

**GENETIC DIVERSITY AND RELATIONSHIPS IN JUTE (*CORCHORUS* SPP.)
REVEALED BY SSR MARKERS**

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Abstract

Characterization of sixteen jute genotypes, from *Corchorus olitorius* L. and *Corchorus capsularis* L. using jute specific SSR marker attained a high polymorphism value of 92.20%. A total of 171 different alleles were amplified by 27 primer pairs with a mean of 6.33 ± 2.04 alleles per locus. The genetic diversity was also relatively high (0.81 ± 0.06). The Un-weighted Pair-group Method with Arithmetic averages (UPGMA) cluster analysis of the 16 jute genotypes produced a dendrogram, which was in concordance with known information. The study reinforces the utility of SSR primers for providing useful and high levels of markers for individual plant genotypes even with a narrow genetic base.

Introduction

Information on the genetic diversity within and among closely related crop varieties is essential for crop improvement and to meet the diverse goals like producing cultivars with increased yield (Joshi and Dhawan 1986), wider adaptability, desirable quality, pest and disease resistance, etc. (Nevo *et al.* 1982).

Simple Sequence Repeats (SSRs), also known as microsatellites, have been proven as an excellent tool for cultivar identification, pedigree analysis and the evaluation of genetic distances among many plant species (Priolli *et al.* 2002). These have been reported to detect high level of polymorphism even amongst closely related plant germplasms (Hokanson *et al.* 1998). SSR markers found to be more variable within genomes of olive than other molecular marker types (Mace *et al.* 2006). SSRs have become a tool of choice for investigations of unique genetic identities or fingerprints, determination of genetic relatedness between accessions, and the assessment of genetic diversity contained within a collection due to their co-dominant inheritance and amenability to high throughput analysis (Hokanson *et al.* 1998).

Jute (*Corchorus* species, $2n=14$) is a natural bast fibre crop and has considerable commercial significance. It includes 40 species, distributed in warm regions of the world (Purseglove 1968). However, for commercial purposes, jute fibre is obtained mostly from two cultivated species, *C. capsularis* L. and *C. olitorius* L. Studies on genetic diversity and relatedness at its molecular level have been surprisingly scarce. Analysis on jute genetic diversity has been reported by Hossain *et al.* (2002) and Qi *et al.* (2003a) using RAPD; Basu *et al.* (2004) using SSR and Roy *et al.* (2006) using STMS, ISSR and RAPD markers. Hossain *et al.* (2003) characterized cold-tolerant and cold-sensitive jute germplasms and Qi *et al.* (2003b) classified wild jute species using Inter Simple Sequence Repeat (ISSR) marker. Recently Akter *et al.* (2008) and Mir *et al.* (2008) reported the utility of studying genetic variability for different traits in jute genotypes using jute specific SSR markers.

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The present study was undertaken to determine the genetic diversity and genetic relationships using SSR markers among 16 elite jute genotypes collected from BJRI.

Materials and Methods

Plant materials: The gene bank of Bangladesh Jute Research Institute (BJRI) has one of the world's largest jute and allied fibre (JAF) germplasm collection of about 6000 accessions. Eleven popular jute varieties (four from *C. oltorius* and seven from *C. capsularis*) and 5 advanced lines (these will be released within couple of years as varieties for commercial cultivation) used in this study were selected for their superior fibre yield, quality and other characteristics, collected from the Physiology Department, Bangladesh Jute Research Institute (BJRI), Dhaka. Details of the genotypes along with their breeding pedigree and geographic origins are given in Table 1.

Table 1. Breeding pedigree, geographic origin and superior characteristics of the jute genotypes used in the study.

