Plant Tissue Cult. **14**(2) : 143-148, 2004 (December)

PTC

Method for Quality DNA Isolation from Different Parts of Jute Plant: *Corchorus capsularis* L. and *C. olitorius* L.

Samiul Haque[1], Nadim Ashraf, Aleya Awal, R. H. Sarker[2], Selina Begum[1] and Haseena Khan[*]

Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka-1000, Bangladesh

Key words: Jute, *Corchorus capsularis*, *C. olitorius*, DNA extraction, Polysaccharides, Metabolites

Abstract

A suitable method for isolating quality DNA from two cultivated species of jute (*Corchorus capsularis* and *C. olitorius*), which are rich in polysaccharides is described. Four types of plant materials such as seed, seedling, young leaf and old leaf from each species were used. Two isolation methods were followed with some modifications. The basic difference between the two methods was the presence or absence of phenol in the extraction protocol. The method lacking phenol failed to isolate DNA from seed samples of both species. The other method (using phenol) was capable of extracting DNA from all the samples but the quality of DNA was inferior to the other one.

Introduction

Many standard protocols are available for isolation of DNA from plants but they do not work consistently in plant tissues that are rich in polysaccharides and phenolics. These contaminants can directly affect the quality as well as the quantity of nucleic acid isolated. Polysaccharides often interfere with downstream applications, thereby making the nucleic acid unstable (Khan et al. 2004). Since different plants contain different amounts of nucleic acids, a single nucleic acid isolation method is not likely to be suitable for all plants (Loomis 1974) or even all plant parts such as seed, seedling, young or old leaves of a particular plant. Therefore, the biochemical composition of plant tissues in different species is expected to vary considerably and a single isolation protocol is unlikely to be equally effective for all species (Weishing et al. 1995).

*Author for correspondence. E-mail: haseena@bangla.net [1]Agronomy Division, Bangladesh Jute Research Institute, Dhaka-1207, Bangladesh. [2]Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh.

Jute is a major cash crop of Bangladesh. The jute plant is an annual shrub belongs to the family Tiliaceae and has seven pairs of chomorosomes. The commerce fiber is obtained from the secondary phloem tissue in the stem of cultivated varieties of the two species, *Corchorus capsularis* and *C. olitorius*. Jute is the second most important fiber crop next to cotton on a global scale and yet very little information about its genome is known, as it is grown mainly in developing countries like Bangladesh, India, China, Thailand, Myanmar etc. Natural genetic variability is limited in both species, due to limited cross-pollination (Hossain et al. 2002). So molecular techniques could be an important tool for jute crop improvement and for this, isolation of quality DNA is an important prerequisite.

Materials and Methods

Plant material: The basic plant materials were the seeds of the two cultivated species of jute varieties D-154 and O-9897 from *C. capsularis* and *C. olitorius,* respectively. The seeds were obtained from Bangladesh Jute Research Institute. The other plant materials were derived from the seeds, which were seedlings (four days old germinated seedlings on moist filter paper in Petri dish at 30^{o}C), young leaves (three - seven days old) and old leaves (35 - 40 days old) for both the species.

Solutions and reagents: For the modified Dellaporta et al. (1983) protocol (Method-I): Cetyltrimethylammonium bromide (CTAB), 2-mercaptoethanol (2-ME), CTAB extraction solution, 24 : 1 (v/v) chloroform/isoamyl alcohol (CI), CTAB precipitation solution, high salt tris EDTA (TE) buffer, 80% ethanol, TE buffer, DNase free RNase, morter and pestle and screw cap glass tubes etc. For modified Doyle and Doyle`s (1990) protocol (Method-II): The additional reagents were phenol, isopropanol and 3M Na-acetate.

DNA isolation protocol: *method-I:* One g of each four fresh samples (seeds, seedlings, young and old leaves) was ground in liquid nitrogen to a fine powder with a mortar and pestle. Each of these samples were transferred to a 15 ml screw cap glass tube containing 5 ml preheated (65^{0}C) CTAB extraction solution and mixed thoroughly before incubating for 30 minutes at 65^{0}C in a water bath with occasional mixing. The homogenate was extracted with an equal volume of 24 : 1 chloroform/isoamyl alcohol (CI). After centrifuging for 20 min at 4000 rpm at room temperature, the top (aqueous) phase was recovered in to a fresh screw cap tube. This step was repeated three more times for seed and seedling and four more times for young and old leaf samples with equal volume

