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EXTRACTION OF JUTE GENOMIC DNA: DIFFICULTIES AND SOLUTIONS

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Abstract

The plant parts of jute are composed of highly viscous substances and phenolic compounds which make nuisance to extract good quality genomic DNA (gDNA). Here different methods viz., phenol chloroform isoamylalcohol extraction and ethanol precipitation method; potassium acetate method and Cetyl trimethylammonium bromide (CTAB) method were applied for isolating the jute gDNA from leaves of 14 and 40 day's plants. All of these methods were unable to extract good quality gDNA from jute leaves. That is why, in this experiment, the concentration and chemical compositions of CTAB method were modified for obtaining good quality jute gDNA. Polyvinylpyrrolidone (PVP) was added in the CTAB extraction buffer and β mercaptoethanol was used while grinding the leaf tissues with CTAB extraction buffer. It was found that good quality gDNA was obtained using modified CTAB method from 14 day's plant's leaves but low quality gDNA was obtained from 40 day's plant's leaves. These were confirmed by 0.8% agarose gel electrophoresis. Considering the visual quality of the banding patterns and their reproducibility MHR24 (5'-TTCCCTCCCA-3') and MHR21 (5'-CCC GAAGCGA-3') primers were selected out of 8 *Random Amplified Polymorphic DNA* (RAPD) primers for PCR. A total number of 11 loci were identified by these RAPD primers. Popgen32 software was used for analyzing the RAPD data. The number of polymorphic loci was one and the percentage was 9.09 which stated that a low level of genetic variations was existed among the jute accessions. In the dendrogram, jute accession CC875 (*C. capsularis*) was grouped in one cluster while CC894 (*C. capsularis*) and CC896 (*C. capsularis*) accessions were grouped in another cluster.

Key Words: Jute; DNA extraction; phenolic compounds; RAPD.

Introduction

Jute (*Corchorus* spp., Malvaceae) is one of the major cash crops of Bangladesh (Haque *et al.*, 2004). Around 10% of the total labor force of Bangladesh earns their livelihood from activities related to the production and processing of jute and allied fibers (Saha, 2006). In the Gene Bank of Bangladesh Jute Research Institute (BJRI) there are 5936 accessions of jute and allied fiber germplasms comprising 15 species of *Corchorus* and 22 species of *Hibiscus* (Haque *et al.*, 2007). A number of methods have already been developed to extract gDNA from plant tissues such as phenol chloroform isoamylalcohol extraction and ethanol precipitation method (Rahman *et al.*, 2006), potassium acetate method (Dellaporta *et al.*, 1983), CTAB method (Doyle and Doyle, 1990). Isolation of gDNA is necessary for molecular characterization of plants, facilitating marker assisted breeding, and also for genomic studies. However, the traditional protocols of plant's gDNA extraction are not suitable for jute gDNA extraction because the plant materials of jute contain high level of polysaccharides,

phenolics (Haque *et al.*, 2004), mucilage (Ogunkanmi *et al.*, 2010). These substances hamper extraction of jute nucleic acids and make the nucleic acids unstable (Haque *et al.*, 2004). Polyphenols act as powerful oxidizing agents which can reduce the yield and purity of extracted DNA (Porebski *et al.*, 1997). To overcome the problems associated with jute gDNA extraction of, CTAB method has been modified.

Materials and Methods

Potassium acetate method (Dellaporta *et al.*, 1983); phenol chloroform isoamylalcohol extraction and ethanol precipitation method (Rahman *et al.*, 2006); CTAB method (Doyle and Doyle, 1990) were used to extract jute gDNA but good quality gDNA was not obtained using these methods. Finally, CTAB method was modified for obtaining good quality gDNA. The details of the modification of CTAB method and genome extraction protocol are given below.

Composition of CTAB extraction buffer and preparation

CTAB extraction buffer was comprised of CTAB powder (0.02 g/ml), EDTA (0.5 M, pH: 8.0), Tris HCl (1 M, pH: 8.0), NaCl (5M), PVP (0.01 g/ml) and ddH₂O. NaCl, EDTA and Tris HCl were autoclaved before mixing all together and CTAB powder was also preheated in incubator for 5 minutes at 60^o C.

Leaf collection and preparation

Leaves were taken from 14 day's and 40 day's old jute plants. Leaves were cut into small pieces. On an average of 25 mg leaf sample was taken. Leaf samples were submersed in 100% alcohol and then washed in ddH₂O and soaked in tissue paper.

