Australian Journal of Crop Science
1(3): 97-107 (2008)
ISSN: 1835-2707

Southern Cross Journals© 2008
www.cropsciencejournal.org

Microsatellite markers for determining genetic identities and genetic diversity among jute cultivars

Jesmin Akter*, Md. Shahidul Islam [2], Abu Ashfaqur Sajib [1], Nadim Ashraf [3], Samiul Haque [2], Haseena Khan**

**Plant Molecular Biology Lab, Department of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh*
[1] Department of Genetic Engineering and Biotechnology, University of Dhaka, Bangladesh
[2] Bangladesh Jute Research Institute, Dhaka, Bangladesh
[3] University of Nottingham, U. K.
***Corresponding author: pmblab@gmail.com*

Abstract

DNA fingerprint was generated using jute specific SSR markers on 10 jute cultivars from two *Corchorus* species (C. *olitorius* and *C. capsularis)* and the genetic relatedness among the cultivars was estimated. A total of 106 alleles were identified using 23 primer pairs among the 10 cultivars with an average of 4.61 ± 1.92 alleles per locus, and a mean genetic diversity of 0.68±0.16. Four *C. olitorius* cultivars could be easily distinguished with 6 markers using 5 primer pairs and six *C. capsularis* cultivars with 7 markers using 6 primer pairs. The UPGMA analysis enabled the grouping of the cultivars into two major clusters, which matched with the known information on jute. This experiment provides proof that in spite of low levels of genetic polymorphisms among jute cultivars, SSR markers can reliably distinguish among them. This finding reinforces the utility of SSR primers for providing unique genetic identities or fingerprints of various jute cultivars.

Keywords: *Corchorus,* jute, markers, microsatellite, polymorphism, SSR

Introduction

Jute cultivars are currently distinguished by morpho-physiological characters such as pigmentation pattern in plant, leaf shape, stipule, seed coat colour of mature field-grown plants (Anon, 1997). This method is slow and unreliable (Cooke, 1999) and phenotypic identification based on morphological traits is subjected to environmental variation (Nielsen, 1985). Genetic diversity in improved jute cultivar has been narrowed (La Farge et al., 1997), due to the existence of a strong sexual incompatibility barrier between the two cultivated species *Corchorus capsularis* L. and *Corchorus olitorius* L as well as secondary gene pool. Cultivars that are closely related or have low genetic variability cannot be readily distinguished by morphological indices (Deng et al., 1994; Degani et al., 1998). Identification of crops has become increasingly important with commercial interest in Plant Breeders Rights and cultivar registration. PCR-based molecular markers, such as randomly amplified polymorphic DNA (RAPDs), Simple Sequence Repeats (SSRs) and amplified fragment length polymorphisms (AFLPs), have an apparent advantage as cultivar descriptors in that they are unaffected by environmental or physiological factors (Bowditch et al., 1993). Among different molecular markers, Simple Sequence Repeats (SSRs) are more abundant, ubiquitous in presence, hyper variable in nature and have high polymorphic information content (Gupta et al., 1996). These markers have been used for genotype identification in many crop plants such as barley (*Hordeum* vulgare L.) (Russell et al., 1997), and soybean (Rongwen et al., 1995; Song et al., 1999). Microsatellites have been shown to be almost twice as informative as dominant markers (RAPD and AFLP) and much more informative than RFLPs in

Table 1. Jute cultivars used in this study along with their pedigrees

Species	Cultivar name	Pedigree	Characteristics of the varieties
Corchorus olitorius	O-4	Pure line selection from local[1] land race	High yielding. Late maturing type. Maximum yield 4.51 ton/ha.
	O-72	{(O-9897 X O-2012) X O-9897}	High yielding. Maximum yield 4.96 ton/ha
	O-9897	O-5 X BZ-5	High yielding. Can be sown early. Proper sowing time: 30 March 30 April. Maximum yield 4.61 thon/ha
	OM-1	Pure line selection from Uganda collection	High yielding. Can be sown early. Less photo sensitivity. Proper sowing time: 20 March to 30 April. Maximum yield 4.62 ton/ha
Corchorus capsularis	D-154	Pure line selection from local land race	High yielding.
	CVL-1	Pure line selection from local land race	High yielding variety. Very popular. Maximum production 5.16 ton/ha.
	CVE-3	Pure line selection from Thailand collection	Early maturing type. Quick growing. Suitable for 3 crop rotation per year. Maximum yield 4.51 ton/ha
	CC-45	Pure line selection from Egypt collection	Suitable for early sowing. Quick growing. Suitable for 3 crop rotation. Maximum yield 5.16 ton/ha
	BJC-83	CVL-1 X Fuleshwari	Quick growing and early maturing type. Maximum yield 4.72 ton/ha
	BJC-7370	D-154 X CC-45	Short day tolerant type. Suitable for early sowing. Quick growing. Suitable for 3 crop rotation. Maximum yield 5.46 ton/ha

