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MOLECULAR DIVERSITY ASSESSMENT USING SSR PRIMERS CONFIRMING DISTINCT SPECIES OF JUTE GERMPLASM UNDER *Corchorus* GENUS

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ABSTRACT

The aim of the experiment was to find out genetic diversity of some jute germplasm collected from different locations of the world that are stored at the gene bank of Bangladesh Jute Research Institute (BJRI). The experiment was conducted at the laboratory of Cytogenetics department of Genetic Resources and Seed Division of BJRI. Genetic variation among 22 jute germplasm including 9 varieties of BJRI were determined using 33 SSR primers. DNA was extracted from seedlings following mini preparation CTAB method with some modifications. Vertical gel electrophoresis system was used with polyacrylamide gel. Among these, 14 primers were polymorphic and amplified 39 loci. The highest gene diversity value among the polymorphic primers was 0.685 and the lowest was 0.177 with an average of 0.507. The highest genetic distance value (1.0) was found between *Corchorus capsularis* (white jute) and *C. olitorius* (dark jute/tossa jute) germplasm. Among the *C. olitorius* germplasm, the highest genetic distance value (0.4) was found between accession no. 1039 and accession no. 1347. Among the *C. capsularis* germplasm, accession no. 4484 showed the highest genetic distance value (0.214) with varieties CVL-1 and BJRI Deshi Pat Shak-1. UPGMA dendrogram, based on genetic differences separated the germplasm of two different species in two main clusters and the cluster of *C. olitorius* is subdivided into two sub-clusters. We found no genetic similarity between the two species of jute. Plant breeders can consider the studied germplasm of *C. olitorius* as more potential for crossing programs as they have good genetic diversity.

Key words: Genetic polymorphism; Genetic variation; Jute; Molecular markers; SSR Markers; UPGMA dendrogram

Introduction

The jute genus *Corchorus*, consists of around 100 species having both annual and perennials (Saunders 2001-2006; Benor *et al.* 2010). There are only two cultivated jute species, viz., *C. capsularis* L. (white jute) and *C. olitorius* L. (dark jute) (Ghosh 1983) where the latter one is being cultivated around 80% jute growing areas in Bangladesh (Islam *et al.* 2009). Jute is a self-pollinated crop with limited genetic variation among the germplasm (La Farge *et al.* 1997). Over that, the number of germplasm is declining in jute in a alarming rate (Heywood *et al.* 2007). Therefore, it is urgent to identify and conserve the endangered genotypes of jute for their future use in jute improvement program.

Jute genotypes are mainly characterized by qualitative morphological characters like pigmentation pattern in different parts of a plant, various shapes of leaf, stipule, seeds and many other characters (Begum and Kumar 2011). Closely related cultivars cannot be distinguished through this method (Deng *et al.*1994). Even though, a large environmental influence on these traits makes this morphological characterization less reliable (Nielsen 1985; Cooke 1999). These limitations forced scientists to use molecular marker for characterization of plants.

PCR (ploymerase chain reaction) based DNA markers, amplified fragment length polymorphisms (AFLPs), randomly amplified polymorphic DNA (RAPD), and simple sequence repeats (SSRs) are unaffected by environmental factors (Bowditch *et al.* 1993). Among these, SSRs are more abundant in plant genome and have high polymorphic information content in various plants like soybean and poplar (Gupta *et al.* 1996), suggesting their suitability in separating individuals at molecular level. Jute genetic diversity has been reported using SSRs for few Bangladeshi varieties and advanced lines by Huq *et al.* (2009). Indian and other countries wild jute genotypes and varieties was also characterized by Mir *et al.* (2008), Ghosh *et al.* (2014), Zhang *et al.* (2015), Ghosh *et al.* (2017), and Nag *et al.* (2018). Therefore, A comprehensive analysis of genetic diversity of Bangladeshi wild jute accessions along with the released varieties is inadequate. Thus, the current research is undertaken to develop genetic fingerprints and to estimate genetic relatedness of selected 22 jute (*C. olitorius* and *C. capsularis*) germplasm from the Gene Bank of Bangladesh Jute Research Institute (BJRI) using SSR markers.

Materials and Methods

We collected seeds of 22 jute genotypes (Table 1) from the Gene Bank.

Table 1. Twenty-two accessions of Jute with their country of origin

Sl. no.	Accession Number/Variety	Species	Country of Origin
1	O-5	*Corchorus olitorius*	BJRI Variety
2	O-9897	*C. olitorius*	BJRI Variety
3	O-72	*C. olitorius*	BJRI Variety
4	OM-1	*C. olitorius*	BJRI Variety
5	O-795	*C. olitorius*	BJRI Variety
6	A.1039	*C. olitorius*	Bangladesh
7	A.1234	*C. olitorius*	Bangladesh
8	A.1245	*C. olitorius*	Bangladesh
9	A.1338	*C. olitorius*	Philippine
10	A.1347	*C. olitorius*	Uganda
11	A.1480	*C. olitorius*	Srilanka
12	A.1362	*C. olitorius*	Bangladesh
13	CVL-1	*C. capsularis*	BJRI Variety
14	BJC7370	*C. capsularis*	BJRI Variety
15	BJC2197	*C. capsularis*	BJRI Variety
16	BDPS1	*C. capsularis*	BJRI Variety
17	A.552	*C. capsularis*	Bangladesh
18	A.568	*C. capsularis*	Bangladesh
19	A.628	*C. capsularis*	Bangladesh
20	A.646	*C. capsularis*	Bangladesh
21	A.4484	*C. capsularis*	Thailand
22	A.4618	*C. capsularis*	Brazil

