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DNA Fingerprinting of Jute Germplasm by RAPD

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The genotype characteristic of cultivars was investigated, along with varieties of both of the jute species, *Corchorus olitorius* and *Corchorus capsularis*, in the germplasm collection at the Bangladesh Jute Research Institute (BJRI). DNA fingerprinting was generated for 9 different varieties and 12 accessions of jute cultivars by using random amplified polymorphic DNA (RAPD). A total of 29 arbitrary oligonucleotide primers were screened. Seven primers gave polymorphism within the varieties, and 6 primers detected polymorphism within the accessions that were tested. A dendrogram was engendered from these data, and this gave a distinct clustering of the cultivated species of jute. Therefore, we generated RAPD markers, which are species-specific. These primers can distinguish between *C. olitorius* and *C. capsularis*. From the dendrogram that we generated between the various members of these two species, we found the existing genetic classification that agrees with our molecular marking data. A different dendrogram showed that jute accessions could be clustered into three groups. These data will be invaluable in the conservation and utilization of the genetic pool in the germplasm collection.

Keywords: Jute germplasm, Genetic diversity

Introduction

The jute plant is an annual shrub. It contains 7 pairs of chromosomes and belongs to the plant family *Tiliacea*. It is a natural inhabitant of the tropical and subtropical regions of the world. The commerce fiber is obtained from the phloem tissue in the stem of cultivated varieties of the two species, *Corchorus olitorius* and *Corchorus capsularis*. Commercially *Corchorus olitorius* is known as tossa. *Corchorus capsularis* is known as white jute. Genetic variability is limited in both species, due to self-pollination.

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Bangladesh has the largest gene bank of JAF (Jute and Allied Fibers) crops. Although a number of varieties have been experimentally developed from intervarietal crosses, none were stable enough to be commercially released (Islam *et al*., 1992).

In 1998, a devastating flood damaged virtually all of the Bangladesh jute plants, which left no seeds for sowing in the following years. To meet the demand of the local jute farmers, most of the jute seeds were imported. Unscrupulous businessmen supplied the farmers with low-quality seeds that had an appearance that was identical to the high-quality ones. This resulted in a poor harvest in 1999.

In the case of jute, no published data on the genome of any cultivars or wild species is available. In order to solve the problems, like the one mentioned previously, it is time to consider DNA marking technology for the study of these plants. This knowledge will be useful for designing and accelerating breeding programs, as well as to test seeds for their authenticity.

Random-amplified polymorphic DNA (RAPD) markers (Williams *et al*., 1990) have been successfully used for cultivar analysis in a number of plant species. These include the following: broccoli and cauliflower (Hu and Quiros 1991; Kresovich *et al*., 1992), cocoa (Wilde *et al*., 1992), banana (Kaemmer *et al*., 1992; Howell *et al*., 1994), papaya (Stiles *et al*., 1993), apple (Koller *et al*., 1993), celery (Yang and Quiros *et al*., 1993), onion (Wilkie *et al*., 1993), cranberry (Novy *et al*., 1994), sunflower (Lawson *et al*., 1994), soybean (Powel 1996; Lin, 1996), rice (Takenchi, 1994), rose (Denbener *et al*., 1996), maize (Yazaki *et al*., 1994), and *Brassica juncea* (Fujushiro *et al*., 1994).

RAPD primers have been used for the germplasm characterization of kenaf (Cheng *et al*., 2000) and roselle (Hanboonsong *et al*., 2000), two members of the JAF family. Kenaf varieties can be clearly distinguished, based on their RAPD pattern. Workers found that RAPD markers are sufficient to establish variability. The dendrogram of kenaf varieties shows a large genetic diversity among the varieties of different origins, as compared to those with close origins (Cheng *et al*., 2000). To conserve the roselle genetic pool for breeding purposes, the genotype characteristics of both the

wild and cultivar varieties in the germplasm collection were investigated by Hanboonsong *et al.* (2000). By using RAPD, a DNA fingerprint of 55 roselle varieties was generated. The results showed that 4 groups of roselle varieties could be clustered. However, the variation was minimal, which suggests a close relationship between all varieties.

In the present study, polymorphism was generated using RAPD primers. RAPD patterns were analyzed to identify jute varieties and accessions. A comparative analysis of the genetic variability of the jute plants was also conducted.