BZ-5 = Collected from Brazil through International Jute Organization (IJO); O-5 = Collected from local land race; Fuleshwari = Local farmer's variety

[1]variety developed from single plant collected from Bangladeshi farmer's field based on superior phenotypic characteristics

soybean (Powell et al.1996), and approximately six times more informative than RAPD and nine times more informative than allozymes in poplar (Rajora and Rahman, 2003); emphasizing these markers as ideal for discriminating individuals and for parentage determinations. Due to these properties, SSRs have become a powerful and popular tool for determining unique genetic identity or fingerprint, and establishing genetic relatedness as well as diversity among various crop cultivars. Recently Mir *et al* (2007) has reported the utility of studying genetic variability for different traits in jute genotypes using jute specific SSR markers.

Jute (*Corchorus* species, 2n=14) is a natural bast fibre crop, similar to flax, kenaf, roselle and is comprised of two cultivated species, *C. capsularis* L. and *C. olitorius* L. Jute fibre is completely bio-degradable and environment friendly. It is the second most important plant fibre after cotton, in terms of usage, global consumption, production, and availability (Singh, 1976). As jute fibre is also being used to make pulp and paper (Mohiuddin et al., 2005), and with increasing concern over forest destruction, the importance of jute for this purpose may increase with time. Jute is an annually renewable resource of biomass, requiring only 120 to 180 days for its growth. This as a result, has stimulated growing interest among various developed and developing countries toward jute as a widely available alternative source of pulp. Single jute accession/germplasm have been reported (Hossain et al. 2003) which can tolerate temperatures (around 16°C), below the base temperature for jute. Such accession/germplasm could be used in breeding programmes for developing new varieties for mild winters of tropical countries, the natural abode for jute. This would therefore meet round the year demand for jute in paper industries. A substantial number of new cultivars of jute with better combinations of characteristics have been developed in the last couple of decades. Along with the development of new cultivars, there has always been a keen interest in the genetic characterization of jute for aiding in various purposes including commercial protection provided by the Plant Variety Protection Act.

Although jute is the world's second most important fibre crop, very little effort has been given to its molecular investigation. Only few research references are available on the genetic information of jute cultivars. Using RAPD, genetic diversity analysis of jute has been reported by Hossain et al., (2002) and Qi et al., (2003a). Genetic diversity analysis has also been reported by Basu et al., (2004) using SSR markers. Characterization of cold-tolerant germplasms has also been reported by Hossain et al., (2003). Wild jute species has been classified using Inter Simple Sequence Repeat (ISSR) marker by Qi et al., (2003b) and the genetic diversity has been evaluated by STMS, ISSR and RAPD markers by Roy et al., (2006). To further enrich the present information, status of jute genetic SSR markers were analyzed with three specific objectives: i) identification SSR markers useful for distinguishing 10 cultivars of jute, ii) developing genetic fingerprints for these cultivars and iii) estimation of the genetic relatedness among these cultivars.

Materials and Methods

Plant material

Depending on the yield and fibre quality ten popular varieties of jute, four from *C. olitorius* and six from *C. capsularis,* were used in this study. Table 1 lists the detail of the cultivars along with their breeding parents and places of origin.

DNA Extraction

DNA was extracted from seedlings of each variety following the modified protocol by Doyle and Doyle (1990) of the CTAB (Hexadecyl trimethyl ammonium bromide) method (Murray and Thompson, 1980). The quality of the extracted DNA was examined under UV light following agarose gel electrophoresis (0.8% gel containing 0.5µgml^{-1} ethidium bromide) (Sambrook et al., 1989). DNA concentration was estimated by extrapolation by Kodak Scientific Imaging System software using known concentrations of lambda DNA as standard. The final DNA concentration of each template stock was adjusted to 50 ng/µl.

Microsatellite primers

Twenty three microsatellite primers (Table 2) were used for PCR amplification. The primers flanking the SSR sequences were designed by a Canadian company, Vizon Sci. Inc., Canada BC Research Complex 3650, Wesbrook Mall, Vancouver, British Columbia based on the construction of a genomic library by the same company from the DNA of jute variety, namely 0-4. Primers were designed manually using standard rules: 20-25 nucleotides in length, 40-60% GC content, and non-complementary 3´ nucleotides. The expected size of the amplification products was nearly between 100 and 300 bps. Annealing temperatures were

Table 2. Microsatellite primer sequences used in the study.

