng μL^{-1} was used for PCR.

Polymerase chain reaction (PCR) and data analysis:

DNA samples were subjected to PCR amplification with sixteen RAPD primers (Table 1). The total volume of the PCR reaction was 25 μL , which contained 5 μL of template DNA, 2.5 μL of 10 x buffer (10 mM), 2.00 μL MgCl_2 (25 mM), 2.5 μL of 5% Tween 20, 1.0 μL of 2.5 mM dNTPs, 0.4 μL of Taq polymerase (Bioline), 1.0 μL of RAPD primer and the remaining 10.6 μL was made up of ultra pure water. The PCR cycle was carried out with a Techne gradient cycler with the initial denaturation at 94°C for 3 min followed by 45 cycles of 94°C for 20 sec, 37°C annealing for 40 sec and 72°C for 1 min. It was followed with 72°C for a 10 min extension and stored at 4°C. The PCR products were viewed on 2% UltraPure agarose

Table 1: List and sequence of primers used for the amplification of DNA from the two species *C. olitorius* and *C. incisifolus*

Primer code	Nucleotide sequence 5' to 3'	No. of scorable bands
OPAE 10	CTCAAGCGCA	9
OPAG 20	TGCGCTCCTC	5
OPAF 20	CTCCGCACAG	5
OPAC 20	ACGGAAGTGG	6
OPAE 01	TGAGGGCCGT	11
OPAE 03	CATAGACGG	9
OPAF 02	CAGCCGAGAA	7
OPAC 15	TGCCGTGAGA	12
OPAE 02	CTCTGGCGA	7
OPAE 15	CACGAACCTC	4
OPAE 02	TCGTTACCC	7
OPAC 07	GTGCCGATC	3
OPAC 1	GACGCGATTG	8
OPAD 20	TCTTCGGAGG	3
OPAE 10	TGTCAGTGGC	13
OPAC 10	AGTCCGCTG	7
Total No. of bands scored		116

(invitrogen) gels stained with ethidium bromide. Clear polymorphic bands were scored as 1- present and 0- absence of bands. From sixteen primers, one hundred and sixteen bands were scored and this was analyzed using NTSYS version 2.02j to generate the dendrograms. Statistical packages from excel was used in calculating the mean, standard deviation and Coefficient of Variation (CV).

RESULTS AND DISCUSSIONS

Morphological study: Phenotypic variability and interrelationship among the characters studied show the mean values for stem length and four other traits in each of the two species, *C. olitorius* and *C. incisifolus*, having continuous distribution. However, *C. incisifolus* revealed a higher mean in all the traits except the number of leaves per plant where the other species has a higher value for mean (Table 2). This point to the fact that *C. olitorius* may be better as vegetables than *C. incisifolus*. Higher mean and higher upper range values for all the five traits in *C. incisifolus* indicated its superiority over *C. olitorius* and confirmed results of an earlier study (Ali *et al.*, 2002; Ali, 1994).

The relative variability for the five traits also differed with highest variability (CV) for petiole length in *C. olitorius* followed by number of leaves per plant in *C. incisifolus*. The variability for two (leaf length and width) of the remaining three traits was relatively low. Among these five traits, *C. incisifolus* has a higher CV in two (stem length and number of leaves per plant) while the other species has a higher CV in three traits. Since stem length is an important component of fibre yield, *C. incisifolus* may be a desirable species to look for, for fibre yield and perhaps for improvement of plant height (Reyazul *et al.*, 2008; Haque *et al.*, 2008). It is also known that tossa jute (*C. olitorius*) is relatively more tolerant to diseases and pests with low resistance to floods and drought (Reyazul *et al.*, 2008; Roy *et al.*, 2006). Therefore, interspecific genetic variability needs to be exploited for developing genotypes with desirable attributes of both species. In the qualitative traits, both species have the

same attribute in plant habit and type of cotyledon which are erect and epigeal respectively. However, they differ in the other four traits; *C. olitorius* has light green glossy leaves, crenated leaf margin, late flowering and elliptic leaf shape while the other species has dark green leaves, serrated leaf margin, early flowering and hastiform leaf shape.