of CI mixture. One-tenth (1/10th) volume of 65^{0}C CTAB/NaCl solution was added to the recovered aqueous phase and mixed well by inversion before extracting with an equal volume of CI. The tubes were mixed well, centrifuged (4000 rpm) and the top phase recovered. Exactly 1 volume of CTAB precipitation solution was added, mixed well by inversion. If a precipitation was visible then proceeded to next step, if not, the mixture was incubated for 30 min at 65^{0}C. The solution was then centrifuged for 10 min at 4000 rpm at room temperature, the pellet was collected and resuspended in high-salt TE buffer (2 - 3 ml per gram of starting material). The suspended extract was treated with DNase free Rnase (1/100 vol) and incubate for 20 min at 37^{0}C. The suspend was transferred to microcentrifuge tube (1.5 ml) and the DNA was precipitated by adding 0.6 volume ice-cold isopropanol. After mixing well by inverting slowly and then centrifuging for 10 min at 10000 rpm at 4^{0}C. The pellet was washed with 80% ethanol, dried and resuspended in a minimal volume of TE (Tris 10 mM, EDTA 1 mM) buffer (usually 100 - 500 µl per gram of starting material) and stored at – 20^{0}C.

Method-II: In this method the first step is similar to the previous one. Then 1 volume of 25 : 24 : 1 phenol/chloroform/isoamyl alcohol (PSI) was added. The mixture was mixed and centrifuged at 4000 rpm for 20 min at room temperature. The supernatant was transferred to a new tube; the PCI extraction was repeated three - four times (depending on samples) until a clear supernatant was obtained. The DNA was precipitated with ice-cold isopropanol. The DNA pellet was washed in 80% ethanol (ice-cold), dried, dissolved in a TE buffer. After the RNase treatment the DNA was treated with one volume of PCI. The mixture was then centrifuged at 10000 rpm for 10 min at 4^{0}C. The DNA was precipitated with 1/10 volume of 3M Na-acetate and double volume of ice-cold 99% ethanol. After washing with 80% ethanol, the DNA was dried, dissolved in a TE buffer and stored at – 20^{0}C.

Quantification of DNA: Two methods were followed for quantification of DNA (a) Spectrophotometric method: The optical density (OD) of the isolated DNA was measured at 260 and 280 nm wavelength (Specord 50, analytikjena) to assess the quality and quantity. (b) Gel analysis : The genomic DNAs were run through 0.8% agarose gel, the gel was stained by ethidium bromide solution and visualized using UV illumination and documented by Kodak Bio doc system. The DNA were quantitate by comparing the known concentration of λ DNA.

Results and Discussion

In any molecular biology work, the quality of DNA is more important than its quantity. But isolation of quality DNA from jute is very difficult because of the presence of polysaccharides and other metabolites. In this study, two modified methods (cited here as Method I & II, Dellaporta et al. 1983, Doyle and Doyle 1990), and four types of plant materials (seed, seedling, young leaf and old leaf) from two species of jute (*C. capsularis* var. D-154 and *C. olitorius* var. O-9897) were used. The basic difference between the two methods is that in one was used phenol and in the other it was excluded. DNA extracted following Method-I (without phenol), showed (Table 1) that, the ratio of the optical density (OD) at 260 and 280 nm ranged from 1.71 to 2.11. These values were very close to the ratio 1.8 indicating that it was pure DNA. It is interesting to note that no DNA

Table 1. Quality and quantity assessment of DNA isolated from different plant parts and methods.

Genotype and Method	Plant parts	$OD_{260/280}$	Total DNA (µg/g of tissue)
***C. capsularis* var. D-154** Method-I	Seeds	No DNA	No DNA
	Seedlings	1.75	68.23
	Young leaves	2.11	58.76
	Old leaves	2.06	66.91
C. olitorius var. O-9897 Method-I	Seeds	No DNA	No DNA
	Seedlings	1.72	68.67
	Young leaves	1.89	88.23
	Old leaves	1.71	98.97
C. capsularis var. D-154 Method-II	Seeds	1.73	97.30
	Seedlings	1.78	86.80
	Young leaves	1.35	47.47
	Old leaves	1.30	50.20
C. olitorius var. O-9897 Method-II	Seeds	1.60	110.00
	Seedlings	1.68	63.38
	Young leaves	1.27	45.50
	Old leaves	1.29	49.00