Species	Genotypes	Variety/ advanced line	Pedigree	Geographical origin	Superior characteristics
<i>C. oltorius</i>	O-4	Variety	Pure line selection from local land race	Bangladesh	High yield and good fibre quality
	O-9897	Variety	O-5 X BZ-5	O-5 (Bangladesh) and BZ-5 (Brazil)	High yield and good fibre quality
	Acc-1805	Adv.line	Pure line selection against cold stress	Egypt	Cold tolerant
	OM-1	Variety	Pure line selection	Bangladesh	High yield and good fibre quality
	O-72	Variety	(O-9897 X O-2012) X O-9897	Bangladesh	High yield and good fibre quality
	SDLT-1*	Adv. line	Pure line selection	Bangladesh	High yield and good fibre quality, tolerant to short day and low temperature
	SDLT-2*	as above	as above	Bangladesh	as above
	SDLT-3* Line7/95	as above as above	as above O-4 X Uganda red	Bangladesh O-4 (Bangladesh) and Uganda red (Uganda)	as above High yield and good fibre quality, mite tolerant
<i>C. capsularis</i>	D-154	Variety	Pure line selection from local land race	Bangladesh	High yield and good fibre quality
	CVL-1	Variety	as above	Bangladesh	High yield and good fibre quality
	CVE-3	Variety	Pure line selection	Thailand	High yield and good fibre quality
	CC-45	Variety	Pure line selection	Egypt	High yield and good fibre quality, less photosensitive
	BJC-83	Variety	CVL-1 X Fuleshwari	Bangladesh	High yield and good fibre quality, early maturation
	BJC-7370	Variety	D-154 X CC-45	Bangladesh	High yield and good fibre quality
	BJC-2142	Variety	CC-45 X S-718	Bangladesh	High yield and good fibre quality, fibre colour snow-white

*Although these three lines were selected for tolerance to short day and low temperature, these were different in phenotypes.

DNA extraction: 1g of four-day old seedlings were ground in presence of liquid nitrogen. DNA was extracted from the ground sample using the CTAB following a protocol modified from Doyle and Doyle's method (1990). DNA quality and quantity were checked following agarose gel

electrophoresis (0.8% gel containing 0.5 µg/ml ethidium bromide) using known concentrations of uncut lambda DNA as standard. The final DNA concentration of each template stock was adjusted to 50 ng/µl.

Designing of SSR primers: Twenty seven primer pairs (Table 2), each flanking specific jute SSR sequences were used. The primers were designed from a SSR specific genomic library (developed by Vison Sci. Inc., Canada) generated from an elite Bangladeshi jute variety O-4 that belongs to the species *C. olitorius*. Primers were designed following standard rules: 20-25 nucleotides in length, 40-60% GC content, and non-complementary 3' nucleotides. Annealing temperatures for the primers were between 55°C-65°C.

Table 2. Repeat motifs and primer sequences of microsatellite loci.

Primer name	Forward	Reverse	Repeat motif
HK-1	CTTCTTGACCCAAACAATGCCA	GGATGATGAAAAACGAAGTGCCTA	(AG)17G3(AG)20
HK-2	GTTTATCCAACCAATACCAACCA	TGCCTCGTTGCTGGACATTGCA	(CT)11(GT)14
HK-4	CAAAAGTAGTGAAGAACATGAGCA	GCCAAATCTGATATACGCCTGA	(GT)28
HK-5	AGTGACTTATAGTCTAATTAGTGA	ACAGATAGGATGTTAACGGGA	(GT)24
HK-6	CTATCTCCCATTGTACCTGCA	GGCAGATTGTGTGAGACTATCA	(GT)10
HK-7	AATGATTATGAACCATAGTGGTACA	TTATCACAAAAGTAGCAGACTAACA	(GA)25
HK-9	TTACATTATATAATGTCCAGCCA	AGTGGCTACTGGTTCCTACA	(TC)21(T)33
HK-10	GAACATCAAGACTGAGTAAGACCTA	TTGAGGATTTTCATATGCATGCA	(TG)16
HK-12	CGCTCGCCTAAGTGAAGGCA	ATAAAATACAAGGGACACTTAGCA	(CA)19
HK-13	TTAGGAGTCATTTCTAACAAGAC	AATCCCTCCAGCTTCTCGA	(GA)28
HK-15	GAGAGGAATGATGCTGAGATTCA	GACACCCTCCGCCTATCTCA	(GA)15
HK-16	TGGAACCTGAGCATCTCTCCAGA	CTTTTCTTGTTTCAGGGACCTGA	(CT)21
HK-17	AGAGTTTGCAACAAGGTAGCCA	TGGTACTTAAACTTAGTTGTGTGCA	(GT)21
HK-18	GCTGTTGTCTCTCTATTGGTGA	TTCCACGCTCCTGTTGCCA	(AG)16
HK-19	TATGAAGGTGAACTACTTGTCCACA	AGCTTCCATTTTCGAACATTCCA	(CA)21(AG)10
HK-20	GTAAAGCACAGGATTAGTCCCA	GGAAAGTGAACCTCTAGTAGATGA	(CT)30
HK-21	AATCAAATTGAGAATGGACATGCA	GAAAGGCAAATGCGCTTGTGA	(CA)12
HK-22	CTGTTTGTCAATCTCTTTTGAGTCA	GTCCAAAACATCGTGCAGTGTGA	(GA)25
HK-23	GGCCCTTCTAATTAACCTCCA	AGTTTGTTTCCAGATATTGCTCA	(GA)17
HK-27	TTGTGTGCAAACACGAGTGCA	GGTAGCCATGTTTACTTCCTGA	(CA)26
HK-28	AGAGACGAGTAAAACATAAAAGTCC	CTGGCGAAGCCTTAAAAATGCA	(AG)21
HK-29	CTGAATGAAAGATTGCTTTTAATCC	CATGCATCATTTCATTGCATGCA	(GA)39
HK-30	GAGTGATTAGAGGGCAGCCA	TGCAACAAAGTATCCAAATCGAC	(CA)21
HK-33	GTTGATATCTTTACGGTGTATAGA	GTGATTTGGTTACTATGACCCA	(CA)16(TA)6
HK-37	AATGTGAAATCCAATTAAGCACA	CCAAGATTGTAGCAACAAGCCA	(GATA)13
HK-38	ACCAAGTATGATCTGACCTCT	AGCTAAAAACAACACAAAAATATCT	(CTAT)16
		TGC	
HK-41	GGAAAACAGCCTATTGGGGACA	CATTCTCCATTGTTGAAGGA	(CAA)15