Extraction and purification of gDNA

500 µl CTAB extraction buffer was taken in each eppendorf tube containing 25 mg of leaf samples. Then 2 µl of β mercaptoethanol was added in each tube. After that the samples were grinded by the homogenizers. Again, 300 µl CTAB extraction buffer was added in each tube and then mixed by short centrifugation for 1 minute. The tubes were then heated for 5 minutes in water bath at 65^o C. Tubes were then vortexed for 20 seconds followed by heating in water bath for 10 minutes at 65^o C. The tubes were then centrifuged for 5 minutes at 14000 rpm. 600 µl supernatant was then taken to a new tube. Then, 600 µl phenol: chloroform: isoamylalcohol (25:24:1) was added to each tube. The solution was mixed properly by inverting the tubes and vortexed for 20 seconds and then centrifuged again for 10 minutes. 400 µl supernatant was then transferred to a new tube. Then, 400 µl chloroform: isoamylalcohol (24:1) was added to each tube and mixed well by inverting the tubes and vortexed for 20 seconds. The tubes were then centrifuged for 10 minutes at 14000 rpm. 400 µl supernatant was taken to a new tube. Then, 50 µl sodium acetate (pH 5.2) was added to each tube. Sodium acetate was autoclaved before adding in each tube. Then, 900 µl ice cold absolute ethanol was added to each tube. Thread like DNA was observed in each tube by simply inverting the tubes. Then the tubes were kept in a freezer at -20^o C for at least 20 minutes. The tubes were then centrifuged for 15 minutes. The DNA pellet was attached to the wall of the tubes, ethanol was discarded from the tubes. Then, 1 ml ice cold isoamylalcohol was added to each tube. The tubes were centrifuged for 15 minutes. The DNA pellets were attached to the wall of the tubes. Then, ethanol

was discarded from the tubes and air dried. Finally, 50 µl Tris-EDTA (TE) buffer was added to each tube.

Removal of RNA

50 µl of DNA stock solution was dissolved in 340 µl sterilized ddH₂O. 20 µl RNase was added to degrade RNA. Mixture was incubated for 20 minutes at 25^oC. 420 µl phenol: chloroform: isoamylalcohol (25:24:1) was added and mixed properly. Mixture was centrifuged for 10 minutes at 14000 rpm and then transferred the supernatant (500µl) into a new eppendorf tube. Again, 420 µl phenol: chloroform: isoamylalcohol (25:24:1) was added and vortexed for 20 seconds. Then, tubes were centrifuged for 10 minute at 14000 rpm and transferred the supernatant (500 µl) into a new eppendorf tube. Then, 40 µl 5M sodium acetate was added in each tube and mixed properly. After that, 1000 µl of 100% ice cold ethanol was added and mixed gently. Then, the mixture was centrifuged for 20 minutes at 14000 rpm and ethanol was removed. After that, 1000 µl of 70% ice cold ethanol was added and mixed gently. Then, mixture was centrifuged for 10 minutes at 14000 rpm. Again, ethanol was removed and DNA pellets were air dried for 3 to 5 hours. Finally, the pellets were suspended in 60µl of 1X TE buffer and stored at -20^oC.

Quantification of DNA (Spectrophotometric method)

The optical density (OD) of the isolated gDNA was measured at 260 nm wavelength to assess the quantity of gDNA (Table 1).

PCR conditions and data analysis

Two primers were selected among eight RAPD primers for polymerase chain reaction (Table 2). The total volume of PCR cocktail was 10 µl per sample (Table 3). PCR cycling was comprised of five steps (Table 4). PCR products were run in 1.4% agarose gel. Electrophoresis was conducted at 120 volt for 90 minutes. The gel was stained in ethidium bromide solution for 15 minutes. Then, the gel was placed on gel documentation system for visualization of the gDNA bands. DNA fragments that were amplified by the selected primers were scored as present '1' or absent '0' for all of the accessions that were studied and the scores obtained using the primers in the RAPD analysis were then pooled to create a single data matrix. Popgen32 (Yeh et al., 1999) software was used to estimate polymorphic loci, Nei's (1978) gene diversity, gene flow, and to construct a UPGMA dendrogram among the jute accessions.

Table 1: Quantity assessment of gDNA, isolated from 14 and 40 day's plants.