These seeds were placed on wet blotting paper in petri dishes and kept in a dark place. DNA was isolated from 4 days old seedlings using modified CTAB method (Doyle and Doyle 1987). Approximately 1.5 g tissue was ground to a fine powder with the mortar and pestle in liquid nitrogen and was taken in a 2ml centrifuge tube and 1ml extraction buffer (2% CTAB, 1.4M NaCl, 20mM EDTA, 100 mM Tris base, 100mM β-mercaptoethanol, 2% Polyvinylpolypyrrolidon) was added. The centrifuge tubes were kept in a water bath with 65°C for 10 minutes and then centrifuged with 13000rpm for 10 minutes. The supernatants were collected and transferred to fresh 1.5ml centrifuge tubes. DNA was purified by Phenol: Chloroform:Isoamyl alcohol (25:24:1) and was precipitated using ice-cold isopropanol in the presence of 0.3 M sodium acetate. Finally, DNA was pelleted and washed with 70% and 100% ethanol. The pellets were dried with vacuum freeze dryer and dissolved in 100µl TE buffer (10 mM Tris-HCl, 1mM EDTA pH-8.0) and stored at -20°C. For making working solutions, extracted DNA solutions were diluted to 10 times in new centrifuge tubes. Thirty three SSR primers (Table 2) were collected from the Basic and Applied Research on Jute Project, Bangladesh Jute Research Institute, Manik Mia Avenue, Dhaka 1207, Bangladesh and were used in this experiment as molecular markers. PCR was performed in 10 µl reactions containing around 25 ng of DNA template (3 µl DNA with 10X dilution factor), 1 µl 10X TB buffer (containing 200 mM Tris-HCl pH 8.3, 500 mM KCl), 1.35 µl 25 mM $MgCl_2$, 0.2 µl of 10 mM dNTP, 0.5 µl each of 10 µM forward and reverse primers and 0.1 µl of Taq DNA polymerase (5 U/µl) using thermal cycler

(Neeraja *et al*. 2007). After initial denaturation for 5 min at 94°C, each cycle comprises 45 sec denaturation at 94°C, 45 sec annealing at 55°C, and 2 min extension at 72°C with a final extension for 7 min at 72°C at the end of 35 cycles. 2.5 µl of 10x loading buffer (Bromophenol Blue 0.4%, Xylene cyanol 0.4%, Glycerol 50%) was added to each PCR product.