Molecular study

Evaluation of genetic diversity: The cluster analysis separated all the genotypes of *C. olitorius* (Fig. 1) except five genotypes at 1.00 similarity level while they were grouped as one cluster at similarity coefficient 0.74. Levels between these two extremes gave rise to different clusters in the dendrogram. As the similarity index decreases, the number of clusters and sub clusters formed reduces with the merging of clusters and sub clusters. However, in cluster 1, five genotypes (B12, B13, B8, B9 and B15) could not be differentiated, as they are similar at 100% similarity level and hence needs further clarification with more robust marker such as SNP or SSR.. Cluster 2 is small group with 4 genotypes, all differentiated and no tie genotypes. However, the dendrogram (Fig. 2) for *C. incisifolus* showed a different pattern from the above, all the genotypes were clearly differentiated with no single tie among them at 1.00 similarity coefficient while they were grouped in more than one clusters at similarity index 0.64 with some genotypes clearly separated from the clusters. There are 3 clusters, cluster 1 with seven genotypes, cluster 2 with nine while cluster 3 with just

Table 2: Summary of data on range, mean and coefficient of variation (CV) for five morphological traits based on genotypes from two *Corchorus* species (*C. olitorius* and *C. incisifolus*)

Statistics	Species	Stem length (m)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	No. of leaves per plant
Range	<i>C. olitorius</i>	0.8-1.4	2.7-4.7	1.8-2.8	0.5-1.6	8.0-13.0
	<i>C. incisifolus</i>	0.8-1.4	3.0-5.0	2.4-3.0	1.1-2.0	5.0-11.0
Mean	<i>C. olitorius</i>	1.00	3.64	2.3	1.16	9.3
	<i>C. incisifolus</i>	1.04	4.08	3.0	1.44	7.8
CV (%)	<i>C. olitorius</i>	19.94	18.58	17.35	32.92	18.37
	<i>C. incisifolus</i>	20.21	16.48	15.00	24.95	26.60

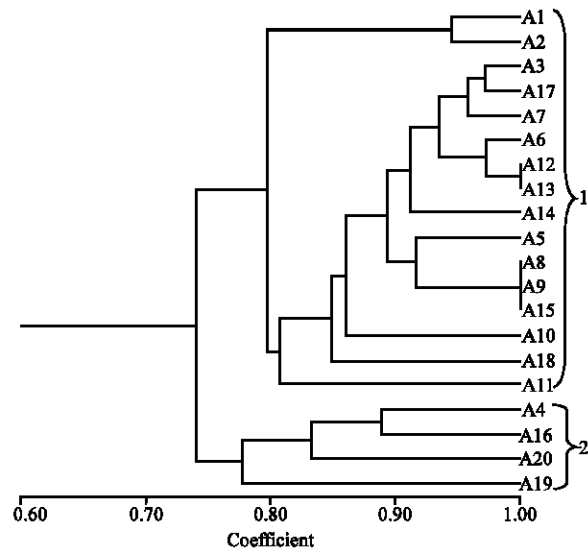


Fig. 1: A UPGMA dendrogram as revealed by sixteen RAPD primers using twenty genotype within *C. olitorius*

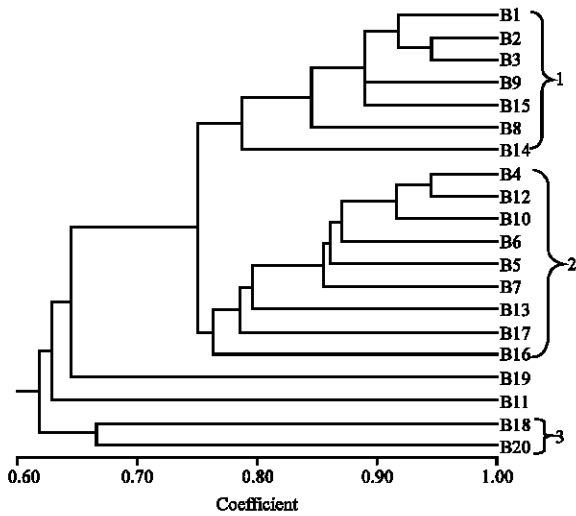


Fig. 2: Dendrogram showing clustering analysis within twenty genotypes of *C. incisifolus* using sixteen RAPD primers

only two genotypes. The genotypes B11 and B19 showed a greater distinction from others as they did not group with any of the clusters. The results of the present study revealed a higher level of allelic diversity in *C. incisifolus* than in *C. olitorius*. This may be attributed to higher level of natural outcrossing in *C. incisifolus* relative to that of *C. olitorius*. This again suggested the need for exploitation of interspecific genetic diversity for jute improvement. Clustering of genotypes from *C. olitorius* with genotypes from *C. incisifolus* (Fig. 3) correlate well with the results from intra genetic study above. Cluster 1 contained all the genotypes from *C. olitorius* with two genotypes (I32 and I27) from *C. incisifolus* grouping with them while cluster 2 comprised mainly *C. incisifolus* species. The inclusion of the two genotypes from *C. incisifolus* in cluster 1 needs further study to ascertain the identity of each one, perhaps it's a mixed up, mishandling or contamination. This again revealed a greater diversity in *C. incisifolus* than *C. olitorius*. It could be noticed that within *C. incisifolus*, genotype 11