Note: The average of at least two readings.

was found from seed samples from both the species. It was suspected that the CTAB extraction buffer failed to disrupt the cell walls of the compact seed tissue to release DNA. To confirm this, the experiment was repeated with increasing lyses period from one to four h but the results remained unchanged. The amount of DNA obtained ranged from 58.76 to 98.97 µg/g of tissue. The quality and quantity of the isolated DNA was also reflected on agarose gel

(Fig. 1). The DNA isolated following Method-II (with phenol) showed (Table 1) that the ratio of $OD_{260/280}$ nm ranged from 1.27 to 1.78, indicating a wide range of quality. The ratio of the DNA isolated from seed and seedlings from both the species was very close (1.60 - 1.78) to the ratio 1.8, but DNA obtained from young and old leaves was poor in quality, which was also reflected on the gel (Fig. 1). It was observed that the DNA in the lanes 11, 12, 15 and 16 failed to migrate in the gel at the same rate as the others. This could be due to the presence of other impurities like protein. Proteins associated with the isolated

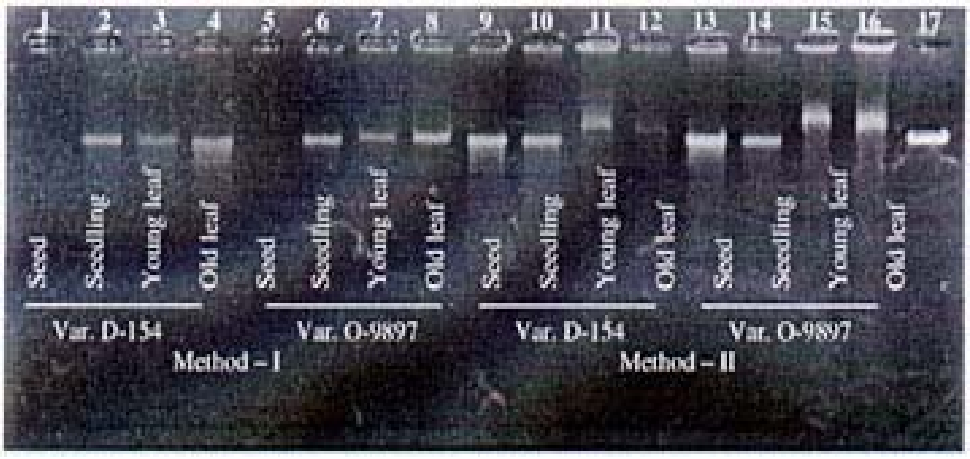

Fig. 1. Profile of DNA on 0.8% agarose gel.

DNA could not be removed with phenol. The presence of such proteins may have retarded the migration rate decreasing the quality of the isolated DNA. The yield of DNA ranged from 45.50 - 110.00 µg/g of tissue (Table 1). The quantity of DNA is also dependent on the amount of grinded tissue harvested and collection of the supernatant. From the study, it may be concluded that Method-I is suitable for isolating DNA from seedlings and leaves but not seeds. On the other hand, Method-II is effective for all samples, however compromising the quality of DNA isolated from leaf samples. If the sample is limiting as in the case of dead seeds, then Method-II is likely to be more suitable.

Acknowledgements

The authors are grateful to the U.S. Department of Agriculture (USDA) for the financial support (Grant No. BG ARS-104), without which this work could not

have been carried out. They are also thankful to Bangladesh Jute Research Institute for supplying the seeds.

References

Dellaporta SL, Wood J and **Hicks JB** (1983) A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. **1**(4): 19.

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

Hossain MB, Awal A, Rahman MA, Haque S and **Khan H** (2002) DNA fingerprinting of jute germplasm by RAPD. J. Biochem. & Mol. Biol. **35**(4): 414-419.

Khan F, Islam A and **Sathasivan K** (2004) A rapid method for high quality RNA isolation from jute: *Corchorus capsularis* and *C. olitorius*. J. Plant Tissue Cult. **14**(1): 63-68.

Loomis MD (1974) Overcoming problems of phenolics in the isolation of plant enzymes and organelles. Meth. Enzymol. **31**: 528-545.

Weishing K, Nybom H, Wolff K and **Meyer W** (1995) DNA isolation and purification. *In:* DNA fingerprinting in plants and fungi, CRC Press, Boca Raton, Florida. pp. 44-59.