Table 2. SSR Primers used for diversity analysis of 22 jute germplasm

Sl	Primer		Sequence	Repeat Motif	Status
1	JMBD 142	Forward Primer	ATGAAATGGAGGTGCTACGG	(CGC)6	Not amplified
		Reverse Primer	CACTTCCTTCGACTTCTCCG		
2	JMBD 148	Forward Primer	ACCCACCAAGTTCATGCTTC	(CAT)5	Polymorphic
		Reverse Primer	GAAGGAAGTGAGCAAGCCAG		
3	JMBD 154	Forward Primer	ATCCACGCCTCCAACATAAG	(TCA)5	Not amplified
		Reverse Primer	GAACAAACCCGCACTTGACT		
4	JMBD 166	Forward Primer	GCAGTTTGTGGTGATGGATG	(TTA)6	Polymorphic
		Reverse Primer	GCCTTAAAGTGCATACAGGAGG		
5	JMBD 238	Forward Primer	CACCGTGCAACTGCAAATAG	(GAA)6	Not amplified
		Reverse Primer	CTGTCTTCTCCTTCCGCTTG		
6	JMBD 423	Forward Primer	CACAGCCAAGCTGATGAGAA	(GAT)5	monomorphic
		Reverse Primer	CCTCACGCTCTGGAGACTTC		
7	JMBD 434	Forward Primer	TTGTGGGAGTAACAGGAGGG	(AAG)5	monomorphic
		Reverse Primer	GAGCTGATATTGGCGGTGTT		
8	JMBD 492	Forward Primer	AACCAAAGCACCACCACTTC	(ACC)5	monomorphic
		Reverse Primer	CGCTGACGACGATATCTTGA		
9	JMBD **563**	Forward Primer	TGGGCTTGTAACCAAGGAAG	(GTA)5	Polymorphic
		Reverse Primer	CAAACAAATGTGCCATTCCA		
10	JMBD 598	Forward Primer	CCTAATTTCCACCACCAACG	(CTT)5	Polymorphic
		Reverse Primer	CGGGTTAAGGGTCTTGTTGA		
11	JMBD 616	Forward Primer	AGCATCCCATTCTTCAGGTG	(CTT)5	monomorphic
		Reverse Primer	GTCATCTCGCTCTGCTCTCC		
12	JMBD 639	Forward Primer	TCATCCTCCACCTCCTCATC	(CAT)5	monomorphic
		Reverse Primer	CCTAACCCTAATGCCACCCT		
13	JMBD 643	Forward Primer	TAATAACTGCGCCTTCGACC	(GCC)5	monomorphic
		Reverse Primer	GCTGTTGTTGCTGCTGGTAA		
14	JMBD 709	Forward Primer	GTTGACCAGGCTTCTTCTGC	(ATC)5	Polymorphic
		Reverse Primer	CAAGCAGCAATCACAGCAAT		
15	JMBD 721	Forward Primer	CCCATCCAAATTAGCCACAC	(TGT)5	Polymorphic
		Reverse Primer	CTCTCTCCAAACTGCCCAAG		
16	JMBD 880	Forward Primer	GCTCCTACTTTCATTGAATGGC	(TCT)5	Polymorphic
		Reverse Primer	CCTGTTCTTGTTGCTGCTGA		
17	JMBD 929	Forward Primer	ACCCTTTCCTTGGATTACGC	(GAA)5	monomorphic
		Reverse Primer	GCTTCTTCAATTCGCGAGAG		
18	JMBD 969	Forward Primer	ATTCTTGCATGGAAACGGAG	(AAT)5	Polymorphic
		Reverse Primer	CCTTTGTTCATCTGCTGCAA		
19	JMBD 989	Forward Primer	GGAAGAGATCAGGCTCAACG	(GGA)5	Polymorphic
		Reverse Primer	GATCTCATTCCCTTGCCAAA		
20	JMBD 1150	Forward Primer	CGCTATCTCCTCTGCTCCTG	(TTC)6	monomorphic
		Reverse Primer	CATTTCGACGATCGGATTCT		
21	JMBD 1579	Forward Primer	TCAATCTTCACCAGCAGCAG	(ACA)5	monomorphic
		Reverse Primer	GCCGTCTCCTATTTCCATGA		
22	JMBD 1667	Forward Primer	AGTTCACTTGGGATCGGTTG	(GTG)5	monomorphic
		Reverse Primer	GATAAAGCCACAGGAAGCCA		
23	JMBD 1774	Forward Primer	TCTTTCTGGTCCACCTTTGG	(GAG)5	monomorphic
		Reverse Primer	CTGGATTCGTCCACTCCCTA		
24	JMBD 1793	Forward Primer	TTCCGACTTCCGCAATAAAC	(TCT)5	monomorphic
		Reverse Primer	GTGTCGGACGAGGAAACACT		

Sl	Primer		Sequence	Repeat Motif	Status
25	JMBD 1806	Forward Primer	TGAGTCACTTCTTGATGCCG	(GAT)5	Not amplified
		Reverse Primer	CTTGGCCTGGATAATAGGCA		
26	JMBD 1824	Forward Primer	TTTACGAAACCTGCCACTCC	(GAA)5	Polymorphic
		Reverse Primer	CGCTCACAACTCCTTTCTCC		
27	JMBD 1912	Forward Primer	ACTACGTCCCGTCAGTCACC	(CTG)5	monomorphic
		Reverse Primer	GCACATTCTTCCGACCATCT		
28	JMBD 1950	Forward Primer	AGTGAACCTCCACAAATGCC	(AAC)6	Polymorphic
		Reverse Primer	GGCGAATTCGAAATGGTAGA		
29	JMBD 2032	Forward Primer	AAAGCATTGGATCTTCGTGG	(TGA)5	Polymorphic
		Reverse Primer	GTTGCATACTGGTGCATTGG		
30	JMBD 2045	Forward Primer	GGACAGAAGTTCGAGCCAAG	(CGG)5	Polymorphic
		Reverse Primer	GTTTCCCACCAGTAGTCCGA		
31	JMBD 2047	Forward Primer	CATACAAATGCAGACGGTGG	(AAG)5	Not amplified
		Reverse Primer	GCTCTCCTTCATTTGGCTCA		
32	JMBD 2054	Forward Primer	TTGGGAAGCAAGATGGAAAC	(GAA)5	Not amplified
		Reverse Primer	CGCACTTCCACCCATCTTAT		
33	JMBD 2064	Forward Primer	ACGAGATGGATTCTGATGCC	(GAC)5	Polymorphic
		Reverse Primer	CTCCAGCTTTGCTTGGAAAC		

PCR products were run on 8 % polyacrylamide gel electrophoresis system. Electrophoresis was conducted at 130 volts for 90 minutes. DNA ladder (1 kb^+) were electrophoresed alongside the PCR products. After completion of the electrophoresis, the gel was stained in ethidium bromide solution for 25 minutes. Then the gel was rinsed carefully with the tap water and placed on a gel documentation system for visualization of DNA bands and the images were taken and saved in a computer.

Data analysis: Molecular weight for each amplified allele was measured in base pair using Alpha EaseFC 4.0 software. The allele frequency data from Power Marker version 3.25 (Liu and Muse. 2005) was used to export the data in binary format (allele presence = 1 and allele absence = 0) for analysis with NTSYS-pc version 2.2 (Rohlf, 2002). The summary statistics including the number of alleles per locus, major allele frequency, genetic diversity, polymorphism information content (PIC) values were determined using Power Marker version 3.25 (Liu and Muse, 2005). A similarity matrix was calculated with the Simqual subprogram using the DICE coefficient, followed by cluster analysis with the SAHN subprogram using the UPGMA clustering method and implemented in NTSYS-pc to construct a dendrogram showing a relationship among the genotypes.