PCR amplification: PCR was performed in GeneAmp® PCR system 9700 (Applied Biosystems). Each of the 25µl reaction mixtures contained 50 ng of jute genomic DNA, 10 µM of each primer pair, 1× PCR buffer, 2.5 mM MgCl₂, 2 mM dNTPs, 1 unit Taq DNA polymerase (Invitrogen, India). The thermal cycler was programmed as follows: preheating for 5 min at 95°C; 35 cycles, each for 30 s at 95°C (denaturation), 40 s at the annealing temperature of a particular primer pair, and 30 s at 72°C (extension) and a final extension at 72°C for 5 min, followed by cooling at 4° C for infinite period.

Separation and staining of PCR products: Before loading in gels, PCR products were denatured by adding equal volume of tracking dye (95% formamide, 10 mM EDTA, 0.23%

bromophenol blue and 0.23% xylene cyanol) and heating at 94°C for 5 min. Amplified products were separated in 8% denaturing polyacrylamide gel in 1×TBE buffer adjusted to pH 8.3 at 2000 V for 2.5-3 h, using sequencing gel apparatus (Adjustable nucleic acid sequencing unit, Model SG-400-33, C.B.S. Scientific Co. Del Mar, California). Silver staining of the gels was performed according to Streiff *et al.* (1998). Fragment sizes were calculated using the computer programme SEQAID II (Fragment Sizer) by comparing with fragments of 1kb-plus ladder marker DNA (Invitrogen, India).

Data analysis: Amplified PCR products were scored to create a binary matrix. Effective allele per locus (A_{ep}) were calculated according to Weir (1990) using formula $1/(1 - H_{ep})$, where H_{ep} (the genetic diversity per locus) is equal to $1 - \sum P_i^2$ and P_i is equal to the frequency of the i^{th} allele of this locus.

Shannon's diversity index was calculated according to Martynov *et al.* (2003) with the formula $H = -\sum P_i \ln(P_i)$, where P_i is the frequency of a particular allele i and \ln is the symbol of natural logarithm. A pair-wise distance matrix was generated based on total and mean character differences in phylogenetic analysis (Swofford 2002). A dendrogram was constructed to identify genetic relatedness among the cultivars based on the distance matrix by applying the Un-weighted Pair-group Method with Arithmetic averages (UPGMA) cluster analysis using the software STATISTICA.