Materials and Methods

Plant materials Nine jute varieties, which were collected from 3 countries and 12 accessions that were collected locally (Table 1), were included in the present analysis. From these varieties, seeds and leaves were used for DNA isolation.

DNA extraction By grinding in the presence of liquid nitrogen before DNA was extracted using the CTAB procedure, which is a modified version of Doyle and Doyle's method (1990), 1.0-1.5 g of tissue was homogenized. 5.0 ml CTAB extraction buffer was added and incubated at 60°C for 30 min. One volume of phenol : chloroform : isoamylalcohol (25 : 24 : 1) was added, the mixture was shaken, then centrifuged at 14000 rpm for 10 min at room temperature. The supernatant was then transferred to a new tube. The DNA was precipitated with ice-cold isopropanol. The DNA pellet was washed in 70% ethanol, dried, and dissolved in a TE buffer (Tris-HCl_{10mM}, $EDTA_{1mM}$). After RNase treatment, the DNA was treated with one volume of phenol:chloroform:isoamylalcohol (25 : 24 : 1). The same procedure was also followed for the supernatant. The DNA was precipitated with $1/10^{th}$ volume of 3M Na-acetate and a double volume of ice-cold 99% ethanol. After washing with 70% ethanol, the DNA was dried, dissolved in a TE buffer, and stored at −20°C.

Table 1. The Jute Samples used in this study

No.	Species	Variety	Source
1	*C. olitorius*	O-4	Bangladesh
2	*C. olitorius*	O-9897	Hybridization between var. O-5 and Brazilian var. BZ-5
3	*C. olitorius*	*C. olitorius* var. Incisifolius	Egypt
4	*C. capsularis*	CC-45	Egypt
5	*C. capsularis*	CVE-3	Thailand
6	*C. capsularis*	BJC-7370	Bangladesh
7	*C. capsularis*	D-154	Bangladesh
8	*C. capsularis*	CVL-1	Bangladesh
9	*C. capsularis*	BJC-83	Bangladesh

N.B. All the accessions were collected from Rangpur, Bangladesh and classified as *Corchorus capsularis* using taxonomic studies by Gene Bank, Bangladesh Jute Research Institute.

Amplification conditions For DNA amplification, the following primers were used: 29 arbitrary decamer primers from Operon Technologies, USA; 6 primers from Set OPAB (01, 03, 05, 06, 12 and 18); 3 primers from Set OPE (01, 13 and 17); 6 primers from Set OPG (03, 05, 06, 07, 09 and 15); 6 primers from Set OPH (02, 07, 12, 13, 14 and 15); 2 primers from Set OPN (02 and 13); 4 primers from Set OPQ (01, 05, 07 and 17); 1 primer from Set OPBC-09; and 1 primer from Set OPS-14 (Table 2). The reaction mixture (25 µl) contained the following: 1Xreaction buffer (20mM Tris-HCl pH 8.4, 50 mM KCl), 0.2 mM dNTPs, 2 mM $MgCl_2$, 60 ng primer, 1.0 unit of Taq DNA polymerase, and 25-40 ng genomic DNA. DNA was amplified in a thermal cycler (Eppendorf Mastercycler Gradient) that was programmed as follows: after preheating for 5 min at 94°C; 45 cycles of 0.45 min at 94°C (denaturation), 1 min at 37°C (annealing) and 1.50 min at 72°C (extension), and a final extension at 72°C for 7 min that was followed by cooling to 4°C.

RAPD analysis and determination of relatedness Amplified DNA samples were analyzed by electrophoresis on 2% agarose gel in a 1Xelectrophoresis buffer (Tris-$acetate_{40mM}$-$EDTA_{1mM}$). The gels

Table 2. Name and the sequences of the RAPD primers (Operon Technologies, USA).