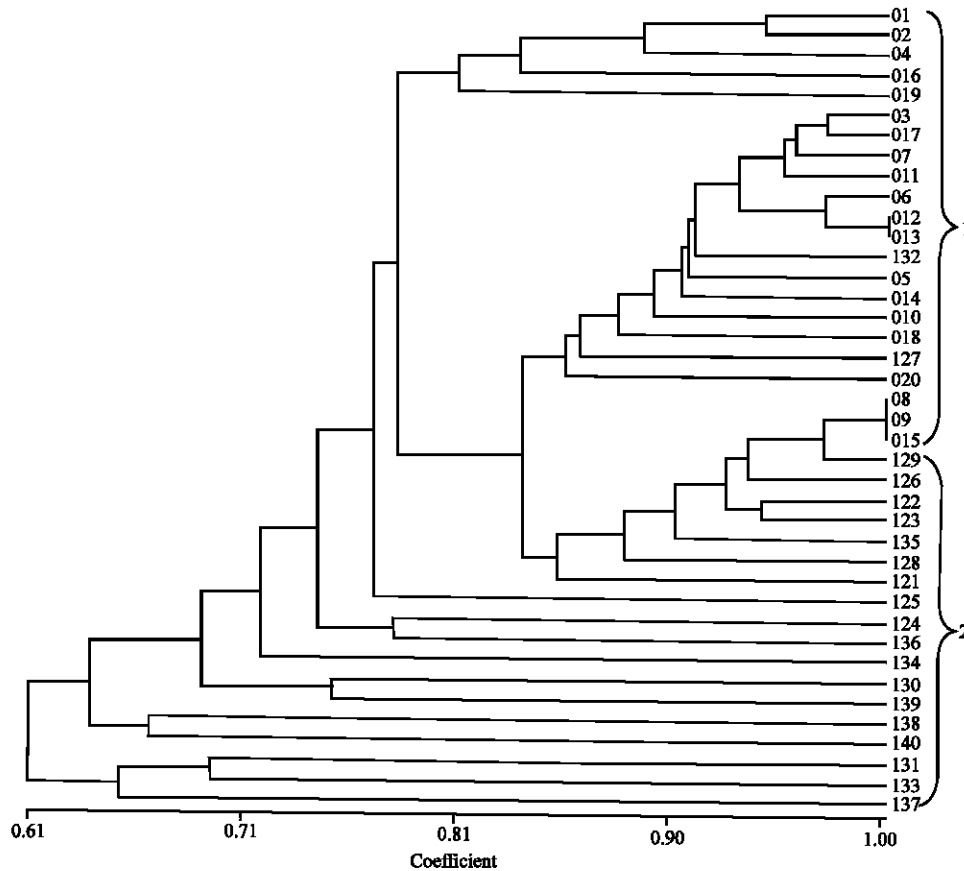


Fig. 3: A dendrogram generated from sixteen RAPD primers with forty genotypes from both species *C. olitorius* and *C. incisifolus*. Codes O1-O20 represent genotypes from *C. olitorius* while I21-I40 represent genotypes from *C. incisifolus*

and 19 (Fig. 2) and genotypes 31 and 39 (Fig. 3) were most divergent. These pairs of divergent genotypes may be involved in intraspecific crosses for getting improved cultivars of white and tossa jutes. Interspecific crosses between these two diverse genotypes with *C. oltorius* may also help combining desirable attributes from the two species, since interspecific hybridization has been successfully attempted and utilized in the past (Islam and Rashid, 1960; Swaminathan *et al.*, 1961; Islam, 1964; Sinha *et al.*, 2004).

CONCLUSION

It is hoped that in future present results will afford breeders opportunity to make selection from diverse genotypes of the two species studied and use them as parents for crosses that are designed for breeding improved jute cultivars and for producing mapping populations. The ongoing development of SSR and SNP markers may be a better tool in resolving the tied genotypes and utilization of these genotypes in breeding programme for construction of a genetic linkage map, QTL mapping and marker assisted selection in jute cultivars.

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