Results and Discussion

Studies on 16 elite jute genotypes, twenty seven SSR primer pairs yielded a total of 171 different alleles with an average of 6.33 ± 2.04 alleles per locus (Table 3), which differed from 4.61 ± 1.92 and 3.04 found by Akter *et al.* (2008) and Mir *et al.* (2008), respectively. Differences in average number of alleles might arise from using different combination of genotypes and different loci in present study. Only reproducible and distinct bands were counted in the analysis. Though SSRs are generally believed to be locus specific and expected to amplify single or twin bands with a single SSR primer, we observed multiple alleles. Similar results were obtained by Gupta *et al.* (2003). This might arise from amplification of more than homeolocus as was explained by Holton *et al.* (2002). The observed allele sizes ranged from 110 bps (HK-30) to 480 bps (HK-28). Di-nucleotide repeats had more alleles (average 6.5) compared to tri and tetra-nucleotide repeats (average 5). 158 of the total 171 alleles amplified were polymorphic giving an average 5.85 ± 2.12 polymorphic alleles per locus (Table 2). Although jute is self-pollinating and incompatible for inter-specific cross hybridization which results in a narrow genetic base, this study attained a high polymorphism of 92.20% with SSRs which was similar to Akter *et al.* 2008 (92.45%) and Mir *et al.* 2008 (91.11%). Similar levels of polymorphisms for SSRs were also reported in previous studies with other plant species (Rajora and Rahman 2003). This study, therefore, reinforces the utility of SSR primers for providing useful and high levels of markers for individual plant genotypes even with a narrow polymorphic genetic background. The present study differs significantly from Mir *et al.* (2008) in using varieties that are widely used in Bangladesh, and from Akther *et al.* (2008) in having more genotypes including five advanced lines, which are on the verge of commercial release soon. In addition, this study involves loci, different from previous studies. The more markers are available for a species the more helpful it might be in future for various analysis such as gene identification, MAS, etc.

The minimum and maximum numbers of alleles obtained at different loci were 3 and 10, respectively (Table 3). The effective number of alleles per locus was smaller than the observed numbers of alleles in the corresponding locus except for locus HK-21 and HK-41. A similar pattern was observed in other plants such as orchid (Gustafsson 2000), neotropical tree *Swietenia humilis* Zucc (White *et al.* 1999) and grapes (Martinez *et al.* 2006). However, the differences

between the observed number of alleles and expected number of alleles were more pronounced in these plants than jute. Genetic diversity (GD), which is indicative of the effectiveness of SSR loci information, was relatively high, ranging from 0.66 (HK-13) to 0.89 (HK-4), with a mean value of 0.81 ± 0.06 , which was slightly higher than Akter *et al.* (2008). This discrepancy may have arisen due to the inclusion of more genotypes and loci in the present study. Another reason for this could

Table 3. Range of allele size, number of allele, number of effective allele, genetic diversity (\pm SE) and polymorphism at 27 SSR loci (defined by primer pair) in sixteen jute genotypes

Locus	Range of allele size (bp)	Number of allele (A)	Number of effective allele (A_{ep})	Genetic diversity (GD)	Polymorphic band (%)	Shannon's diversity index (I)
HK-1	150-300	6	5.29	0.81	83.33	1.73
HK-2	140-290	7	6.03	0.83	100.00	1.86
HK-4	130-390	10	9.50	0.89	100.00	2.28
HK-5	110-280	6	5.16	0.81	83.33	1.71
HK-6	130-370	9	7.54	0.87	88.89	2.10
HK-7	230-310	4	3.95	0.75	100.00	1.38
HK-9	160-400	6	4.92	0.80	83.33	1.70
HK-10	150-430	8	6.12	0.84	100.00	1.93
HK-12	100-390	9	8.36	0.88	100.00	2.15
HK-13	340-390	3	2.96	0.66	100.00	1.09
HK-15	140-280	7	6.40	0.84	71.43	1.90
HK-16	150-320	5	4.63	0.78	60.00	1.57
HK-17	200-400	4	3.74	0.73	75.00	1.35
HK-18	110-400	8	6.44	0.84	100.00	1.94
HK-19	120-190	7	6.28	0.84	85.71	1.88
HK-20	110-260	9	8.61	0.88	100.00	2.18
HK-21	170-240	3	3.00	0.67	100.00	1.10
HK-22	220-350	6	5.47	0.82	100.00	1.73
HK-23	130-310	8	7.31	0.86	75.00	2.03
HK-27	180-360	6	5.22	0.81	83.33	1.72
HK-28	280-480	5	4.43	0.77	100.00	1.54
HK-29	210-380	6	5.03	0.80	100.00	1.67
HK-30	100-210	10	7.89	0.87	100.00	2.17
HK-33	200-400	4	3.37	0.70	100.00	1.28
HK-37	140-390	4	3.72	0.73	100.00	1.34
HK-38	100-400	6	5.54	0.82	100.00	1.74
HK-41	110-310	5	5.00	0.80	100.00	1.61
Mean		6.33 ± 2.04	5.62 ± 1.74	0.81 ± 0.06	92.20	1.73 ± 0.32

