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Research Article

Efficient isolation and long-term storage of genomic Deoxyribonucleic acid (DNA) from the leaves of some tropical trees

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Abstract
Analysis of the genetic diversity of tropical trees can contribute to forming active approaches for their conservation and future use. High-quality Deoxyribonucleic acid (DNA) is required for many molecular applications used in the analysis of genetic diversity. The present study standardized efficient DNA isolation and long-term storage protocol using the 268 leaf samples from 8 tropical tree species such as *Cullenia exarillata* (Malvaceae), *Dalbergia latifolia*, *Dalbergia sissoides*, *Kingiodendron pinnatum* (Fabaceae), *Dysoxylum malabaricum* (Meliaceae), *Mesua ferrea* (Calophyllaceae), *Myristica malabarica* (Myristicaceae) and *Tectona grandis* (Lamiaceae). The high DNA quality resulted in the present method as Mean A260/280 = 1.90 ± 0.05. The amount of extracted DNA ranged from 23.4 ng/µL (in the mature leaf of *Myristica malabarica*) to 1922.7 ng/µL (in the young leaf of *Dalbergia sissoides*), while the yield ranged from 4.7 to 384.5 µg per 100 mg leaf sample. The extracted DNA samples of 8 species stored with 70% ethanol in the pellet form showed less DNA degradation and a maximium storage life of up to one year than those stored with TE buffer and sterile water. The integrity of one year of DNA stored in 70% ethanol was suitable for PCR amplification with RAPD and ISSR markers. The present method also facilitated efficient molecular characterization and validation of tropical tree species.

Keywords: DNA damage, DNA smearing, DNA preservation, Endemic species and RNase treatment

INTRODUCTION

Genetic investigation of tropical tree species can create operative plans for their protection and future usage. At present, molecular markers have been demonstrated to be precious tools for evaluating the genetic resources of tropical tree species and the level of genetic variation within and between species (Alhasnawi *et al.,* 2024). High-quality extraction and purification of genomic DNA to evaluate tropical trees using PCR-based molecular markers, i.e., RAPD (Random amplified polymorphic DNA), ISSR (Inter simple sequence repeat), RFLP, AFLP, SSR, etc., was a restrictive factor in genetic analysis. The leaves of tropical plants contain an abundance of subordinate metabolites such as phenolic compounds, tannins, latex and polysaccharides. Taking out and cleansing high-quality DNA is tough due to the occurrence of mechanically stable cell walls with high levels of polysaccharides, proteins, and DNA polymerase inhibitors such as tannins, alkaloids, and polyphenols. The presence of these compounds in the isolated DNA showed improper amplification. Therefore, the standardization of DNA isolation protocols is an important factor in the field of plant molecular biology (Krishnan *et al.,* 2024).

Tropical tree species have economic and ecological

importance and crucial in biodiversity (Pijut *et al*., 2012). *Cullenia exarillata* A. Robyns (commonly known as Wild Durian) is an endemic tree of the Western Ghats (Anoop *et al*., 2021) and belongs to the Malvaceae family (World Flora Online Plant List, 2022). Flowers and fruits of *C. exarillata* are eaten by many mammals and birds (Ganesh and Davidar, 1997). *Dalbergia sissoides* Wight & Arn. and *D. latifolia* Roxb. are closely related, valued trees of Fabaceae family. They are distributed naturally in moist deciduous, dry deciduous and semi-evergreen forests in India. *D. latifolia* is commonly acknowledged as Indian Rosewood and occurs in the lower Himalayas from Oudh due east to Sikkim, Bihar, Orisha, Central, Western and South India. *D. sissoides* is known as Malabar Blackwood and is found in the Western Ghats of Tamil Nadu, Karnataka, and Kerala (Vasudevan *et al*., 2023). *Kingiodendron pinnatum* (DC.) belongs to the Fabaceae family and it is endemic in the Western Ghats (Pareeth *et al*., 2023). Commonly known as Malabar Mahogany. *Dysoxylum malabaricum* Bedd. ex C. DC. is an economically important, endangered, endemic species of the Western Ghats. It is commonly known as White Cedar and belongs to the Meliaceae family (Bodare *et al*., 2017). *Mesua ferrea* L. has medicinal and wood values. It is known as Ceylon Ironwood, Cobra's Saffron and Indian Rose Chestnut. It belongs to the Calophyllaceae family (Asif *et al*., 2017). *Myristica malalabarica* Lam. also has medicinal and timber value. It is known as Malabar nutmeg, which belongs to the Myristicaceae family. It is endemic to the Western Ghats (Chelladurai and Ramalingam, 2017). Teak (*Tectona grandis* L. f.) is an economically important tree species that is naturally distributed in India, Lao PDR, Myanmar and Thailand. It is a member of the Lamiaceae family and has been introduced to many tropical countries in America, Africa, and Asia for timber production (Moya *et al*., 2014). The present study pointed to progress in general genomic DNA extraction and long-term storage protocols for the leaf samples of the aforementioned tropical tree species.