Primer No	Forward sequence	Reverse sequence	Repeat motif
HK - 46	ATTTTCAGCCAATGGAGCTCA	TATCACATTACTTCCAGCACAC	(CAA)7
HK - 47	TGAAAATCTGGTCAAAATGCTATC	TGTACTCATGATAAGTTGCCTAC	(GTT)11
HK - 50	TTCCTGTACCTTTGGGCCTCA	AAACACACTCAAGTAGTTCGCA	(GA)13
HK - 51	ATTTAAGATGCCAGCCATTCCA	AAACACACTCAAGTAGTTCGCA	(GT)17
HK - 52	GGCCAATAAAATACAAGGGACA	GATGGTTATATCACCTGAGGCA	(TG)19
HK - 53	GTACAAACAACTTTATTAACATAC	CCTATAACCCAAATTTGATACTAC	(CA)13
HK - 55	TGATGATAAACCATCCTTCACCA	GTCTACACTCTGAAGTAGCTTCA	(T)12(TC)17
HK - 56	GCCAAAATTGTGGGAAGCAC	TGGTGTCGATTCGTTTCTAC	(GTT)8
HK - 59	CTTTTCGAGCTTGATCAGTTACCA	GACTTTACTTGTACCCATCTCCA	(CTAT)18
HK - 60	CCATACTTGCGTTCTGAGGTGCA	AATCCTTCCCATACTGGAGATGA	(GATA)17GA>82
HK - 61	TCAGTTGAGGAGGCAGAACC	CACAATATCGACCACAGTATATCC	(TA)5(AG)13
HK - 63	AATCAGAGTCAGACAGAAGGGA	GTCTTACCCATCATCTCAGACA	(CT)14
HK - 64	GATTGAATGGTTCTGGGTTTCA	CAATGTAAGCGCATTCATCAATAG	(AG)21
HK - 65	TCATTGTGGTTAATTTGCTTGCAAC	TTCCATGCATGGTTGGCTAAAGAC	(AG)19
HK - 67	GTTACCAACATAAAAATAGCAATCAC	ACCATGAAAGATTGTTGCTGGAC	(AG)20
HK - 68	CTCTGTTTTTACATGGTTACTTCGC	TCAGCGATTGATGCATATAGTCC	(AG)22
HK - 69	CCTTCCCATACTGGAGATGAGA	TACTTGCGTTCTGAGGTGCA	(AG)23
HK - 71	GATCTGGCTATCGGATTACTTCA	CTTCAAAACGGAGCTATTGTGTC	(AG)25
HK - 73	GGATTTGGTGAGGAGAATATATTC	TCCCGTCACTCTCACCTTCA	(AG)27
HK - 75	CACTTTGCATTAAAGAACACCA	AAGTCTCTCTATATATAGCAGCA	(AG)29
HK - 83	CTTTGTTGAGTTTGAAGCCGC	GAGAGTAGACAATGATTTACCA	(CT)12
HK - 85	TATGATGTCATGTAGGTGAAC	GGTCATATTTACACTCCTGAC	(GA)12
HK - 87	CTACAGAGAAATGCTGTTCCCA	GAAATGTTGCAATGGAAGCCA	(CT)13