Results

Our used 33 SSR primers (Table 1) amplified a total number of 52 loci. Out of these 33 primers, we found 14 primers polymorphic (Tables 3-4) for the germplasm and they amplified 39 loci (Table 4). Thirteen primers were monomorphic (Table 5) for the germplasm and 6 primers did not amplify properly. The SSR profiles of 22 Jute accessions using SSR primers JMBD563 and JMBD598 are shown in Fig. 1. Table 3 shows the major allele frequency of 14 polymorphic primers and genetic diversity values. The highest gene diversity value (0.685) and PIC (Polymorphism Information Content) value (0.635) were found for primer JMBD1824. The second highest gene diversity value (0.640) and PIC value (0.567) were found for the primer JMBD563. The lowest gene diversity value (0.177) and PIC content (0.169) among the 14 primers were found for the primer JMBD709. The result suggests that genetic variations exist among these jute germplasm. The overall gene diversity value and PIC value for all polymorphic primers were estimated as 0.507 and 0.413 respectively. The highest (0.90) Major Allele Frequency was found for allele size 185bp for JMBD709 (Tables 3-4). The frequency and variance of all 39 alleles from 14 polymorphic markers for twenty-two jute genotypes are presented in Table 4. $JMBD989_{170}$ allele $JMBD989_{185}$ showed the highest variance for all 22 genotypes (Table 4).

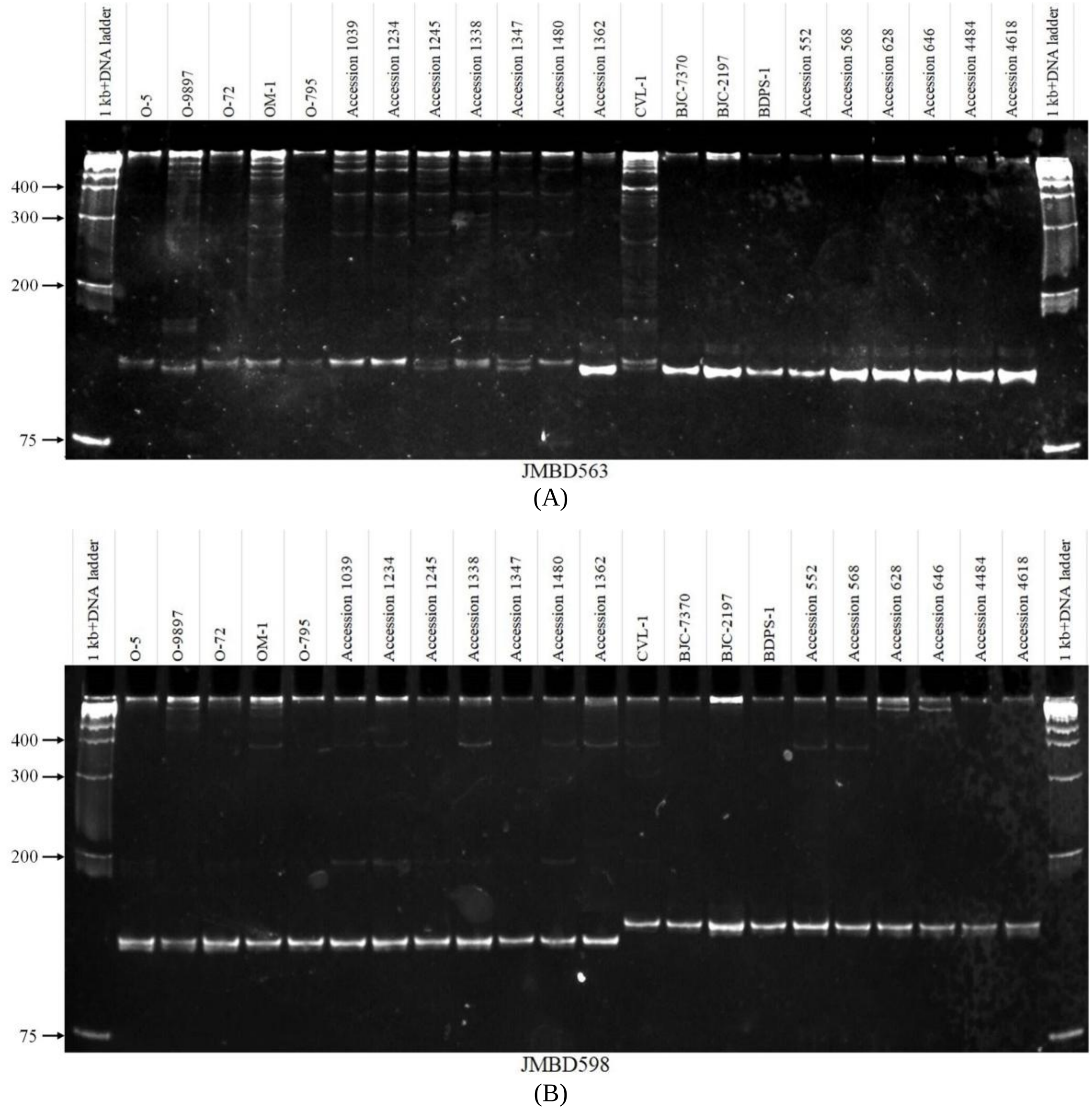

Fig.1. DNA profile of the 22 jute genotypes with the SSR marker JMBD563 (A) and JMBD 598(B)