MATERIALS AND METHODS

Collection of leaf samples

The young and mature leaves of tropical eight tree species viz., *C. exarillata*, *D. latifolia*, *D. sissoides*, *K. pinnatum*, *D. malabaricum*, *M. ferrea*, *M. malabarica* and *T. grandis* in the forest areas of Kerala and Tamil Nadu were collected through field visits (Fig. 1). Collected leaves were rinsed with fresh water to avoid desiccation and packed in zip lock containers. The leaf samples were then properly labeled and stored in a refrigerator at -20^{o}C.

Preparation of reagents

Cetyltrimethylammonium bromide (CTAB) extraction buffer was prepared by mixing 20 mM EDTA (Ethylenediamine tetraacetic acid), 1.4 M NaCl (Sodium chloride), and 2 mM CTAB in 100 mM Tris-Hcl solution (pH-8.5). The mixture was incubated in a water bath at 65°C for 1 hour. After cooling, 2% v/v BME (β-mercaptoethanol or 2-mercaptoethanol) was add on and blended well. Final pH of the extraction buffer should be between 8 and 8.2.

Chloroform: Isoamyl alcohol mixture was prepared by mixing 1 ml of isoamyl alcohol with 24 ml of chloroform. The above mixture was vortexed for 3 hours with an equal volume of 10X TE buffer (pH 8). Discard the supernatant solution. An equal volume of 1X TE buffer (pH 8) was mixed with the above mixture and vortexed for 2 hours. The pH of the supernatant mixture was checked with pH indicator paper. The final pH of the extraction buffer should be 8 (if the final pH is below 8, discard the supernatant solution and repeat the last

Fig. 1. *Leaf collection of selected tropical tree species in Tamil Nadu and Kerala; A – Dalbergia latifolia, B – Dalbergia sissoides, C – Dysoxylum malabaricum, D – Tectona grandis, E – Cullenia exarillata, F – Mesua ferrea, G – Kingiodendron pinnatum and H – Myristica malabarica*

step until the desired pH is reached).

Methodology used for DNA extraction

The fresh leaves were cleaned with sterile water to remove dust and other unwanted particles. By using a sterile surgical knife, the interveinal portions of the leaf were taken for DNA extraction. The leaf materials were kept with distilled water to avoid the oxidation of secondary metabolites. 100 mg of interveinal leaf portions were weighed and ground using a mortar and pestle (autoclaved at 120 °C for 30 mins) with 1.5 ml of freshly prepared and preheated CTAB extraction buffer. Approximately 1.5 ml of the paste was transported to a new 2 ml microcentrifuge tube. The tube was incubated in a water bath at 65°C for 1½ hours; then, the tube was allowed to cool for 15 minutes. 0.5 mL of chloroform: isoamyl alcohol mixture was added to the tube and blended well. After a 15-minute rest, the tube was centrifuged at 10,000 rpm for 10 minutes at 25 ° C. The upper aqueous layer (about 1.2 ml) was collected in a new 2 ml microcentrifuge tube. Another 0.5 ml of 24:1 ratio isoamyl alcohol was added on to the tube and blended well. After a 15-minute rest, the tubes were centrifuged at 10,000 rpm for 10 minutes at 25 ° C. The upper aqueous layer (about 0.8 ml) was carefully collected in a new 2 ml microcentrifuge tube. An equivalent volume of isopropanol (stored at room temperature) was added and blended well. After resting for 15 minutes, the tubes were centrifuged at 10,000 rpm for 10 minutes at a temperature of 25 ° C. The supernatant was removed and 1 ml of ethanol (70%) was added to the pellet (Fig. 2), which was kept overnight (Ginwal and Maurya, 2010).