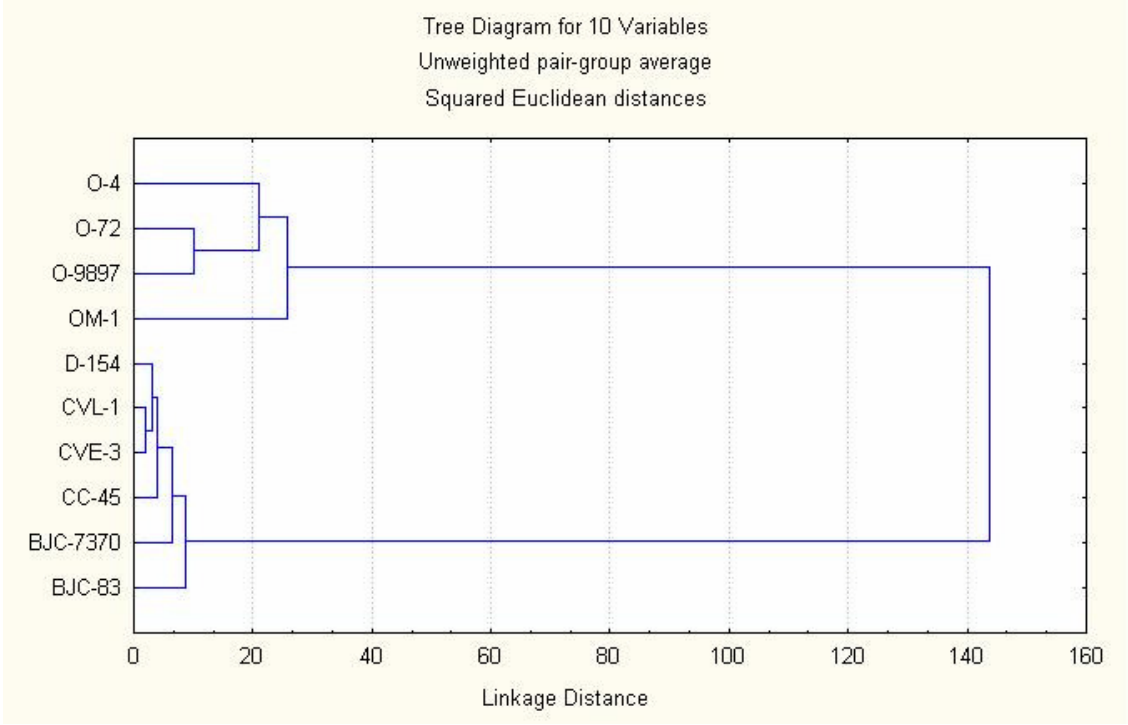

Fig 1. Dendrogram of the jute variety obtained from SSR data.

approximately 55°C-65°C with no greater than 3˚C difference in Tm between primers of each pair.

PCR amplification

PCR amplification was carried out in a 25µl reaction mixtures containing 50ng of jute genomic DNA, 10µM of each primer pair, 10X PCR buffer, 50 mM $MgCl_2$, 2mM dNTPs, 0.2 unit Taq DNA polymerase using a GeneAmp® PCR system 9700 (Applied Biosystems). The amplification 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

The PCR products were pretreated according to Streiff et al. (1998) and run in a 8% denaturing polyacrylamide gel (Rotiphor 40, 38:2 acrylamide: bisacrylamide, Invitrogen Life Technologies), using sequencing gel apparatus (Adjust nucleic acid sequencing unit, Model SG-400-33, C.B.S. Scientific Co. Del Mar, California). The gels were run in 10X TBE buffer adjusted to pH 8.3 at 150 V for 2.5-3 h. Silver staining of the gels was performed according to Streiff et al. (1998). Fragment sizes were calculated using the computer programme SEQAID II (Fragment Size calculator) by comparing with fragments of 1 Kb+ ladder marker DNA (Invitrogen Life Technologies).

Data analysis

DNA fragments that were amplified by a given primer pair were scored for presence '1' and absence '0' for all cultivars that were studied. Only intense bands and those that were repeatedly amplified were scored visually. Effective allele per locus (A_{ep}) were calculated according to Weir (1990) with the formula $1/(1 - H_{ep})$, where H_{ep}, the genetic diversity per locus, is equal to $1 - \Sigma P_i^2$ and P_i is equal to the frequency of the i^{th} allele at the locus. A pair-wise distance matrix was generated based on total and mean character differences in phylogenetic analysis (Swofford, 2001).

Table 3. Number of allele, range of allele size and frequency of allele of 23 SSR loci in ten jute cultivars

Locus	Range of allele size (bp)	Number of allele (A)	Effective allele (A_e)	Frequency of alleles	Genetic Diversity
HK – 46	140-160	5	4.08	0.30, 0.20, 0.05, 0.15, 0.30	0.75
HK – 47	210-270	4	3.46	0.20, 0.40, 0.133, 0.267	0.71
HK – 50	220-280	5	3.98	0.286, 0.19, 0.048, 0.143, 0.333	0.75
HK – 51	250-270	3	2.67	0.25, 0.50, 0.25	0.63
HK – 52	210-240	5	4.01	0.187, 0.063, 0.187, 0.375, 0.187	0.75
HK – 53	170-270	6	5.17	0.20, 0.233, 0.033, 0.20, 0.20, 0.133	0.81
HK – 55	265-290	4	3.46	0.133, 0.20, 0.40, 0.267	0.71
HK – 56	120-240	7	5.06	0.25, 0.125, 0.042, 0.125, 0.291, 0.042, 0.125	0.80
HK – 59	170-190	2	1.99	0.462, 0.538	0.50
HK – 60	195-250	2	1.85	0.643, 0.357	0.46
HK – 61	340-400	4	2.99	0.45, 0.05, 0.30, 0.20	0.67
HK – 63	110-140	4	3.77	0.19, 0.19, 0.333, 0.286	0.74
HK – 64	160-190	7	6.52	0.167, 0.167, 0.167, 0.083, 0.167, 0.167, 0.083	0.85
HK – 65	110-150	2	1.60	0.75, 0.25	0.38
HK – 67	110-200	3	1.78	0.714, 0.214, 0.072	0.44
HK – 68	415-425	3	2.38	0.40, 0.10, 0.50	0.58
HK – 69	110-190	6	5.33	0.125, 0.125, 0.125, 0.125, 0.25, 0.25	0.81
HK – 71	110-150	6	5.08	0.16, 0.12, 0.20, 0.24, 0.04, 0.24	0.80
HK – 73	210-640	9	8.00	0.105, 0.105, 0.105, 0.053, 0.158, 0.105, 0.158, 0.056, 0.158	0.88
HK – 75	150-360	6	4.57	0.166, 0.042, 0.042, 0.25, 0.25, 0.25	0.78
HK – 83	80-160	7	6.53	0.071, 0.143, 0.214, 0.143, 0.143, 0.143, 0.143	0.85
HK – 85	135-165	2	1.47	0.20, 0.80	0.32
HK – 87	135-190	4	3.11	0.273, 0.182, 0.454, 0.091	0.68
Mean		4.61±1.92	3.86±1.74	-	0.68±0.16

