JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2015. Vol. **9**(3), p. 2271-2274

Use of CTAB Method for Isolation of Good Quality and Quantity of DNA

D. Ashwini[1] and S.P. Tiwari[2]

[1]Department of Plant Pathology, JNKVV, Jabalpur, India.
[2] Principal Scientist, Department of Plant Pathology, JNKVV, Jabalpur, India.

(Received: 09 April 2015; accepted: 13 June 2015)

Isolation of good quality and quantity of DNA is essential for the purpose as well as for preserving the same to a considerable period of time. Although the DNA needed per reaction in PCR based makers is very low, the number of PCR reactions to be performed is large and hence agood quality and quantity of DNA would be needed for such studies. We have CTAB (Cetyl tri methyl ammonium bromide) method for isolation of DNA from plant tissue. The method is suitable for isolating DNA from a small to medium number of plant samples. The DNA can be stored for a longer duration. The method involves extraction of DNA using a buffer (pH 8.0) containing Tris HCL (100 mM), EDTA (20mM), 5 M NaCl, 10% CTAB and 2% β- mercaptoethanol, followed by purification of DNA with phenol, chloroform and Isoamly alcohol and finally precipitation of DNA by sodium acetate and isopropanol. The protocol is simple and does not require expensive chemicals such as proteinase K, liquid nitrogen etc.

Key words: Isolation of DNA, CTAB method.

The quality and quantity of DNA required depends upon the objective. In marker assisted selection the number of samples are high but the number of PCR reactions to be performed are few and hence the amount of DNA needed is small and need not be stored for a long duration[1, 2]. On the other hand in case of Quantitative Trait Loci (QTL) mapping or population studies of Recombinant Inbred Lines (RILS), F2 population etc., the number of PCR reactions to be performed are large and therefore considerable amount of purified DNA would be needed which can be stored for a longer duration.

In this paper we describe a DNA isolation using CTAB method suited for isolating reasonably pure DNA in sufficient amount from fresh leaves that can be stored for a longer duration and lasting for several PCR reactions. The method, which is rapid and versatile, gives high molecular weight DNA of good quality. A method for isolating DNA from the leaves of various species using CTAB (cetyl tri methyl ammonium bromide) as a detergent[3]. It is a relatively quick and inexpensive method for extraction of DNA from leaf materials containing large quantities of mucilage. The modification reduced markedly the viscosity of the mucilage and thus in the final purification step yielded a larger quantity of mucilage-free DNA suitable for subsequent PCR-based detections[4]. Genomic DNA from young leaves of 17 ecotypes of *Gymnema sylvestre* with three different extraction methods i.e., modified CTAB method (method 1), phenol-chloroform method (method 2), and Dellaporta method (method 3). Among the 3 methods tried, CTAB method was found to be suitable for PCR amplification with high purity and yield of genomic DNA[5]. CTAB method was tested in eight plant species, wheat, sorghum, barley, corn, rice, *Brachypodium distachyon*, *Miscanthus sinensis* and tung tree reported that high-yield and good-

* To whom all correspondence should be addressed.
E-mail: aparna1625@gmail.com

quality DNA samples could be obtained by using this new method in all the eight plant species. This method could be used in multiple plant species and can be integrated with multiple conventional DNA isolation methods[6].

MATERIALS AND METHODS

Fresh as well as frozen leaf tissue was used in this method. The DNA was isolated from leaves of different annual and perennial plants which included vegetables like chilli, okra, tomato; fruit trees like guava, papaya; Plantation crop like betel vine; Medicinal plants like tulsi, neem; Aromatic plants like marigold, rose, jasmine, hibiscus.

Leaves from one-month-old field grown plants were harvested and brought on ice to laboratory. The leaves were thoroughly washed with tap water and rinsed with distilled water, blot dried and weighed. The leaves were either stored at –20°C or used directly for extraction.

The chemicals needed for isolation of DNA viz. Tris, EDTA were obtained from Sigma and Sodium chloride, Isopropanol, sodium acetate, chloroform, Isoamlyalcohol and phenol were of the analytical grade. The agarose used for gel electrophoresis was also obtained from Sigma.

DNA from the samples was isolated as follows. Around 100 milligram of leaves were grinded in autoclaved mortar and pestle in the presence of ice flakes to fine powder and pre-warmed DBE (DNA extraction buffer) was added to the powder in the 1.5 ml eppendorf autoclaved tubes. All the reagents are autoclaved before use expect CTAB, phenol. The extraction buffer (pH 8.0) consisted of 100 mMTris, 20 mM EDTA,5 M NaCl, 10% CTAB and 2% â-mercaptoethanol. The eppendorf tubes with grinded sample are incubated at 65°C for 30 min and mixing is done 3-5 times by inverting in between. 0.7 to 0.8 volume of chloroform: isoamyl alcohol (24:1) was added after incubation and mixing is done gently by inverting the tubes 10-20 minutes to form emulsion (800 µl). centrifugation was carried out @ 10,000rpm at 4°C temperature for 10 min.