Genotypes	OD ₂₆₀ (14 day's)	gDNA Conc. (ng/µl) (14 day's)	OD ₂₆₀ (40 day's)	gDNA Conc. (ng/µl) (40 day's)
CC875 (<i>C. capsularis</i>)	0.007	350	0.006	300
CC894 (<i>C. capsularis</i>)	0.009	450	0.007	350
CC896 (<i>C. capsularis</i>)	0.007	350	0.006	300

N.B.: All the accessions were collected from Bangladesh Jute Research Institute, Dhaka

Table 2: RAPD primers that were screened in the study

Primer's code	Base sequence	Melting temperature (T _m)
MHR17	CTGCATCGTG	25 °C
MHR18	CCTAGGTCAG	25 °C
MHR19	ACGCGCATGT	25 °C
MHR20	GACGCCACAC	27 °C
MHR21*	CCCGAAGCGA	27 °C
MHR22	GTGGCTTGGA	27 °C
MHR23	GTTACGGACC	25 °C
MHR24*	TTCCCTCCCA	25 °C

* Selected primers for final RAPD analysis of jute accessions.

Table 3: Components of PCR cocktail (for 40 Cycles)

Reagents	Amount per sample (µl)
gDNA (50 ng/µl)	2
Primer (10 µM)	2
Taqbuffer (10 X)	1
dNTPs	1
MgCl ₂	0.3
ddH ₂ O	3.5
Taq DNA polymerase	0.2
Total	10

Table 4: PCR cycling conditions

PCR cycling step	Temperature	Protocol runs (Minutes)
Enzyme Activation	94°C	3
Denaturation	94°C	1
Annealing	35°C	1
Extension	72° C	2
Final extension	72° C	7

Results and Discussion

The quantity of gDNA depends on the amount of leaf tissues taken. The experiment was performed on three jute accessions (CC875, CC894, and CC896) using existing methods viz. phenol chloroform isoamylalcohol extraction, potassium acetate method (Dellaporta *et al.*, 1983), and ethanol precipitation method (Rahman *et al.*, 2006) and CTAB method (Doyle and Doyle, 2000). High pigmentation was found on gDNA while using potassium acetate method; phenol chloroform isoamylalcohol extraction and ethanol precipitation method and CTAB method. This was because the phenolics and viscous substances were not completely removed that were mingled with the gDNA. Electrophoresis was hampered by these

disturbing substances. As a result, no bands were seen in the gel documentation using these methods, gDNA was extracted from the leaves of both 14 and 40 day's plants. Finally, the concentration and chemical compositions of existing CTAB method were modified for obtaining good quality gDNA. PVP was added in the CTAB extraction buffer and β mercaptoethanol was added during homogenizing the leaf samples with CTAB extraction buffer. These two chemicals worked very well for removing the phenolics and viscous substances from the leaves of 14 day's plants. However, PVP and β mercaptoethanol were unable to remove these substances from the leaves of 40 day's plants. The fact was that old leaves accumulated high amount of phenolics and viscous substances than the younger ones. The gDNAs of 14 day's plants were presented in lanes 1-3 while gDNAs of 40 day's plants were in lanes 4-6 (Fig. 1). It was found that good quality gDNA bands were obtained from 14 day's plants. The highest level of gene diversity value and Shannon's Information Index (Table 5) was found in the locus mhr24-4. Overall gene frequency of three jute accessions was presented in Table 6. The lowest gene frequency (0.3333) was obtained in case of locus mhr24-4. Only one polymorphic locus was found among these three jute accessions which stated that a very low level of genetic variations existed among them. In the dendrogram (Fig. 4), jute accession CC875 grouped one cluster while CC894 and CC896 grouped another cluster.

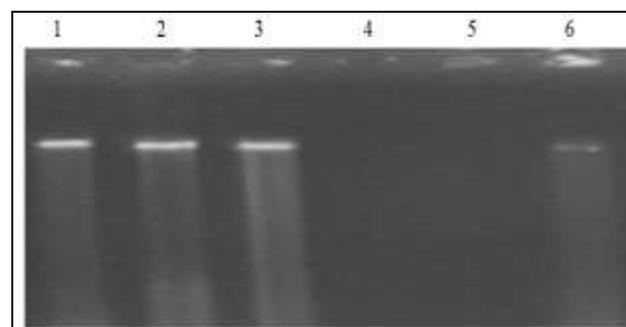


Fig. 1: DNA profile of jute. gDNAs were run on 0.8% agarose gel. Lane 1-3: gDNA samples of 14 day's plants and Lane 4-6: gDNA samples of 40 day's plants. The optical density was ranged from 0.006 to 0.009.