Long-time storage and DNA integrity checking

After resting overnight, the ethanol was detached. Again, 1 ml of 70% ethanol was add on to the pellets. Then, the DNA sample was stored at room temperature for 1 year. After a period of one year, DNA particles were permitted to dry in a BOD incubator at 40°C for 30 minutes (Fig. 3). After complete ethanol evaporation, the particles were dissolved in 100 µl of 1X TE buffer (pH 8.0). Agarose gel (0.8%) blended with ethidium bromide (0.5 µg/ml) was used for electrophoresis to determine the integrity of the extracted DNA sample. This gel was viewed and documented using BIO-RAD gel Doc™ XR+ system. The band percentage was calculated using Image Lab™ software (Molecular Imager software) to determine the integrity of the extracted DNA sample. The band percentage of DNA sample stored in ethanol at room temperature was compared with the band percentage of the DNA sample band stored in 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and double distilled water at -20°C (Abdel-Latif and Osman, 2017).

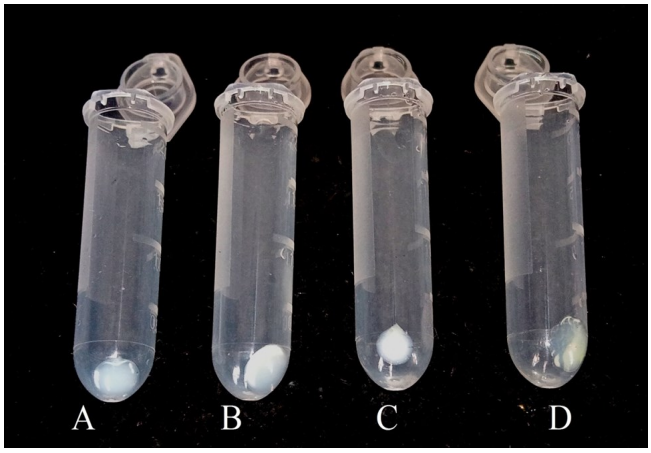

Fig. 2. *White color DNA pellets obtained at the time of DNA extraction from the leaves of some tropical tree; A – Cullenia exarillata, B – Dalbergia latifolia, C – Mesua ferrea and D – Tectona grandis*

Quantification of genomic DNA

Extracted DNA was counted by a Microvolume spectrophotometer (NanoDrop™ Lite, Thermo Scientific). The bottom and top surfaces of the sample container were cleaned with 3 µl of sterile water 3 times using a sterile laboratory wipe. Then, 1 µl 1X TE buffer was used as a blank and repeated twice. After spectrophotometer optimization, 1 µl of carefully extracted DNA solution was added. The quality and quantity readings of DNA were noted for each sample. The yield of DNA was calculated by multiplying the DNA quantity with the total volume of extracted DNA (Moyo *et al*. 2008).

Purification of genomic DNA

At the time of PCR test, the RNA particles present in the extracted DNA solution were removed by RNase A (Ribonuclease A) treatment. The RNase A (GeNei™) solution (2µg/µl) was heated at 100°C for 15 min using a thermal cycler (BIO-RAD T100™) to remove DNase (Deoxyribonuclease) contamination. Heat-treated RNase A (0.5 µg/µg DNA) was add on to each DNA sample. The mixture was vortexed and incubated at 37°C for 30 min using a thermal cycler. After RNase A treatment, the excellence and amount of DNA samples were again measured using a microvolume spectrophotometer (NanoDrop™ Lite, Thermo Scientific) (Heikrujam *et al.,* 2020; Agbagwa *et al.,* 2012).