be the inclusion of more genotypes from *C. olitorius* which have higher genetic diversity than those of *C. capsularis*. This has also been observed by Akter *et al.* (2008) and Mir *et al.* (2008). This high GD value indicates that SSRs were highly informative for jute. Genetic diversity and number of alleles were positively correlated (Fig. 1). GD values increased proportionally with increasing heterozygosity at any locus. The frequency of 84% of the 171 detected alleles was

lower than 0.25, and that of the remaining 16% was equal to or higher than 0.25. Only three alleles amplified with primer pairs HK-6, HK-12 and HK-29 showed a frequency lower than 0.05 (Data not shown).

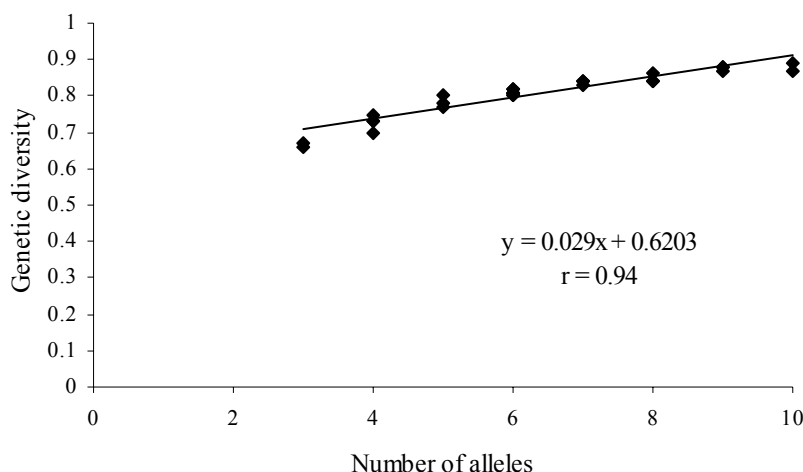


Fig. 1. Relationship between number of alleles at individual locus and genetic diversity among jute genotypes.

Two frequently used statistical tools for genetic diversity studies are percentage of polymorphic bands (P) and Shannon's diversity index (I). In this study, 17 out of 27 loci had 100% polymorphic alleles (Table 3) with HK-16 locus having the least polymorphism (60%). Shannon's diversity index (I) of the jute cultivars ranged between 1.09 (HK-13) and 2.28 (HK-4) with an average of 1.73 ± 0.32 (Table 3) and was directly proportional to the number of polymorphic alleles at corresponding loci.

The UPGMA cluster analysis produced two meaningful groups with genotypes belonging to *Corchorus olitorius* and *C. capsularis* in two different major clusters I and II (Fig. 2). The first group (cluster I) included all the genotypes from *C. olitorius* and the second group (cluster II) included all the genotypes from *C. capsularis*. This pattern of clustering validates the authenticity of the experiment and reliability of data, which was in concordance with available taxonomical information and previous studies by Hossain *et al.* (2002) using RAPD, Basu *et al.* (2004) using AFLP, Roy *et al.* (2006) using STMS, ISSR and RAPD markers, Akther *et al.* and Mir *et al.* (2008) using jute specific SSR markers. Both the clusters were further subdivided into three sub-clusters. Sub-cluster Ia comprised three genotypes namely, O-4, O-9897 and accession 1805, Sub-cluster Ib comprised four genotypes namely, OM-1, O-72, SDLT-1, and SDLT-2 and Subcluster Ic comprised two genotypes namely, SDLT-3 and Line 7/95. Sub-cluster IIa comprised two genotypes namely, D-154 and CVL-1, Sub-cluster IIb comprised three genotypes namely, CVE-3, CC-45 and BJC-83 and Sub-cluster IIc comprised two genotypes namely, BJC-7370 and BJC-2142. The present study revealed that the two jute species are distantly related. Palit *et al.* (1996) also reported presence of distinct patterns of diversity between the two species. This analysis, thus, strengthened the earlier concept that the two species originated from two different geographical locations; *C. capsularis* originated from the Indo-Burma region and *C. olitorius* from Africa (Kundu 1951).