The aqueous supernatant was transferred to a fresh 1.5 ml eppendorf tube.0.8 volume of cold isopropanol was added and mixing is done by inversion to precipitate nucleic acid.Centrifugation was carried out @ 10,000 for 10 min (temperature 4°C). Pellet was washed with 70% cold ethanol and centrifugation was carried @ 10,000 for 10 min (4^{0}C) and the ethanol was completely removed and DNA pellet was dried at 37^{0}C.The pellet was dissolved in 50-70 µl sterile distilled water.To remove RNA 5µl of DNAse free RNAseA (10 mg/ml) was added to the DNA.

Estimating the quality of DNA using agarose gel electrophoresis

One gram of agarose powder was weighed and added into the clean flask containing 100ml of Tris Acetate EDTA (TAE) (1X) buffer and was dissolved by melting at 100 °C in the microwave oven, the solution was cooled to 50 °C; about 5µl of the ethidium bromide (0.5 mg/ml final concentration) was added to the gel and then poured into the gel frame and allowed to set. After setting the gel, it was transferred to the gel tank such that the wells were towards the negative pole. The gel tank was filled with TAE buffer just enough to cover the surface of the gel.

Using a micropipette 2µl of 6X loading dye, 2µl of DNA, 3µl of 1X TAE was loaded into the wells made by the comb. The electrophoresis apparatus was connected to the power supply and electrophoresis was carried out at 60 Volt for three hours or up to deep blue dye migrated to the end of the gel. It was then visualized under UV transilluminator and documented using gel documentation unit. The quality of DNA was checked by electrophoresing an aliquot of DNA. A clear band above > 2.2 kb without sharing indicated good quality DNA.

Estimating the quantity of DNA using Spectrophotometer

The concentration of DNA in a sample, and its condition, are often estimated by running the sample on an agarose gel. Such concentration estimates are semi-quantitative at best and are time consuming and confounded when numerous bands or a 'smear' of DNA are observed. For a more accurate determination of the concentration of DNA in a sample, a UV spectrophotometer is commonly used for DNA solutions (or a fluorometer can be used if the solution is known to be pure DNA).

The purity of a solution of DNA can be determined using a comparison of the optical density values of the solution at various

wavelengths. For pure DNA, the observed 260/280 nm ratio will be near 1.8. Elevated ratios usually indicate the presence of RNA, which can be tested by running the sample, ~1µg, on an agarose gel. 260/280 ratios below 1.8 often signal the presence of a contaminating protein or phenol. Alternatively, protein or phenol contamination is indicated by 230/260 ratios greater than 0.5. The nitrogen bases absorb light at 260 nm and hence this wavelength is used to determine the DNA conc. Pure preparations of DNA and RNA have OD_{260}/ OD_{280} = 1.8 to 2 respectively.

RESULTS AND DISCUSSIONS

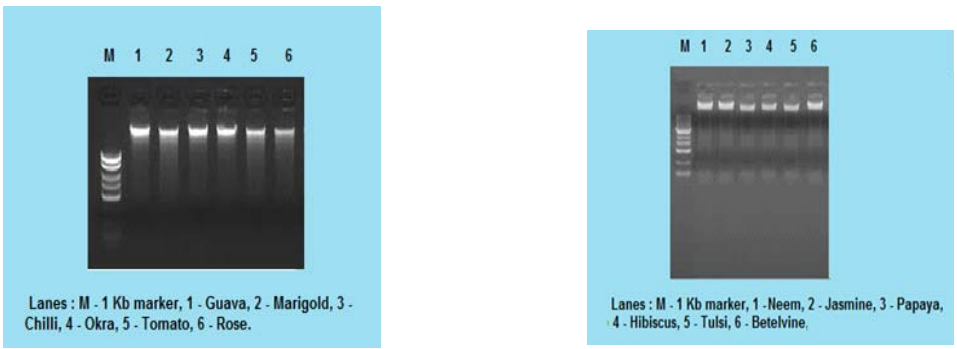
To check the quality and quantity of DNA, it was isolated from leaves of different annual and perennial plants which included vegetables like chilli, okra, tomato; fruit trees like guava, papaya; Plantation crop like betel vine; Medicinal plants like tulsi, neem; Aromatic plants like marigold, rose, jasmine, hibiscus.

