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GENETIC DIVERSITY AND RELATIONSHIP AMONG SIX WILD *CORCHORUS* SPECIES BY RAPD AND SSR ANALYSIS

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Abstract

In this investigation thirteen RAPD and eight SSR primers were used that generated 69 and 27 loci with 100% and 96.30% polymorphism, respectively for six wild *Corchorus* species *viz. C. brevicornatus*, *C. septentrionalis*, *C. urticifolius*, *C. hirtus*, *C. siliquosus* and *C. pinnatipartitus*. After RAPD analysis, *C. septentrionalis* was distantly related from other 5 wild species and formed a separate cluster at the highest genetic distance with *C. pinnatipartitus* (1.1431). On the other hand, *C. hirtus* and *C. pinnatipartitus* were close to each other at the lowest genetic distance 0.2638. SSR analysis showed *C. urticifolius* was distantly related from other 5 wild species and formed a separate cluster at highest genetic distance with *C. septentrionalis* (1.3499), while *C. siliquosus* and *C. pinnatipartitus* were close to each other at the lowest genetic distance 0.0770. Therefore, genetic diversity and relationship among 6 wild *Corchorus* species could be analyzed authentically by RAPD and SSR analysis.

Introduction

Jute (*Corchorus* spp.) is one of the most important natural bast fiber distributed throughout the tropical and sub-tropical regions (Kubitzki and Bayer 2003). To improve the quality and quantity of jute fiber inter species improvement is more desirable. Therefore, wild species can be a good pedigree of variation and can be used as parent in breeding programs during inter-specific hybridization. For meaningful breeding program to improve the cultivated *Corchorus* species wide variation within and between gene pool is first criteria.

In this case, random amplified of polymorphic DNA (RAPD) and simple sequence repeats (SSR) analyses were used by many workers in different plant species (Mir *et al*. 2008, Sultana and Alam 2016). RAPD markers were used to observe genetic diversity and relatedness among different accessions, lines, variety of two cultivated jute species and SSR markers were also used for characterization, studying genetic diversity and cross- species transferability of cultivated jute species (Mir *et al*. 2008, Huq *et al*. 2009, Ghosh *et al*. 2014, Satya *et al*. 2016). Unfortunately, genetic diversity and evolutionary relationship of wild jute taxa are scary. Therefore, in this study RAPD and SSR analysis were used for determination of genetic diversity and relationship among six wild *Corchorus* species from Bangladesh.

Materials and Methods

Six wild *Corchorus* L. species *viz. C. brevicornatus* Vollesen (Acc. 3719), *C. septentrionalis* Planch (Acc. 3122), *C. urticifolius* Wight and Arn. (Acc. 3707), *C. hirtus* L. (Acc. 1474), *C. siliquosus* L. (Acc. 1475) and *C. pinnatipartitus* Wild (Acc. 4541) were investigated in the present study which were collected from Bangladesh Jute Research Institute (BJRI) and maintained in Botanical Garden, Department of Botany, University of Dhaka. This research work was carried out during the period of 2020-21 at Department of Botany, University of Dhaka. In this research, Sultana and Alam (2016) techniques were followed for RAPD and SSR method and data analysis procedure, respectively.

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Results and Discussion

A total of 69 loci were observed with 13 RAPD primers. All the bands were polymorphic in nature due to absence of common bands. So, the polymorphism was 100% among them indicating the highest level of polymorphism. The size of bands ranged from 150-5000 bp following amplification with all primers (Fig. 1a-m, Table 1).