PCR assay of extracted DNA

DNA samples were diluted to a concentration of 20 ng/µl using 1X TE buffer and stored at -20°C. A total of 15 µl of PCR reaction mixture was prepared by mixing 7.5 µl of sterile water with 1.5 µl of 10X Taq buffer (GeNei™), 1.5 µl of 25 mg MgCl2 (GeNei™), 1.5 µl of 10 mM dNTP (GeNei™), 1.5 µl primer 10 µM, 3U Taq DNA polymerase (GeNei™) and 1.0 µl genomic DNA (50ng/µl). A thermal cycler (BIO-RAD T100™) is designed for DNA amplification according to the following

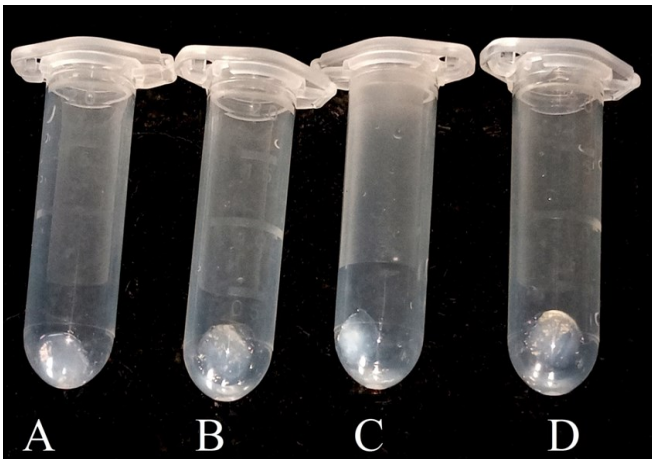

Fig. 3. *After one year of storing with 70% ethanol, transparent DNA pellets were obtained from the leaves of some tropical trees;* ***A*** *– Cullenia exarillata,* ***B*** *– Dalbergia latifolia,* ***C*** *– Mesua ferrea and* ***D*** *– Tectona grandis.*

program. The first cycle was programmed as initial denaturation at 94°C for 1 min. This was observed by 40 cycles of denaturation at 94°C for 20 seconds, tempering for 1 minute at the optimal temperature, and extension at 72°C for 2 minutes. A last extension of 5 minutes at 72°C was performed to complete the reaction. After completing the above steps, horizontal gel electrophoresis successfully sustained the developed PCR products. The electrophoresis tank was filled with 1X TAE buffer. 1.2% agarose gel mixed with ethidium bromide (0.5 µg/ml) was used for electrophoresis. A 100 bp DNA ladder was used on agarose gel to find the genomic DNA bands. The PCR product was mixed with 3 µl 6X DNA loading dye and added to the appropriate well. Then, electrophoresis is done at 80 volts. This gel was visualized and documented using the BIO-RAD Doc™ XR+ gel documentation system and Image LabTM (Molecular Imager software) (Ginwal and Maurya, 2010).

Statistical analysis

DNA quality, quantity and yield values of leaf samples were used for statistical analysis using IBM-SPSS software (version 20). ANOVA (Analysis of variance) with/ without Duncan's Multiple Range test was made to find out the significant changes between mean values of DNA purity, quantity, and yield according to different categories.

RESULTS AND DISCUSSION

The present study observed high DNA quality (A260/280 value) in all leaf samples (Table 1). Before RNase treatment, the quality of extracted DNA was around 2.0, which indicated RNA contamination in the extracted DNA samples. After removing the RNA molecule, the DNA quality value decreased significantly ($P < 0.01$). It approached 1.8 and 1.9, indicating the high purity of the extracted DNA with the absence of contaminants (Abdel-Latif and Osman, 2017). After RNase treatment, *D. latifolia*, *D. sissoides, M. ferrea,* and *M. malabarica* showed significantly good quality of DNA compared with *C. exarillata, D. malabaricum, K. pinnatum,* and *T. grandis* ($P<0.05$). However, the quality difference did not affect the PCR amplification with RAPD and ISSR markers. Mature leaves showed a significantly high quality of extracted DNA compared with the young leaves ($P<0.05$) (Table 2). This may be due to the smaller amount of RNA molecules in mature leaves than in young ones (Laksana and Chanprame, 2015).