Although 12 plants belonging to perennials and annuals were sampled for estimation of DNA. The observations were presented in Table – 1 indicated that maximum DNA was observed in guava (2.775 µg), neem (2.775 µg), papaya (2.775 µg), marigold (2.701µg), chilli (2.701µg), okra (2.701µg), jasmine (2.701µg), hibiscus (2.701µg), tulsi (2.701 µg), betel vine (2.701 µg), rose (2.664 µg), tomato (2.59 µg).

DNA isolated from plants often contains certain compounds that inhibit PCR amplification reactions [7,1]. In this method Sodium chloride and β-mercaptoethanol were added in the extraction buffer to take care of the polysaccharides and the polyphenols in the leaf tissue which are the compounds which could contribute to the inhibition of the DNA amplification during PCR reactions. Hence there were no additional steps

Table 1. Estimation of yield and quantity of DNA samples of perennial and annual plants

Sl. No.	Host plant	230 nm	260 nm	280 nm	260/280	260/230	DNA(µg/ml)	RNA(µg/ml)
01.	Guava	0.106	0.075	0.037	2.0	0.70	2.775	3
02.	Marigold	0.114	0.073	0.039	1.87	0.64	2.701	2.92
03.	Chilli	0.142	0.073	0.038	1.92	0.51	2.701	2.92
04.	Okra	0.148	0.073	0.039	1.86	0.49	2.701	2.92
05.	Tomato	0.138	0.070	0.038	1.84	0.50	2.59	2.8
06.	Rose	0.150	0.072	0.039	1.83	0.48	2.664	2.88
07.	Neem	0.150	0.075	0.039	1.88	0.50	2.775	3
08.	Jasmine	0.114	0.073	0.039	1.87	0.64	2.701	2.92
09.	Papaya	0.106	0.075	0.037	2.0	0.70	2.775	3
10.	Hibiscus	0.142	0.073	0.038	1.92	0.51	2.701	2.92
11.	Tulsi	0.142	0.073	0.038	1.92	0.51	2.701	2.92
12.	Betel vine	0.114	0.073	0.039	1.87	0.64	2.701	2.92



A clear band above > 2.2 kb is formed without sharing which indicates good quality DNA

Fig. 1. Photograph showing bands of DNA isolated from the hosts plants

needed for the removal of these compounds[1,8,9,10]. The presence of the enzyme RNAse A in the DNA solution does not hamper the amplification. Hence repurification of the DNA is not needed. Our experience showed that the DNA isolation protocol could be successfully applied to a broad range of plant species.

REFERENCES

1. McCarthy, P.L. and Berger, P.H. Rapid identification of transformed wheat using a half-seed PCR assay prior to germination. *Biotechniques*, 2002; **32**: 560- 564.
2. Guo-Liang Wang, Rod, A.W. and Paterson, A.H. PCR amplification from single seeds, facilitating DNAmarker-assisted breeding. *Nucl. AcidRes*, 1993; **21**(10): 2527.
3. Kump, B., S., Svetek and B., Javornik. Isolation of high molecular weight DNA from plant tissues. Zbornik Biotehniske Fakultete Univerze Edvarda Kardelja v Ljubljani, *Kmetijstvo;* 1992; **59**: 63-66.
4. Ghosh, R., S., Paul, P., Palit, S., Das, A., Das, S., Acharyya, J.I., Mir, S.K., Ghosh and A., Roy. Molecular characterization and computational analysis of coat protein gene of an east Indian isolate of Bhendi yellow vein mosaic virus and associated DNA-beta satellite. *Indian J. Virol.;* 2008; **19**: 173-182.
5. Krishna, R. B., S. R. R., Reddy, D.,Harika Javangula Swapna and K. J., Reddy. An easy and simple method of isolation and purification of genomic DNA from the leaves of *Gymnema sylvestre* - an anti-diabetic plant. *International Journal of Life science & Pharma Research;* 2012; **2**(1): L-15-L-20.
6. Zhang Ling Ling Wang Bo and Pan Lei Peng Jun Hua. Recycling isolation of plant DNA, a novel method. *Journal of Genetics and Genomics;* 2013; **40**(1): 45-54.
7. Krishna, T.G. and Jawali, N. DNA Isolation from single or half seeds suitable for Random Amplified Polymorphic DNA Analyses. *Anal.Biochem,* 1997; **250**: 125-127.
8. Maliyakal, J.E. An efficient method for isolation of RNA and DNA from plants containing polyphenolics. *Nucl. Acid Res,* 1992; **20**(9): 2381.
9. Oard, J.H. and S. Dronavalli. Rapid isolation of rice and maize DNA for analysis by random-primer PCR. *Plant Mol. Biol. Rep,* 1992; **10**:236-241.
10. Shirzadegan, M., Christie, P. and Seemann, J.R. An efficient method for isolation of RNA from tissue cultured plant cells. *Nucl. Acid Res,* 1991; **19**:6055.