Table 4. Fingerprinting key showing distinguishing characteristics of four C. *olitorius* jute varieties as generated using SSR molecular marker profiles

Primer number	Band size	Band status†
	Variety O-4*	
HK-53	250	+
	Variety OM-1*	
HK-53	250	-
HK-53	170	+
HK-46	150	+
	Variety O-72	
HK-53	250	-
HK 64	160	-
	Variety O-9897	
HK-53	250	-
HK-55	280	+
HK-51	250	-

† Band status denoted as presence (+) or absence (-) of a band.

*Amplification of a 250 bp band with HK-53 primer pair could distinguish O-4 from other varieties while the absence of this band and the presence of a 170 bp band with primer HK-53 and a 150 bp band with HK-46 could distinguish OM-1 and so on.

A dendrogram was constructed for identification of 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 (Swofford, 2001). Fingerprinting keys were developed based on the analysis according to Pradhan et al. (2004).

Results

Genetic variability

Twenty-three primer pairs were used for cultivar identification based on the polymorphism these primers produced on PCR amplification. All 23 primers pairs amplified multiple fragments in each cultivar. A total of 106 alleles were identified among the 10 cultivars with an average of 4.61 ± 1.92 alleles per locus. The least variable locus had two alleles and the most variable locus had nine. The effective number of alleles was less than observed alleles in all loci, with an average of 3.86 ±1.74 (Table 2). The frequency of sixty eight percent of the 106 detected allele was lower than 0.25 and the remaining thirty two percent was equal to or higher than 0.25. Only one allele in locus HK-65 showed a frequency higher than 0.75 and four alleles had frequencies lower than 0.05 in locus HK–56. These results revealed the distribution and the representative aspect of the alleles in the studied sample (Table 2). The genetic diversity (GD), which is indicative of the effectiveness of SSR loci information, was also relatively high, ranging from 0.32 to 0.88, with a mean value of 0.68 ± 0.16.

Fingerprinting key

Of the 106 alleles 8 were monomorphic and 98 were polymorphic (data not shown). Each of the 23 loci produced either 2 or more polymorphic bands for each cultivar, which were useful for distinguishing each cultivar. Fingerprinting keys were developed separately for each species. Four *C. olitorius* cultivars could easily be distinguished with 6 markers using 5 primer pairs (Table 4) and six *C. capsularis* cultivars with 7 markers using 6 primer pairs (Table 5).

Genetic relationships

The average genetic distance among cultivars of the *C. olitorius* and cultivars of *C. capsularis* was 21.6 and 6.0 unites, respectively, and distance between the species was 143.67 units (Table 6). The cluster analysis enabled the grouping of all the cultivars

Table 5. Fingerprinting key showing distinguishing characteristics of six *C. capsularis* jute varieties as generated using SSR molecular marker profiles

Primer number	Band size	Band status†
	Variety D-154#	
HK-71	125	-
	Variety BJC-7370#	
HK-60	250	-
	Variety BJC-83#	
HK-60	250	+
HK-64	200	+
	Variety CC-45	
HK-64	190	+
HK-50	280	-
	Variety CVE-3	
HK-50	280	+
HK-87	135	+
	Variety CVL-1	
HK-55	290	-

† Band status denoted as presence (+) or absence (-) of a band.