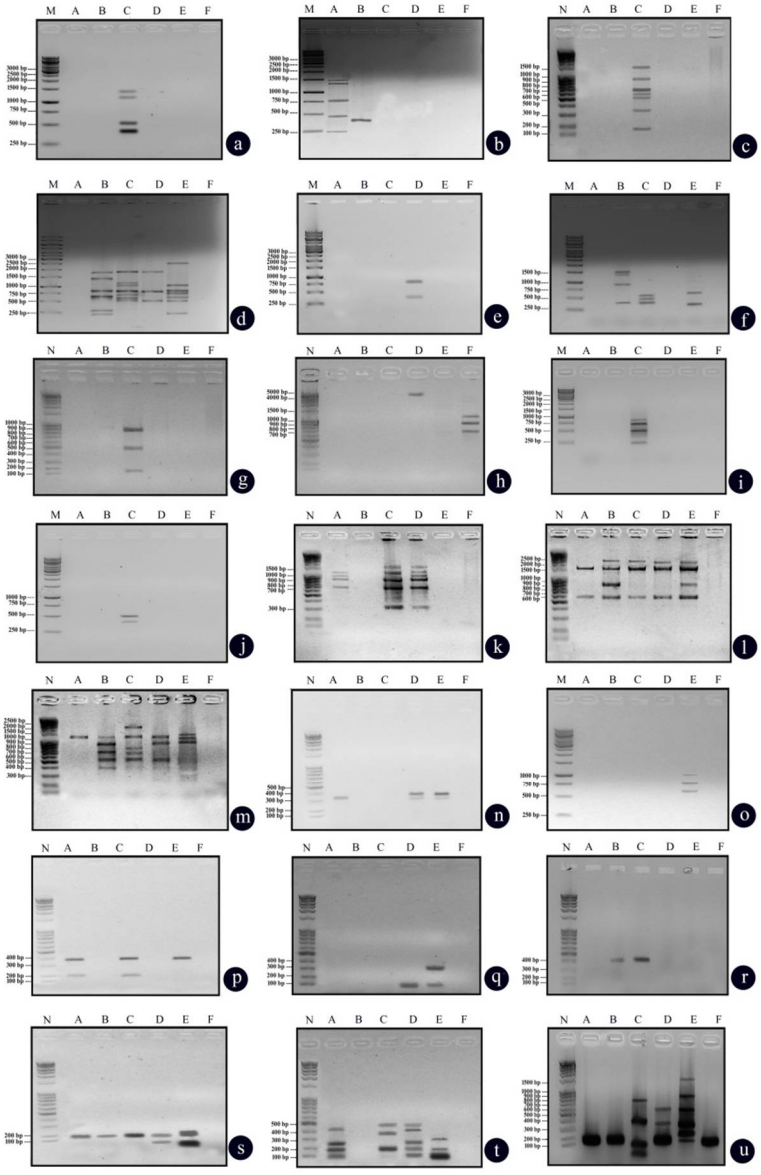

Fig. 1 RAPD and SSR analysis of six wild *Corchorus* L. species. a-m. RAPD profile with 13 random primers, a. OPA-18, b. OPC-10, c. OPC-13, d. OPC-14, e. OPC-16, f. OPC-26, g. OPC-96, h. OPD-20, i. OPD-69, j. OPF-22, k. OPG-03, l. OPG-06, m. OPG-09, n-u. SSR profile with 8 SSR primers, n. AW-584539, o. CED-30, p. CED-56, q. HK-7, r. HK-12, s. HK-16, t. HK-18, u. HK-30, A= *C. hirtus*, B= *C. siliquosus*, C= *C. septentrionalis*, D= *C. brevicornatus*, E= *C. urticifolius*, F= *C. pinnatipartitus*, M = 1 Kb and N = 100 bp DNA ladder.

Diverse polymorphism was reported previously only for two cultivated *Corchorus* spp. (Haque *et al.* 2007, Mir *et al.* 2008, Satya *et al*. 2016) which showed low level of DNA polymorphism. Forty-six unique bands were observed in six wild *Corchorus* that was present with a specific primer of a germplasm but absent in other species (Fig. 1a-m, Table 1). Many scientists used unique band as a parameter to characterize germplasms (Sultana and Alam 2016, Hossain *et al*. 2016). Therefore, unique band could be utilized as a useful tool for characterization of six wild *Corchorus* species used in this investigation.

Table 1. RAPD bands generated by using 13 primers in six wild species of *Corchorus* L.

Primer	Sequence (5′-3′)	Thermocycling condition	Total loci	No. of polymorphic loci	No. of unique bands
OPA-18	AGG TGA CCG T	94°C 2 min; [32 cycles: 94°C 60 s; 48°C 10 s; 65°C 2 min]; 65°C 5 min	4	4	4
OPC-10	TGT CTG GGT G		6	6	6
OPC-13	AAG CCT CGT C		7	7	7
OPC-14	TGC GTG CTT G		12	12	4
OPC-16	CAC ACT CCA G		2	2	2
OPC-26	CAC GTT ATC GCA		7	7	6
OPC-96	ACC AAG AAA GGG		3	3	3
OPD-20	AAG ACC CTA CGA		4	4	4
OPD-69	CGC TCC AAA TCA		4	4	4
OPF-22	AAG ATC AAA GAC		2	2	2
OPG-3	GAG CCC TCC A		6	6	-
OPG-6	GTG CCT AAC C		4	4	-
OPG-9	CTG ACG TCA C		8	8	4
Total			69	69	46