The quantity and yield of DNA depended upon the sample size, part of the plant used, age of the plant, and species type. In the present study, RNase treatment significantly reduced DNA samples' total number and yield ($P<0.05$). After removing RNA molecules, *T. grandis* showed higher DNA quantity than the other seven species ($P < 0.05$). Most of the teak leaves used for the present DNA extraction came under the young leaves category, and the teak leaves were larger than the other species' leaves.

DNA quantity and yield were highly affected by the age of the leaves. Young leaves showed significantly higher DNA content and yield than mature leaves ($P < 0.01$) (Table 2). Comparable outcomes were stated by Fatima *et al*. (2018), who reported on young and mature leaves of *D. latifolia* and *Lagerstroemia lanceolata* using modified HEPES (4-(2-hydroxyethyl)-1-piperazinee thanesulfonic acid) DNA extraction method.

In many protocols, extracted DNA pellets were dried and dissolved with 1X TE buffer or sterile water after washing with 70% ethanol and then stored at -20°C for further studies (Ginwal and Maurya, 2010; Fatima *et al*., 2018; Semagn, 2014). In the present study, DNA pellets were not allowed to dry or dissolve before storage. On the other hand, DNA pellets were kept with 70% ethanol for one year period at room temperature. At the time of PCR amplification, DNA pellets were allowed to dry and dissolved with 1X TE buffer. This modification improves the integrity of extracted DNA samples for a long time and highly reduces DNA smearing or degradation compared with 1X TE buffer and sterile water respectively (Fig. 4 and Fig. 5).

Additionally, 70% ethanol-stored DNA samples showed a significant reduction in the DNA quality compared with 1X TE buffer and sterile water stored DNA samples respectively ($P<0.01$). It may be due to any kind of microbial contamination. However, 70% of ethanol-stored DNA samples did not show any significant difference in DNA quantity and yield compared with sterile water and 1X TE buffer-stored DNA samples ($P>0.05$) (Table 3). The DNA samples stored with 70% ethanol showed good integrity and less degradation after

RNase treatment (Fig. 6). Agarose gel images of PCR products of RAPD and ISSR markers with one year of 70% ethanol stored DNA samples showed clear and intact bands in Fig. 7.

The use of DNA isolation techniques should produce efficient extraction of pure and uncontaminated DNA with good quantity and quality. All molecular amplification studies are highly dependent on the efficiency of the DNA extraction method (Alhasnawi *et al.,* 2024). The leaves of tropical trees are the source of many secondary metabolites and bioactive substances that have medicinal and economic value. Many secondary metabolites and bioactive substances in the leaves complicate DNA extraction (Krishnan *et al.,* 2024). The leaves also showed quick oxidation of secondary metabolites, affecting the DNA quality and changing the DNA pellet color into brown. Sahu *et al*. (2012) also mentioned similar color changes in their protocol, which was standardized for the leaves of mangroves and salt marsh species. However, the complex polysaccharide cell wall and phospholipid membranes of plant tissues were denatured by a cationic detergent, CTAB. A high concentration of NaCl (1.4 M) provides the necessary ionic strength for CTAB to precipitate polysaccharides and proteins. Tris buffer is adjusted to maintain a pH of 8.0, preventing DNA molecule denaturation during extraction. EDTA removes dissociated cations, which are co-factors for many enzymes, such as DNase. BME (β-mercaptoethanol) denatures proteins by reducing disulfide bonds. Chloroform is a non-polar solvent which dissolves proteins and lipids. Isoamyl alcohol prevents chloroform from producing phosgene, which can denature DNA molecules. Isopropanol is used to precipitate DNA molecules and reduce the co-precipitation of RNA molecules and polysaccharides. RNase A treatment removes RNA contamination in the DNA solution, improving the DNA quality (Jadhav *et al*., 2015). Ethanol is commonly used to kill the decomposing microorganisms and prevent DNA by denaturing DNA degrading enzymes. Therefore, ethanol was considered an excellent DNA and tissue fixative of insect species (Marquina *et al*., 2021). Jadhav *et al*. (2015) mentioned that 70% ethanol is also used to wash DNA pellet and remove excess salt. The present study, 70% ethanol was used as a plant DNA fixative for long-term storage.