Name of the primers	Sequence of primers (5′ to 3′)
OPAB-01	CCGTCGGTAG
OPAB-03	TGGCGCACAC
OPAB-05	CCCGAAGCGA
OPAB-06	GTGGCTTGGA
OPAB-12	CCTGTACCGA
OPAB-18	CTGGCGTGTC
OPBC-09	GTCATGCGAC
OPE-01	CCCAAGGTCC
OPE-13	CCCGATTGCG
OPE-17	CTACTGCCGT
OPG-03	GAGCCCTCCA
OPG-05	AGTCGTCCCC
OPG-06	GTGCCTAACC
OPG-07	GAACCTGCGG
OPG-09	CTGACGTCAC
OPG-15	ACTGGGACTC
OPH-02	TCGGACGTGA
OPH-07	CTGCATCGTG
OPH-12	ACGCGCATGT
OPH-13	GACGCCACAC
OPH-14	ACCAGGTTGC
OPH-15	AATGGCGCAG
OPN-02	ACCAGGGGCA
OPN-13	AGCGTCACTC
OPQ-01	GGGACGGATG
OPQ-05	CCGCGTCTTG
OPQ-07	CCCCGATGGT
OPQ-17	GAAGCCCTTG
OPS-14	AAAGGGGTCC

were stained with ethidium bromide and visualized under UV light. Only intense clearly-resolved amplification products that were reproducible in multiple runs were considered for further analysis.

The DNA fragments that were amplified by a given primer were scored as present '1' or absent '0' for all of the cultivars that were studied. A cluster analysis was accomplished using the software STATISTICA: Cluster Analysis (StatSoft 1994).

Results

Two species of jute, *Corchorus capsularis* and *Corchorus olitorius*, were used in this study. Amplification of genomic DNA from each of the 9 jute varieties and 12 accessions using all 29 primers, revealed a variety of RAPD patterns. They had a total of 107 scorable DNA fragments or RAPD markers that were observed, of which 28 (26%) were polymorphic among the jute samples. Among these 29 primers, 7 were able to generate genetic variability between the different cultivars of jute. They were as follows: OPAB 03, OPAB 06, OPG 05, OPG 06, OPG 09, OPG 15, and OPH 13. Six primers gave polymorphism within the jute accessions. They were as follows: OPAB 03, OPAB 05, OPG 03, OPG 05, OPG 06, and OPQ 17 (Tables 3 and 4).

For the different jute samples, Tables 5 and 6 show the differences in the number of bands that were obtained by the RAPD primers that were used in the construction of the dendrogram, as well as for determining their genetic distances. These tables show two triangular sections. The upper portion of triangle shows the number of band differences between the pairs of jute samples, and the lower triangle shows the distances between the jute varieties and accessions, respectively. From these data, we could easily distinguish between the *C. olitorius* and *C. capsularis* varieties. Distances between O-4, O-9897 and *incisifolius* were found to be closer than distances between these *olitorius* varieties with other *capsularis* ones. Another table (Table 6) for distances between the jute accessions showed that Acc. No. 702 and 703 were closer to each other than the other accessions were.

Squared Euclidean distances (Tables 5 and 6) were used to analyze the genetic distances between the jute varieties and accessions for the construction of 2 different dendrograms (Figs. 1 and 2). The resulting dendrogram showed a differentiation of the jute varieties into 2 clusters that represented the 2 different species, *C. olitorius* and *C. capsularis.* Another dendrogram, using a similar approach, was generated for the different accessions of jute (Fig. 2). The jute accessions could also be clearly distinguished, as revealed by their distinctive RAPD patterns. The dendrogram showed a differentiation with 3 clusters - the first with Acc. Nos. 701, 706, and 711, and the second with Acc. Nos. 705 and 710 - the rest were found to be in another cluster.

Discussion

From the present data, the molecular markers that are generated can identify DNA of jute varieties and accessions that are amplified with 13 primers. The experiments were

Table 3. The number of fragments scored (with the polymorphic bands) for jute varieties with 7 RAPD primers

Primer Name	Number of bands										
	O-4	O-9897	Incisi-folius	CC-45	CVE-3	BJC-7370	D-154	CVL-1	BJC-83	Total	Polym-orphic
OPAB-03	3	6	4	6	3	4	5	4	3	38	2
OPAB-06	5	6	6	5	7	7	7	7	7	57	3
OPG-05	2	4	4	6	4	6	2	0	3	31	1
OPG-06	6	6	5	6	6	6	6	6	6	53	2
OPG-09	3	5	5	6	9	9	9	9	6	61	4
OPG-15	6	6	5	5	5	5	6	6	5	49	3
OPH-13	6	8	4	5	5	5	5	4	4	46	3