The values of genetic distance were analyzed by using computer software "popgene32" showed value ranging from 0.2638 to 1.141 (Table 2). *C. septentrionalis* is distantly related from other 5 wild species and formed a separate cluster at the highest genetic distance with *C. pinnatipartitus* (1.1431) (Fig. 2a, Table 2). On the other hand, *C. hirtus* and *C. pinnatipartitus* were close to each other at the lowest genetic distance 0.2638 (Fig. 2a, Table 2). These results indicated diverse genetic variability among six wild *Corchorus* species. Wide genetic diversity in different crops has been reported earlier (Molla *et al.* 2010, Sultana and Alam 2016). The highest genetic distance between *C. septentrionalis* and *C. pinnatipartitus* might be obtained due to the differences in genetic constituent and the lowest genetic distance between *C. hirtus* and *C. pinnatipartitus* due to the highest homology in their genetic constituent.

Table 2. Genetic distances of six wild species of *Corchorus* L. by RAPD.

Species	*C. hirtus*	*C. siliquous*	*C. septentrionalis*	*C. brevicornatus*	*C. urticifolius*	*C. pinnatipartitus*
C. hirtus	****					
C. siliquous	0.4729	****				
C. septentrionalis	0.9760	1.0561	****			
C. brevicornatus	0.3423	0.4274	0.7077	****		
C. urticifolius	0.4499	0.3629	0.7376	0.3629	****	
C. pinnatipartitus	0.2638	0.3840	1.1431	0.3840	0.3629	****

After SSR analysis, a total number of 27 loci were generated from eight primer pair combinations (Fig. 1n-u, Table 3). A common locus (200 bp) was detected with primer pair HK-30 indicating the sharing of similar DNA fragments among six wild *Corchorus* species. As a result, 96.30% polymorphism was recorded for six wild *Corchorus* species (Table 3). These results indicated high level of polymorphisms among studied wild *Corchorus* species. Wide range of SSR polymorphisms were reported both intra-specific and inter-specific levels (Mir *et al.* 2009, Ghosh *et al.* 2014, Zhang *et al.* 2015).

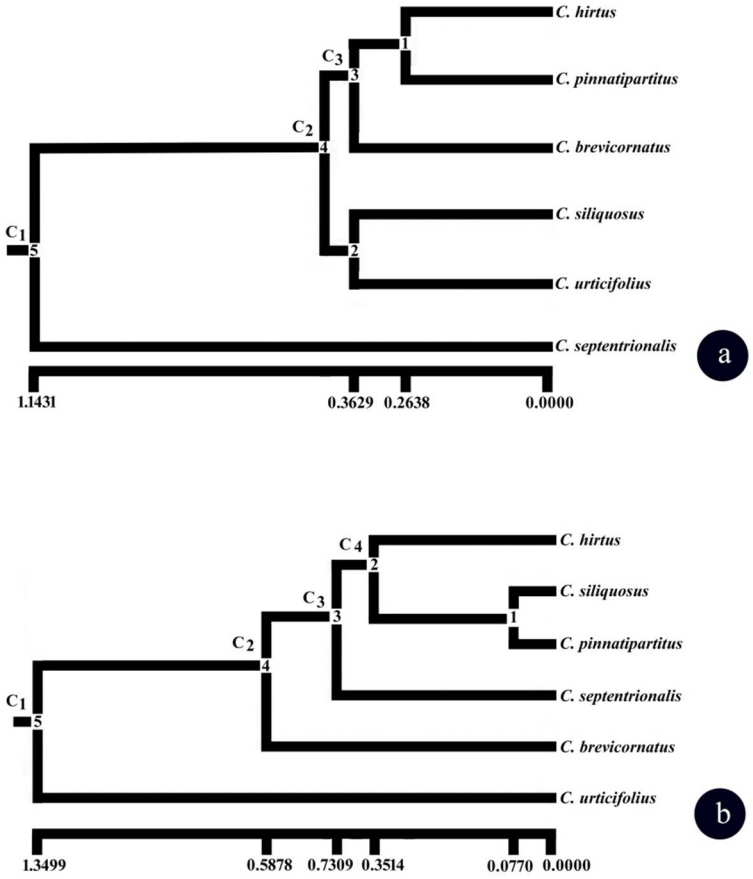

Fig. 2. UPGMA dendrogram constructed based on Nei's (1972) genetic distance summarizing the data on differentiation among six wild *Corchorus* L. species by a: RAPD analysis and b: SSR analysis.