We computed the values of pair wise Nei's genetic distance between accessions from combined data for 14 SSR primers. The values were ranged from 0 to 1 (Table 6). BJRI dark jute variety O-795 showed the highest genetic distance value with white jute accession no. 4484, 4618, 552, 568, 628, 646, BDPS1, BJC2197, BJC 7370, and CVL-1. BJRI Deshi Pat Sak-1 showed the highest genetic distance value with dark jute accession no. 1039, 1234, 1338, 1347, 1362, and BJRI dark jute varieties O-72, O-5, O-9897 and O-795. Dark jute accession no. 1347 showed highest distance value with white jute accession no. 552, 628 and BJRI white jute variety CVL-1, BJC-7370 and BJRI Deshi Pat Sak-1. Among the dark jute germplasm, the highest genetic distance value (0.4) was found between accession no.1039 and accession no.1347. Among the white germplasm, accession no. 4484 showed the highest genetic distance value (0.214) with varieties CVL-1 and BJRI Deshi Pat Shak-1.

Table 3. Summary of the genetic variation statistics for major alleles

Marker	Sample Size	No. of obs.	No. of Allele	Highest frequency allele		Gene Diversity	Polymorphism Information Content
				size (bp)	frequency		
JMBD148	22	22	3	168	0.50	0.541	0.436
JMBD 166	22	22	4	140	0.55	0.566	0.484
JMBD 563	22	22	3	111	0.45	0.640	0.567
JMBD 598	22	22	2	124	0.55	0.496	0.373
JMBD 709	22	21	3	185	0.90	0.177	0.169
JMBD 721	22	15	3	245	0.73	0.418	0.370
JMBD 880	22	20	3	195	0.50	0.545	0.441
JMBD 969	22	22	2	86	0.55	0.496	0.373
JMBD 989	22	16	2	185	0.56	0.492	0.371
JMBD 1824	22	20	5	305	0.45	0.685	0.635
JMBD 1950	22	18	3	293	0.50	0.549	0.448
JMBD 2032	22	22	2	170	0.55	0.496	0.373
JMBD 2045	22	22	2	220	0.55	0.496	0.373
JMBD 2064	22	21	2	225	0.52	0.499	0.374
Mean	22		3		0.56	0.507	0.413

Table 4. Overall Allele Frequency of 14 polymorphic markers for 22 jute germplasm

Sl no.	Marker	Allele Size (bp)	Frequency	Variance	SD
1	JMBD148	168	0.50	0.0114	0.107
2	JMBD148	172	0.05	0.0020	0.044
3	JMBD148	220	0.45	0.0113	0.106
4	JMBD166	135	0.36	0.0105	0.103
5	JMBD166	140	0.55	0.0113	0.106
6	JMBD166	145	0.05	0.0020	0.044
7	JMBD166	188	0.05	0.0020	0.044
8	JMBD563	111	0.45	0.0113	0.106
9	JMBD563	117	0.23	0.0080	0.089
10	JMBD563	124	0.32	0.0099	0.099
11	JMBD598	124	0.55	0.0113	0.106
12	JMBD598	137	0.45	0.0113	0.106
13	JMBD709	185	0.90	0.0041	0.064
14	JMBD709	190	0.05	0.0022	0.046
15	JMBD709	218	0.05	0.0022	0.046
16	JMBD721	236	0.07	0.0041	0.064
17	JMBD721	245	0.73	0.0130	0.114
18	JMBD721	253	0.20	0.0107	0.103
19	JMBD880	195	0.50	0.0125	0.112
20	JMBD880	205	0.05	0.0024	0.049
21	JMBD880	218	0.45	0.0124	0.111
22	JMBD969	86	0.55	0.0113	0.106
23	JMBD969	96	0.45	0.0113	0.106
24	JMBD989	170	0.44	0.0154	0.124
25	JMBD989	185	0.56	0.0154	0.124
26	JMBD1824	305	0.45	0.0124	0.111
27	JMBD1824	315	0.05	0.0024	0.049
28	JMBD1824	320	0.10	0.0045	0.067
29	JMBD1824	325	0.30	0.0105	0.102
30	JMBD1824	333	0.10	0.0045	0.067
31	JMBD1950	198	0.44	0.0137	0.117
32	JMBD1950	293	0.50	0.0139	0.118
33	JMBD1950	299	0.06	0.0029	0.054

Sl no.	Marker	Allele Size (bp)	Frequency	Variance	SD
34	JMBD2032	170	0.55	0.0113	0.106
35	JMBD2032	200	0.45	0.0113	0.106
36	JMBD2045	212	0.45	0.0113	0.106
37	JMBD2045	220	0.55	0.0113	0.106
38	JMBD2064	225	0.52	0.0119	0.109
39	JMBD2064	240	0.48	0.0119	0.109