Table 4. The number of fragments scored (with the polymorphic bands) for accessions of jute varieties with 6 RAPD primers

Primer Name	Number of bands													
	Acc. no.-701	Acc. no.-702	Acc. no.-703	Acc. no.-704	Acc. no.-705	Acc. no.-706	Acc. no.-707	Acc. no.-708	Acc. no.-709	Acc. no.-710	Acc. no.-711	Acc. no.-712	Total	Poly-morphic
OPAB-03	4	5	5	5	4	-	4	5	4	6	3	5	50	2
OPAB-05	7	6	7	7	2	2	7	4	5	1	8	6	62	2
OPG-03	6	6	6	6	3	6	6	6	6	2	6	5	64	1
OPG-05	2	3	3	5	-	4	5	4	5	2	5	5	43	3
OPG-06	1	4	3	4	3	-	5	5	5	5	-	3	38	1
OPQ-17	3	4	5	5	2	5	6	5	4	1	5	4	49	1

Table 5. Number of Band differences and Squared Euclidean distances between Jute Varieties

	Number of band differences between different jute varieties								
Variable	O-4	O-9897	Inc.	CC-45	CVE-3	BJC-7370	D-154	CVL-1	BJC-83
O-4	**.0**	10	2	8	8	11	9	5	3
O-9897	10.0	**.0**	8	2	2	1	1	5	7
Incisifolius	16.0	18.0	**.0**	6	6	9	7	3	1
CC-45	38.0	36.0	30.0	**.0**	0	3	1	3	5
CVE-3	34.0	38.0	32.0	20.0	**.0**	3	1	3	5
BJC-7370	39.0	41.0	33.0	15.0	5.0	**.0**	2	6	8
D-154	38.0	38.0	34.0	18.0	8.0	7.0	**.0**	4	6
CVL-1	35.0	39.0	33.0	23.0	9.0	8.0	5.0	**.0**	2
BJC-83	27.0	31.0	21.0	15.0	17.0	18.0	21.0	18.0	**.0**
	Squared Euclidean distances								

Table 6. Number of Band differences and Squared Euclidean distances between Jute Accessions

	Number of band differences between different jute accessions											
Variable	Acc. No. 701	Acc. No. 702	Acc. No. 703	Acc. No. 704	Acc. No. 705	Acc. No. 706	Acc. No. 707	Acc. No. 708	Acc. No. 709	Acc. No. 710	Acc. No. 711	Acc. No. 712
Acc. No. 701	**.0**	5	6	9	9	6	10	6	6	6	4	5
Acc. No. 702	11.0	**.0**	1	4	14	11	5	1	1	11	1	0
Acc. No. 703	10.0	3.0	**.0**	3	15	12	4	0	0	12	2	1
Acc. No. 704	17.0	8.0	7.0	**.0**	18	15	1	3	3	15	5	4
Acc. No. 705	15.0	18.0	19.0	24.0	**.0**	3	19	15	15	3	13	14
Acc. No. 706	13.0	14.0	15.0	18.0	16.0	**.0**	16	12	12	0	10	11
Acc. No. 707	16.0	9.0	8.0	9.0	23.0	15.0	**.0**	4	4	16	6	5
Acc. No. 708	14.0	5.0	8.0	11.0	17.0	11.0	8.0	**.0**	0	12	2	1
Acc. No. 709	12.0	7.0	8.0	9.0	19.0	15.0	6.0	6.0	**.0**	12	2	1
Acc. No. 710	18.0	15.0	18.0	21.0	9.0	21.0	22.0	14.0	16.0	**.0**	10	9
Acc. No. 711	12.0	13.0	10.0	11.0	23.0	11.0	8.0	14.0	10.0	26.0	**.0**	1
Acc. No. 712	19.0	12.0	11.0	10.0	22.0	20.0	11.0	13.0	13.0	21.0	13.0	**.0**
	Squared Euclidean distances											

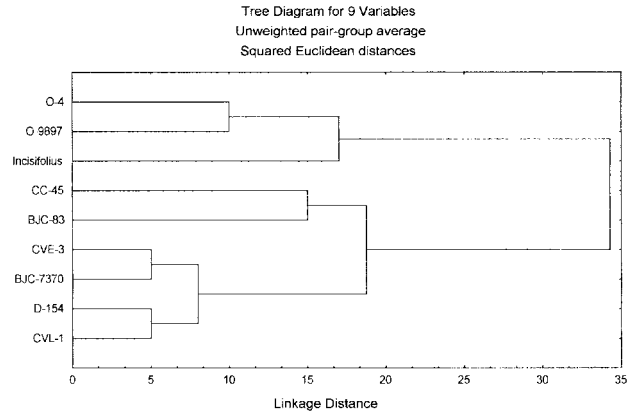

Fig. 1. Genetic relationship of Nine Varieties using STATISTICA Software.