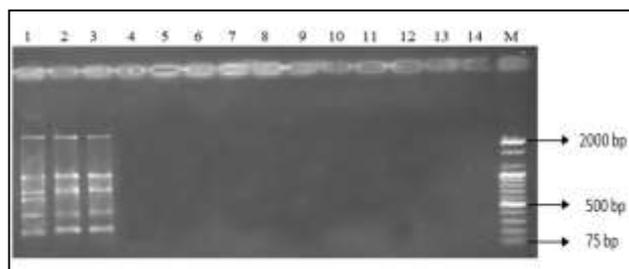
Table 5: Summary of the genetic variation statistics for all loci

Locus	Sample Size	na*	ne*	h*	I*
mhr24-1	3	1.0000	1.0000	0.0000	0.0000
mhr24-2	3	1.0000	1.0000	0.0000	0.0000
mhr24-3	3	1.0000	1.0000	0.0000	0.0000
mhr24-4	3	2.0000	1.8000	0.4444	0.6365
mhr24-5	3	1.0000	1.0000	0.0000	0.0000
mhr24-6	3	1.0000	1.0000	0.0000	0.0000
mhr21-1	3	1.0000	1.0000	0.0000	0.0000
mhr21-2	3	1.0000	1.0000	0.0000	0.0000
mhr21-3	3	1.0000	1.0000	0.0000	0.0000
mhr21-4	3	1.0000	1.0000	0.0000	0.0000
mhr21-5	3	1.0000	1.0000	0.0000	0.0000
Mean	3	1.8889	1.5658	0.3288	0.4888
St. Dev.		0.3015	0.2412	0.1340	0.1919

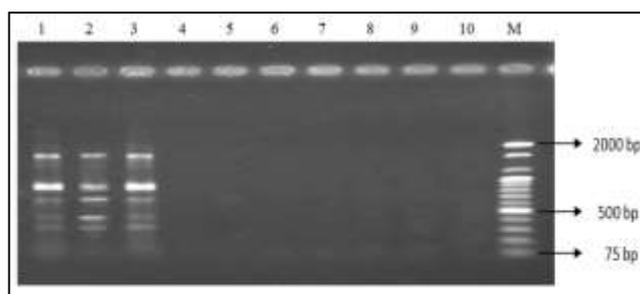
N.B: na *= Observed number of alleles, ne *= Effective number of alleles, h *= Nei's (1973) gene diversity, I *= Shannon's Information index

Table 6: Overall gene frequency

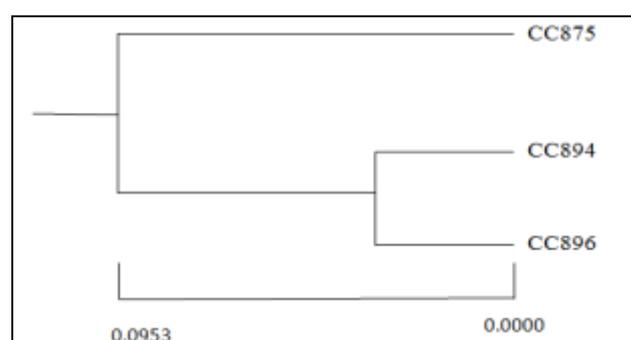
Loci	Frequency
mhr24-1	1.0000
mhr24-2	1.0000
mhr24-3	1.0000
mhr24-4	0.3333
mhr24-5	1.0000
mhr24-6	1.0000
mhr21-1	1.0000
mhr21-2	1.0000
mhr21-3	1.0000
mhr21-4	1.0000
mhr21-5	1.0000

**Fig. 2:** Banding patterns of jute accessions using primer MHR24 (5'-TTCCCTCCCA-3').

Lane 1-3=CC875, CC894, CC896 jute accessions; Lane 4-14= negative controls; Lane M= 1 kbp DNA ladder.

**Fig. 3:** Banding patterns of jute accessions using primer MHR21 (5'-CCCGAAGCGA-3').

Lane 1-3=CC875, CC894, CC896 jute accessions; Lane 4-10= negative controls; Lane M= 1 kbp DNA ladder.

**Fig. 4:** UPGMA dendrogram was based on genetic distances of jute accessions.

Accession CC894 and CC896 shared more genetically similarities than accession CC875.

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