Table 5. Amplified allele size of monomorphic primers for 22 jute germplasm

Sl no.	Marker	Allele Size (bp)
1	JMBD423	203
2	JMBD434	205
3	JMBD492	270
4	JMBD616	380
5	JMBD639	210
6	JMBD643	250
7	JMBD929	315
8	JMBD1150	260
9	JMBD1579d	275
10	JMBD1667	295
11	JMBD1774	168
12	JMBD1793	180
13	JMBD1912	280

Table 6. Nei's (1983) genetic distance values among twenty two jute accessions

	A1039	A1234	A1245	A1338	A1347	A1362	A1480	A4484	A4618	A552
A1234	0.083									
A1245	0.300	0.167								
A1338	0.167	0.071	0.250							
A1347	0.400	0.250	0.182	0.167						
A1362	0.333	0.214	0.167	0.214	0.167					
A1480	0.091	0.077	0.091	0.154	0.273	0.231				
A4484	0.917	0.857	0.750	0.857	0.917	0.857	0.769			
A4618	0.909	0.846	0.750	0.846	0.909	0.846	0.750	0.077		
A552	0.917	0.923	0.818	0.923	1	0.923	0.833	0.077	0	
A568	0.917	0.857	0.750	0.857	0.917	0.857	0.769	0.071	0	0
A628	0.917	0.923	0.818	0.923	1	0.923	0.833	0.077	0	0
A646	0.909	0.846	0.750	0.846	0.909	0.846	0.750	0.077	0	0
BDPS1	1	1	0.917	1	1	1	0.923	0.214	0.154	0.077
BJC2197	0.917	0.857	0.750	0.857	0.917	0.857	0.769	0.071	0	0
BJC7370	0.909	0.923	0.833	0.923	1	0.923	0.833	0.154	0.077	0
CVL-1	0.917	0.929	0.833	0.929	1	0.929	0.846	0.214	0.154	0.077
O-5	0	0	0.222	0	0.222	0.182	0.091	0.909	0.900	0.909
O-72	0.100	0	0.182	0.091	0.300	0.273	0.100	0.909	0.909	0.909
O-795	0.333	0.200	0.200	0.200	0.111	0.200	0.333	1	1	1
O-9897	0.250	0.214	0.250	0.286	0.333	0.286	0.308	0.929	0.923	0.923
OM-1	0.167	0.154	0.364	0.154	0.364	0.231	0.250	0.846	0.833	0.846

Table 6 (Contd.)

	A568	A628	A646	BDPS 1	BJC2 197	BJC7 370	CVL- 1	O-5	O-72	O-795	O- 9897
A628	0										
A646	0	0									
BDPS1	0.143	0.077	0.154								
BJC2197	0	0	0	0.143							
BJC7370	0.077	0	0.077	0.077	0.077						
CVL-1	0.143	0.077	0.154	0.143	0.143	0.077					
O-5	0.909	0.909	0.900	1	0.909	0.900	0.909				
O-72	0.909	0.909	0.909	1	0.909	0.909	0.909	0			
O-795	1	1	1	1	1	1	1	0.222	0.200		
O-9897	0.929	0.923	0.923	1	0.929	0.923	0.929	0.182	0.182	0.200	
OM-1	0.846	0.846	0.833	0.923	0.846	0.833	0.846	0.091	0.182	0.300	0.308

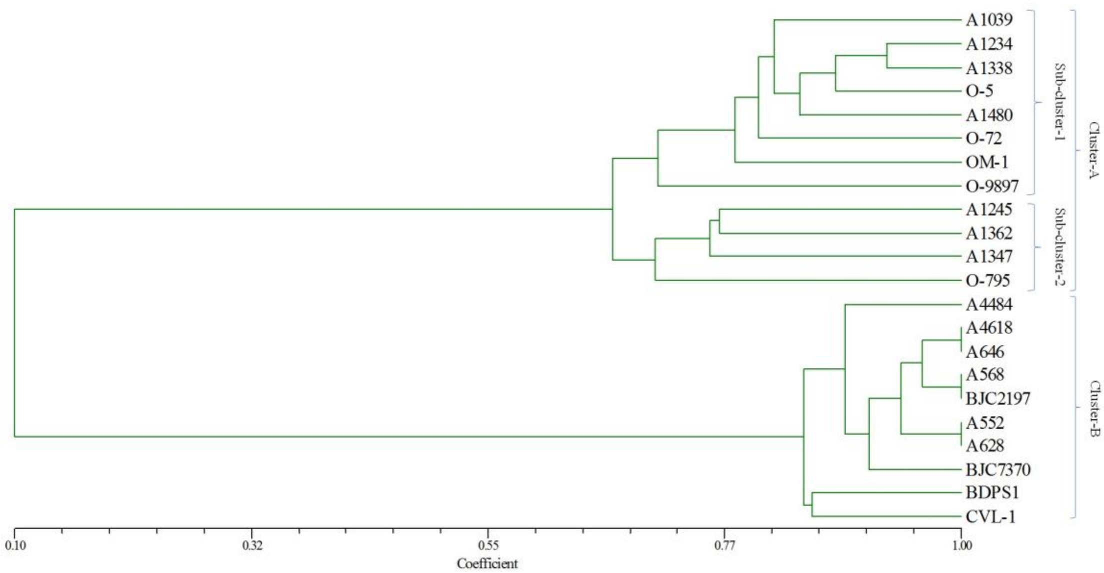

Fig. 2. UPGMA cluster dendrogram showing the Nei's genetic distance (Nei, 1983) among 22 jute genotypes based on the alleles detected by 14 microsatellite markers