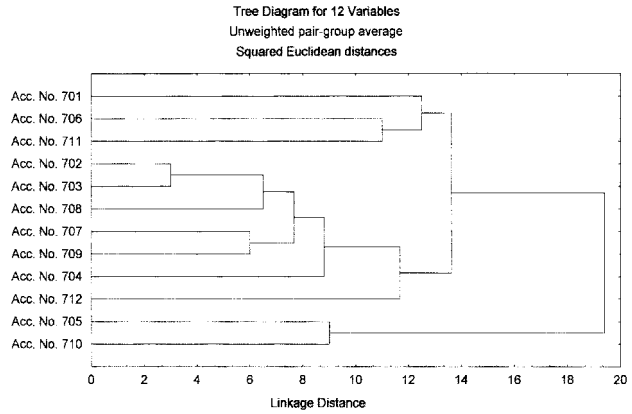

Fig. 2. Genetic relationship of Twelve Accessions using STATISTICA Software.

primarily based on 29 different 10-mer oligonucleotide primers that were scored in 9 jute varieties and 12 different jute accessions. Among the 29 primers, 7 generated substantial differences between the different jute varieties, and 6 primers gave polymorphism within the accessions, and 3 primers gave common bands. Only those fragments that could be reproduced in a second experiment were considered for documentation. Weak and spurious bands were not included in the analysis. DNA fingerprints that were obtained by primers OPAB 03, OPAB 06, OPG 05, OPG 06, OPG 09,

OPG 15, and OPH 13 revealed that *olitorius* varieties (namely O-4, O-9897, and *incisifolius)* were genetically distinct. The other *capsularis* varieties (namely, CC-45, CVE-3, BJC-7370, D-154, CVL-1, and BJC-83) were also genetically distant. The primers OPAB 03, OPAB 05, OPG 03, OPG 05, OPG 06, and OPQ 17 showed polymorphism within different jute accessions.

A dendrogram was created using the STATISTICA: Cluster Analysis (StatSoft 1994), based on the genetic distance matrix (data not shown). The relationship that is portrayed by this clustering also agreed with the available pedigree information. Two major clusters were resolved among the 9 cultivars that were examined in the study. The large cluster comprised 6 cultivars, all capsularis varieties; the other cluster is comprised of 3 varieties. They are as follows: O-4, O-9897, and *incisifolius;* all are *olitorius* varieties. It is interesting to note that *C. olitorius* var. *incisifolius* was classified by taxonomic studies as a variety of *C. olitorius* (Edmonds J. M. 1990). In our study, it clustered with other *olitorius* varieties, namely O-4 and O-9897. Here we have an example of how molecular-marking studies can correlate with established findings that are made by classical taxonomic studies.

DNA fingerprinting profiles of jute accessions from BJRI germplasm were quite similar, suggesting a close genetic relationship. The similarity in amplification profiles of most jute accessions suggested that there is a degree of DNA conservation among the jute accessions. This genetic distance information could be useful in breeding programs in order to introduce agronomically important genes. However, more extensive molecular data is needed in order to make a more general conclusion about the relationship between jute accessions. The accessions with genuine labels are also necessary.

Despite the strong homology that is exhibited by many of the jute genotypes, our work demonstrates that it is now possible to differentiate between closely-related cultivars. Furthermore, most of the cultivars that we studied can be individually characterized with cultivar-specific RAPD markers. Since the pedigree of our material is known, we were able to confirm that the cultivar relationships that were concluded from our RAPD data are extremely reliable.

Our results show that if such an analysis were extended to additional members of the jute germplasm, particularly to lines with unknown pedigree information or from diverse sources, it would be possible to obtain a detailed picture of their genetic relationship.

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