#No amplification of 125 bp band with HK-71 primer pair could distinguish D-154 from other varieties all of which had this specific amplification. Absence of a 250 bp band with HK-60 could identify BJC-7370 while presence of the same band along with a 200 bp fragment amplified with HK-64 could distinguish BJC-83 and so on.

used in the present study into two major clusters based on the 23 SSR loci which appear to be related to their origin (Fig. 1). Four *C. capsularis* cultivars were grouped in one cluster (cluster I) and six *C. olitorius* cultivars were grouped in another cluster (cluster II). Cluster II was further subdivided into two sub-clusters, with sub-cluster IIa containing five cultivars and sub cluster IIb containing a single cultivar, BJC-83.

Discussion

The principal aim of this study was to identify different cultivars by developing SSR markers. The number of alleles detected per locus in our study ranged from 2 to 9, with a mean value of 4.61. The results were similar to those reported for other self-pollinating plant species as wheat with estimated 3.8, 4.6 and 6.2 alleles per locus (Devos et al., 1995; Röder et al., 1995 and Plaschke et al., 1995, respectively). Genetic diversity (GD) estimate was in agreement with values reported by Diwan and Creagan, (1997), who found mean GD values close to 0.69 in a group of 36 commercial soybean lines. The average genetic distance among the *C. olitorius* cultivars (21.6 units) was higher than that of *C.capsularis* cultivars (6.0 units) and the distance between the species was 143.67 units. This result indicates that the genetic base of the *C. capsularis* varieties is narrower than *C. olitorius*. Roy et al., (2006) also observed extremely low levels of diversity in the *C. capsularis* cultivars. In the present study, cluster analysis of the ten most popular varieties produced two meaningful clusters based on the genetic distance matrix. Two major clusters represented two species, cluster I consists four cultivars of *C. olitorius* and cluster II consists six cultivars of *C. capsularis*. These results also revealed that different cultivars within each species are distinctly related with significant level of similarity in genetic content. Similar result was obtained by Hossain et al., (2002) while conducting DNA fingerprinting of jute germplasm by RAPD primers. Presence of distinct patterns of diversity between the two species, based on phenotypic characters, was also reported by Palit et al. (1996). Such distinction further strengthens the previous hypothesis that the two species originated from two different geographical locations; *C. olitorius* originated in Africa and *C. capsularis* originated in the Indo-Burma region (Kundu, 1951). Our result is also supported by Basu et al. (2004) who suggested

Table 6. The genetic distances were calculated by measuring the squared Euclidean distance (numbers in yellow colored boxes indicate the distances between the varieties of *C. olitorius* species, numbers in green colored boxes indicate distances between varieties of *C. capularis* and numbers in blue colored boxes indicate distances between varieties belonging to both species)

Cultivars	O-4	O-72	O-9897	OM-1	D-154	CVL-1	CVE-3	CC-45	BJC-7370	BJC-83
O-4	0	21	21	35	139	140	142	143	144	142
O-72		0	10	26	138	139	141	142	143	141
O-9897			0	16	144	145	147	148	149	147
OM-1				0	144	145	147	146	147	145
D-154					0	3	3	6	11	7
CVL-1						0	2	3	8	6
CVE-3							0	3	10	6
CC-45								0	7	7
BJC-7370									0	8
BJC-83										0

that the two species are indeed allopatric, sharing certain common alleles. This distinction could be due to the strong sexual incompatibility barrier between these two cultivated jute species, which do not cross-fertilize (Patel and Datta, 1960; Swaminathan et al., 1961). The six *C. capsularis* cultivars in cluster II were further grouped into sub-cluster IIa containing 5 cultivars and sub-cluster IIb containing a single cultivar BJC-83, which was derived from a cross between CVL-1 and Fuleshwari. Fuleshwari was not included in this study and that might be the reason why BJC-83 was placed in a solitary sub-cluster. Genetic diversity maintained in a plant species is influenced by specific characteristics of that species (Hamrick and Godt, 1989). Jute is basically a self-pollinated crop and sexually incompatible in inter-specific hybridization (Patel and Datta, 1960; Swaminathan et al., 1961). Taxa with selfing and mixed breeding have diversity than out-crossing taxa (Nybom,2004) follow this pattern.

Out of 23 primers used, 11 were able to differentiate between all 10 cultivars. Among them *C. capsularis* cultivars could be easily distinguished with six markers using 5 primer pairs (Table 4) and *C. olitorius* cultivar could be differentiated easily with seven markers using 6 primer pairs (Table 5). Similar fingerprinting keys were developed in radish cultivars for distinguishing cultivars through molecular techniques (Pradhan et al., 2004).