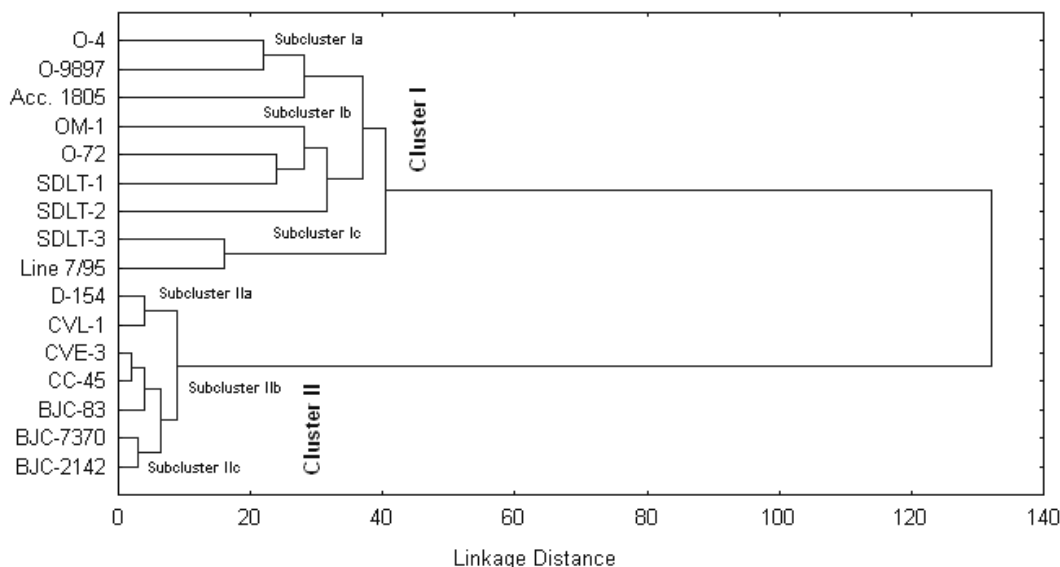


Fig. 2. Dendrogram of 16 jute genotypes based on data on allelic profiles generated using 27 microsatellite primer pairs.

The average genetic distance among the *C. olitorius* cultivars was 35.5 units and *C. capsularis* cultivars was 6.86 units and distance between the species was 138.17 units indicating the low level of diversity in *C. capsularis* than *C. olitorius*. Similar conclusion was drawn in analysis by Akter *et al.* (2008) and Mir *et al.* (2008). This might be correlated to the lower percentage (3 - 4%) of out-crossing in *C. capsularis* as opposed to relatively higher percentage (8 - 12%) of natural cross pollination within *C. olitorius* (Ghose and Gupta 1945). Roy *et al.* (2006) also observed low levels of diversity in the *C. capsularis* varieties.

These SSR markers showed great promise as tools for collection and preservation strategies concerning various jute cultivars *in situ*. The ease of detection and the success of this study with SSR markers to elucidate the diversity among 16 cultivars may encourage the use of SSR markers to characterize all the germplasms (nearly 6000) at BJRI. This will surely reduce duplication in the collection, if any, and will be more useful to the user community. The information on genetic relatedness and characterization of cultivars will also help in making informed choices on selection of parental material based on genetic diversity. In addition, these SSR markers may be positioned on genetic maps, which may further facilitate plant breeders and geneticists to localize agronomically/horticulturally significant genes or gene complexes to specific sites on the genome (Liu *et al.* 1996). These traits could be introduced into desired cultivars by marker-assisted selection or map-based cloning.

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