Eleven unique bands were detected with eight SSR primer pair combinations in six wild *Corchorus* species (Table 3). The bands found with a specific primer of a germplasm but absent in other germplasm considered as unique band (Fig. 1n-u, Table 3). These bands were stable, reproducible and distinct for the particular germplasm. The highest (08) number of unique bands were in *C. urticifolius* that made its distant relationship from the rest 5 species. Unique SSR bands are also considered as a parameter for characterization of germplasm (Sultana and Alam 2016). Therefore, unique SSR bands obtained through this investigation could be utilized as a useful tool for characterization of six wild *Corchorus* species.

Table 3. SSR bands generated through the use of eight primer combinations in six wild species of *Corchorus* L.

Primer	Sequence (5′-3′)	Thermocycling condition	Total loci	No. of polymorphic loci	No. of monomorphic loci	No. of unique bands	Polymorphism (%)
AW-584539	F-TTGATGGGCAATACATGTCG R- GTTGAAGGAAGGTGGTGGTG	94°C 2 min; [32 cycles: 94°C 60 s; 48°C 10 s; 65°C 2 min]; 65°C 5 min	2	2	00	--	100
CEDG-30	F- TGAGGGAATGGGAGAGAGGC R- TCCGCAGATAGAGGCTCACG		3	3	00	3	100
CEDG-56	F- TTCCATCTATAGGGGAAGGG R- GCTATGATGGAAGAGGGCAT		2	2	00	--	100
HK-7	F- AATGATTATGAACCATAGTGGTACA R- TTATCACAAAGTAGCAGACTAACA		2	2	00	1	100
HK-12	F- CGCTCGCCTAAGTGAAGGCA R- ATAAAATACAAGGGACACTT		1	1	00	--	100
HK-16	F- TGGAACCTGAGCATCTCTCCAGA R- CTTTTTCTTGTTCAGGCACCTGA		2	2	00	--	100
HK-18	F- GCTGTTGTCTCTCTATTGGTGA R- TTCCACGCTCCTTGTTGCCA		7	7	00	2	100
HK-30	F- GAGTGATTAGAGGGCAGCCA R- TGCAACAAAAGTATCCAAATC		8	7	1	5	87.5
Total			27	26	1	11	96.30

Table 4. Genetic distances of six wild species of *Corchorus* L. by SSR analysis.

Species	*C. hirtus*	*C. siliquous*	*C. septentrionalis*	*C. brevicornatus*	*C. urticifolius*	*C. pinnatipartitus*
C. hirtus	****					
C. siliquous	0.3514	****				
C. septentrionalis	0.4626	0.3514	****			
C. brevicornatus	0.3514	0.5878	0.7309	****		
C. urticifolius	0.8979	1.0986	1.3499	0.5878	****	
C. pinnatipartitus	0.3514	0.0770	0.4626	0.5878	1.0986	****

The values of genetic distance showed value ranging from 0.0770 to 1.3499 (Fig. 4, Table 4). This high genetic diversity among six wild *Corchorus* species made SSR data highly informative. In the dendrogram, *C. urticifolius* is distantly related from other 5 wild species and formed a separate cluster at the highest genetic distance with *C. septentrionalis* (1.3499) (Fig. 2b, Table 4). On the other hand, *C. siliquosus* and *C. pinnatipartitus* are close to each other at the lowest genetic distance 0.0770 (Fig. 2b, Table 4). The highest genetic distance between *C. urticifolius* and *C. septentrionalis* might be obtained due to the differences in genetic constituents and the lowest genetic distance between *C. siliquosus* and *C. pinnatipartitus* due to the highest homology in their genetic constituents. Therefore, the species having the lowest genetic distance can be considered as parents during improvement of *Corchorus* species through breeding program.

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