Using jute specific SSR markers (Mir et al., 2007) and using tobacco chloroplast specific SSR markers (Basu et al., 2004) studied the utility of these SSRs for evaluation of genetic diversity. However, our study reports the first ever development of DNA fingerprinting keys for distinguishing jute cultivars. This finding added a significant power of resolution or distinctness in the varietal protection of jute. Development of more DNA fingerprinting markers for popular jute cultivars would enable even more precise distinction among cultivars.

Acknowledegements

We would like to thank Dr. Selina Begum (Chief Scientific Officer, Bangladesh Jute Research Institute) for supplying seeds for this study. We are grateful to the U.S. Department of Agriculture (USDA) for the financial support.

REFERENCES

Anonymous (1997) General Recommendation. In: Proceedings of the first workshop on Jute DUS Testing, Organized by Strengthening Seed Certification Agency Project, Seed Certification Agency, Gazipur held in Bangladesh Jute Research Institute, Dhaka, 7 September, 1997.

Basu A, Ghosh M, Meyer R, Powell W, Basak SL and Sen SK (2004) Analysis of Genetic Diversity in Cultivated Jute Determined by Means of SSR Markers and AFLP Profiling, Crop Sci. 44: 678–685.

Botta L, Scott NS, Eynard I and Thomas MR (1995) Evaluation of microsatellite sequence tagged site markers for haracterizing »itis vinifera cultivars. Vitis 34: 99-102.

Bowditch BM, Albright DG, Williams JGK and Braun MJ (1993) Use of randomly polymorphic DNA in comparative genome studies. Methods in Enz 224: 294-309.

Brown SM, Hopkins MS, Mitchell SE, Senior ML, Wang TY, Duncan RR, Gonalez-Candelas F and Kresovich S (1996) Multiple methods for the identiÞcation of polymorphic simple sequence repeats (SSRs) in sorghum [Sorghum bicolor (L.) Moench]., Theor Appl Genet 93: 190-198.

Cooke RJ (1999). Modern methods for cultivar verification and the transgenic plant challenge. Seed Science and Technology 27 : 669-680.

Degani C, Rowland LJ, Levi A, Hortynski JA and Galletta GJ (1998) DNA fingerprinting of strawberry (Fragaria X ananassa) cultivars using randomly amplified polymorphic DNA (RAPD) markers, Euphytica 102**:** 247-253.

Deng LQ, Li JQ and Li AQ (1994) Studies on the agronomic characters of kenaf germplasm and their utilization (in Chinese), China's Fibre Crops 4: 1–4.

Devos KM, Bryan GJ, Collins AJ, Stephenson P and Gale MD (1995) Application of two microsatellite sequences in wheat storage proteins as molecular markers, Theor Appl Genet 90:247-252.

Diwan N and Cregan PB (1997) Automated sizing of fluorescent-labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. Theor Appl Genet 95:723-733.

Doyle, JJ and Doyle JL (1990) Isolation of plant DNA from fresh tissue, Focus 12: 13-15.

Gupta PK, Balyan HS, Sharma PC and Ramesh B (1996) Microsatellites in plants: a new class of molecular markers, Curr Sci 70**:** 45–54.

Hamrick JL and Godt MJW (1989) Allozyme diversity in plant species. *In:* Brown, A. H. D., Clegg, M. T., Kahler, A. L., Weir, B. S., eds. Plant Population Genetics, Breeding and Genetic Resources. Sinauer, Associates Publishers, Sunderland, MA. pp. 43–63

Hossain MH, Haque S and Khan H (2002) DNA Fingerprinting of Jute Germplasm by RAPD, Journal of Biochemistry and Molecular Biology 35(4): 414-419.

Hossain MH, Awal A, Rahman MA, Haque S and Khan H (2003) Distinction between Cold-sensitive and -tolerant Jute by DNA Polymorphisms, Journal of Biochemistry and Molecular Biology 35(5): 427-432.

Kundu BC (1951) Origin of jute. Indian J. Genet. Plant Breed. 11:95-99.

La Farge, T., S.T. Friedman, and C.G. Cock. 1997. Improvement of fiber crops using genetics and biotechnology. p. 39–59. *In* R.M. Powell et al. (ed.) Paper and composites from agro-based resources. CRC Lewis Publishers, Boca Raton, FL.

Lamboy WF and Alpha CG (1988) The utility of simple sequence repeats (SSRs) for DNA Þngerprinting germplasm accessions of grape (*vitis* L.) species, J Am Soc Hortic Sci 123:182-188.