Two species of jute, *Corchorus capsularis* and *C. olitorius* formed two main clusters in the dendrogram (Fig. 2). *C. olitorius* germplasm was divided into 2 sub-clusters. The dendrogram is based on Nei's (1983) genetic distance. Sub-Cluster 1 was comprised of accession no.1039, accession no.1234, accession no.1338, O-5, accession no.1480, O-72, OM-1and O-9897. Sub-cluster 2 was comprised of accession no.1245, accession no.1362, accession no.1347 and O-795. Second main cluster B was comprised of accession no.4484, accession no.4618, accession no.646, accession no.568, BJC-2197, accession no.552, accession no.628, BJC-7370 and, BJRI deshi pat shak-1 and CVL-1. Three pairs of accessions, 4618 and 646, 568 and BJC2197, 552 and 628 were not differentiated through our analysis (Fig. 2).

Discussion

In the present study molecular diversity was assessed in *Corchorus* genus to prove genetic distinctness between the two cultivated jute species, *C. capsularis* and *C. olitorius*. Out tested molecular primers were successfully amplified in jute. These markers showed excellent polymorphism among the jute accessions. The number of alleles per locus generated by each marker varied from 2 to 5, with an average of 3 alleles per locus, which differed as 2.3, 4.61 and 6.33 alleles per locus (Ghosh *et al.* 2017; Huq *et al.* 2009), respectively and similar with 3.04 (Mir *et al.* 2008). Genotypic variation among the genotypes may account for this variation in the number of allels per locus. PIC value indicates the effectiveness of SSR loci information of a given marker locus for the group of genotypes (Cooper *et al.* 2001). The PIC value varied noticeably among the studied SSR loci from 0.169 (JMBD 709) to 0.635 (JMBD 1824) with an average value of 0.413 (Table 3), which was higher than the average PIC of 0.135 and 0.36 as reported by Das *et al.* (2012) and Nag *et al.* (2018) and similar with the result of Ghosh *et al.* 2017. However, higher PIC value of these markers indicating that, these can be used as reliable molecular marker for jute molecular characterization in future. Max. genetic distance was found in between white jute and dark jute (Table 6) because of their high morphological distinctness (Islam 2019). However, accession no. 1039 and 1347 showed he highest genetic distance for dark jute and among the white germplasm, accession no. 4484 showed the highest genetic distance value with varieties CVL-1 and BJRI Deshi Pat Shak-1. The jute accessions were divided into two distinct groups, supporting that there is high genetic variation between white and dark jute. This classification is in accord with the genetic diversity reported in several previous studies (Gao *et al.* 2009). Different geographical origin of these two jute species may classify distinctly white jute from dark jute. This well accepted hypothesis is not valid for the study of Rana *et al*. 2012; Patel and Datta 1960. Taking SSR analysis in consideration, suitable parents was identified for hybridization in a breeding program for generating desirable hybrid within a jute species.

Conclusion

Our experiment revealed considerable variability among these tested 22 jute germplasm. Not only that, we easily distinguish white jute from dark jute based on their genetic distance. Primer JMBD 1824 and JMBD 563 may consider as most suitable for variability study in jute because of their ability to produce high genetic diversity and polymorphism among the studied genotypes. Morphological and biochemical analysis of the dark jute accession no. 1039 and 1347 are required to consider them as suitable parents for a breeding program. High crossing incompatibility between the *C. capsularis* and *C. olitorius* limits hybrid production between dark jute accession no. 1347 with other white jute genotypes as this dark jute accession has the highest genetic distance with white jute genotypes. It is hoped that in future the present results will help scientists to make a selection from the diverse accessions studied to use them as parents for inter-specific crossing that are designed for the breeding.

Authors' contributions

Conceptualization, research planning, research execution: A. B. M. Zahidul Hoque; Discussion and manuscript preparation: A. B. M. Z. Hoque and M. Z. Tareq; Manuscript submission: M. Z. Tareq; Supervision: M. Z. Hossain, S. M. Mahbub Ali and M. R. Islam.

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References

Begum, T. and Kumar, D. 2011. Usefulness of morphological characteristics for DUS testing of jute (*Corchorus olitorius* L. and *C. capsularis* L.). *Span J Agric Res*., 9:473-483.

Benor, S., Blattner, F. R., Demissew, S. and Hammer, K. 2010. Collection and ethnobotanical investigation of *Corchorus* species in Ethiopia: potential leafy vegetables for dry regions. *Genetic Res Crop Evol*., 57: 293-306.