Mir RR, Rustgi S, Sharma S, Singh R, Goyal A, Kumar J, Gaur A, Tyagi AK, Khan H, Sinha MK, Balyan HS, Gupta PK (2007) A preliminary genetic analysis of fibre traits and the use of new genomic SSRs for genetic diversity in jute. Euphytica (Published Online, 30 October, 2007)

Mohiuddin G, Rashid M, Rahman M, Hasib SA and Razzaque A (2005) Biopulping of whole jute plant in soda-anthraqunion (AQ) and kraft processes, TAPPI Journal 4(3):23-27.

Murray M and Thompson WF (1980). Rapid isolation of high molecular weight plant DNA. Nucl. Acids Res, 8: 4321– 4325.

Nielsen G (1985) The use of isozymes as probes to identify and label plant varieties and cultivars. *In:* M. C. Rattazzi, Scandalios, J. G. & Whitt, G. S. (Eds), Isozymes: Current Topics In Biological and Medical Research. Vol 12: pp. 1-32. Alan R. Liss, New York.

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants, Mol Ecol 13:1143–1155.

Palit P, Sasmal BC and Bhattacharyya AC (1996) Germplasm diversity and estimate of genetic advance of four morpho-physio- logical traits in a world collection of jute, Euphytica 90:49–58.

Patel GI and Datta RM (1960) Interspecific hybridization between Corchorus olitorius and C. capsulris and the cytogenetical basis of incompatibility between them, Euphytica 9: 89–110.

Plaschke J, Ganal MW, Röder, MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers, Theor Appl Genet 91:1001-1007.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S and Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis, Mol Breed 2**:**225-238.

Pradhan A, Yan G and Plummer JA (2004) Development of DNA fingerprinting keys for the identification of radish cultivars, Australian Journal of Experimental Agriculture 44**:** 95-102.

Qi J, Zhou D, Wu W, Lin L, Fang P and Wu J (2003a) The application of RAPD technology in genetic diversity detection of Jute, Ying Yong Sheng Tai Xue Bao 30**:**926—932.

Qi J, Zhou D, Wu W, Lin L, Wu J and Fang P (2003b) Application of ISSR technology in genetic diversity detection of jute, Ying Yong Sheng Tai Xue Bao 14: 1473—1477.

Rajora OP and Rahman MH (2003) Microsatellite DNA and RAPD fingerprinting, identification and genetic relationships of hybrid poplar (Populus 3 canadensis) cultivars, Theor Appl Genet 106:470–477.

Röder MS, Plaschke J, König SU, Börner A, Sorrells ME, Tanksley SD and Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat, Mol Gen Genet 246: 327-333

Rongwen J, Akkaya MS, Bhagwat AA, Lavi U, Cregan PB (1995) The use of microsatellite DNA markers for soybean genotype identiÞcation, Theor Appl Genet 90:43-48.

Roy A, Bandyopadhyay A, Mahapatra AK, Ghosh SK, Singh NK, Bansal KC, Koundal KR and Mohapatra T (2006) Evaluation of genetic diversity in jute (Corchorus species) using STMS, ISSR and RAPD markers, Plant Breeding 125:292—297.

Russell, J., Fuller, J., Young, G., Thomas, B., Taramino, G., Macaulay, M., Waugh, R., Powell, W. (1997) Discriminating between barley genotypes using microsatellite markers, Genome 40**:** 442-450.

Sambrook, J., Fritsch, E. F., Maniatis, T. (1989) Molecular cloning. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NewYork

Singh DP (1976) Jute: Evolution of crop plants. In: N.W.Simonds(ed), Longman Publ. Co. London. pp. 290-291.

Song QJ, Quigley CV, Nelson RL, Carter TE, Boerma HR, Strachan JR (1999) A selected set of trinucleotide simple sequence repeat markers for soybean cultivar identification, Plant Var Seeds 12**:** 207-220.

Streiff R, Labbe T, Bacilieri R, Steinkellner H, Glosslè J and Kremer A (1998) Within-population genetic structure in Quercus robur L. and Quercus petraea (Matt.) Liebl. assessed with isozymes and microsatellites. Mol. Ecol 7:317-328.

Swaminathan MS, Iyer RD and Sulbha K (1961) Morphology, cytology and breeding behaviour of hybrids between Corchorus olitorius and C. capsularis, Curr Sci 30:67—68.

Swofford DL (2001) 'PAUP: Phylogenetic analysis using parsimony (4.0b8 edn).' (Illinois History Survey Champaign:Illinois).

Thomas MR, and Scott NS (1993) Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs), Theor Appl Genet 86: 985-990.

Weir BS (1990) Genetic data analysis methods for discrete genetic data. Sinauer Association, Sunderland, Massachusetts, 445 pp.