Bowditch, B. M., Albright, D. G., Williams, J. G. K. and Braun, M. J. 1993. Use of randomly polymorphic DNA in comparative genome studies. *Methods Genet Eng*., 224:294-309.

Cooke, R. J. 1999. Modern methods for cultivar verification and the transgenic plant challenge. *Seed Sci. Technol*., 27:669-680.

Cooper, H. D., Spillan, E. C. and Hodgkin, T. 2001. Broadening the genetic base of crops: an overview. Broadening the genetic base of crop production. CAB International, Wallingford, pp. 1-23.

Das, M., Banerjee, S., Dhariwal, R., Vyas, S., Mir, R. R., Topdar, N., Kundu, A., Khurana, J. P., Tyagi, A. K., Sarkar, D., Sinha, M. K., Balyan, H. S. and Gupta P. K. 2012. Development of SSR markers and construction of a linkage map in jute. *J. Genet.*, 91:1-11.

Deng, L. Q., Li, J. Q. and Li, A. Q. 1994. Studies on the agronomic characters of kenaf germplasm and their utilization. *China's Fibre Crops*, 4:1-4 (in Chinese).

Doyle, J. J. and DOYLE, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull*., 19:11-15.

Gao, Y. L., Hu, R. S., Liu, C. Y., Li, W. F., Jiang, H. W., Li, C. D., Yao, B. C., Hu, G. H. and Chen, Q. S. 2009. Establishment of molecular ID in soybean varieties in Heilongjiang, China. *Acta Agron. Sin*., 35:211-218 (in Chinese with English abstract).

Ghosh, T. 1983. Handbook on jute. FAO, Rome, p.219.

Ghosh, S., Meena, K., Sinha, M. K. and Karmaka, P. G. 2017. Genetic Diversity in *Corchorus olitorius* Genotypes Using Jute SSRs. Proc Natl Acad Sci, India, *Sect B Bio. Sci.*, 87:917-926.

Heywood, V. H., Brummitt, R. K., Culham, A. and Seberg, O. 2007. Flowering plant families of the world. Royal Botanical Gardens Kew publications, London, England.

Huq, S., Islam, Sajib, A. A., Ashraf, N., Haque, S. and Khan, H. 2009. Genetic diversity and relationship: in jute (*Corchorus sp.*) revealed by SSR markers. *Bangladesh J. Bot*., 38:153-161.

Islam, M. M., Begum, S., Alam, A. K. M. M, Ahmed, I. and Alamgir, A. 2009. Study the impact of climatic changes on jute production in Bangladesh. *Bangladesh J. Prog. Sci. Tech*., 7:153-156.

Islam, M. M. 2019. Varietal Advances of Jute, Kenaf and Mesta Crops in Bangladesh: A Review. *Int. J. Bioor. Chem*., 4:24-41.

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

Liu, K. and Muse, S. V. 2005. PowerMarker Integrated Analysis Environment for Genetic Marker Data. *Bioinformatics*, 21:2128-2129.

Mir, R. R., Rustgi, S., Sharma, S., Singh, R., Goyal, A., Kumar, J., Gaur, A., Tyagi, A., Khan, H., Sinha, M. K., Balyan, H. S. and Gupta, P. K. 2008. A preliminary genetic analysis of fibre traits and the use of new genomic SSRs for genetic diversity in jute. *Euphytica*, 161:413-427.

Nag, S., Mitra, J., Satya, P., Kar, C. S. and Karmakar, P. G. 2018. Genetic diversity assessment in jute (*Corchorus species*) utilizing inter simple sequence repeat and simple sequence repeat markers. *Indian J Biotechnol*., 17:316-326.

Neeraja, C. N., Maghirang-Rodriguez, R., Pamplona, A., Heuer, S., Collard, B. C.Y., Septiningsih, E. M., Vergara, G., Sanchez, D., Xu, K., Ismail, A. M. and Mackill, D. J. 2007. A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. *Theor Appl Genet*., 115: 767-776.

Nielsen, G .1985. The use of isozymes as probes to identify and label plant varieties and cultivars, vol 12. Alan R. Liss, New York, pp.1-3.

Patel, G. I. and Datta, R. M. 1960. Interspecific hybridization between *Corchorus olitorius* and *C. capsularis* and the cytogenetical basis of incompatibility between them. Euphytica 9(1):89-110.

Rana, M. K., Arora, K., Singh, S. and Singh, A. K. 2012. Multi-locus DNA fingerprinting and genetic diversity in jute (*Corchorus spp*.) based on sequence-related amplified polymorphism. *J. Plant Biochem. Biotechnol*., 22:1-8.

Rohlf, F. 2002. NTSYS-pc: Numarical taxonomy and multivariate analysis system, 2.2 Depeartment of Ecoligy and Evolution, State University of NY, Stony Brook.

Saunders, M. 2001-2006. Recovery Plan for the Endangered Native Jute Species, *Corchorus cunninghamii* F. Muell in Queensland (Natural Heritage Trust, 2006).

Zhang, L., Cai, R., Yuan, M., Tao, A., Xu, J., Lin, L., Fang, P. and Qi, J. 2015. Genetic diversity and DNA fingerprinting in jute (*Corchorus spp.*) based on SSR markers. *Crop J*., 3:416-422.