In the present method, the usage of liquid nitrogen, Phenol, PVP (Polyvinyl pyrrolidone), Proteinase-K, and ascorbic acid were avoided, which are costly and com-

Table 1. Quality, quantity, and yield of extracted DNA from the leaves of 8 tropical trees

Species	Total number of samples used	Before RNase treatment			After RNase treatment		
		DNA quality ± SD	DNA quantity (ng/µl) ± SD	DNA yield (µg/100 mg of leaf) ± SD	DNA quality ± SD	DNA quantity (ng/µl) ± SD	DNA yield (µg/100 mg of leaf) ± SD
C. exarillata	23	1.98 ± 0.05a	224.1 ± 97.2a	44.8 ±19.5a	1.90 ± 0.06b	220.7 ± 97.2a	44.1 ± 19.4a
D. latifolia	16	2.00 ± 0.05ab	365.0 ± 350.6a	73.0 ± 70.1a	1.86 ± 0.06a	349.8 ± 334.1a	70.0 ± 66.8a
D. sissoides	40	2.02 ± 0.04bc	405.1 ± 296.0a	81.0 ± 59.2a	1.87 ± 0.05a	392.7 ± 283.9a	78.5 ± 56.8a
D. malabaricum	11	2.03 ± 0.06bc	193.9 ± 94.0a	38.8 ± 18.8a	1.91 ± 0.05b	185.2 ± 95.5a	37.0 ± 19.1a
K. pinnatum	10	2.03 ± 0.05bc	299.1 ± 116.0a	59.8 ± 23.2a	1.93 ± 0.04b	293.2 ± 114.8a	58.6 ± 22.9a
M. ferrea	26	2.04 ± 0.04bc	251.4 ± 81.0a	50.3 ± 16.2a	1.87 ± 0.04a	271.7 ± 146.7a	54.3 ± 29.3a
M. malabarica	26	2.03 ± 0.06bc	134.2 ± 64.5a	26.8 ± 12.9a	1.86 ± 0.04a	154.2 ± 80.3a	30.8 ± 16.1a
T. grandis	116	2.04 ± 0.06c	924.2 ± 659.0b	184.8 ±131.8b	1.92 ± 0.03b	679.2 ± 487.0b	135.8 ± 97.4b
Mean ± SD		2.03 ± 0.06	558.0 ± 563.5	111.6 ± 112.7	1.90 ± 0.05	452.3 ± 410.2	90.5 ± 82.0

Column-wise mean values of DNA quality, quantity, and yield with different alphabets are significantly different at <0.05 level by DMRT test.

Table 2. ANOVA between young and mature leaf-extracted DNA quality, quantity, and yield values

ANOVA		Sum of squares	Degrees of freedom	Mean square	F-value	Significance
DNA quantity (ng/µl)	Between young and mature leaves	8127143.2	1	8127143.2	58.598	0.000
	Within young and mature leaves	36892625	266	138694.08		
	Total	45019768	267			
DNA quality (A260/280)	Between young and mature leaves	0.02	1	0.02	8.263	0.004
	Within young and mature leaves	0.632	266	0.002		
	Total	0.652	267			
Total yield (µg/100 mg of leaf)	Between young and mature leaves	325107.67	1	325107.67	58.608	0.000
	Within young and mature leaves	1475552	266	5547.188		
	Total	1800659.6	267			

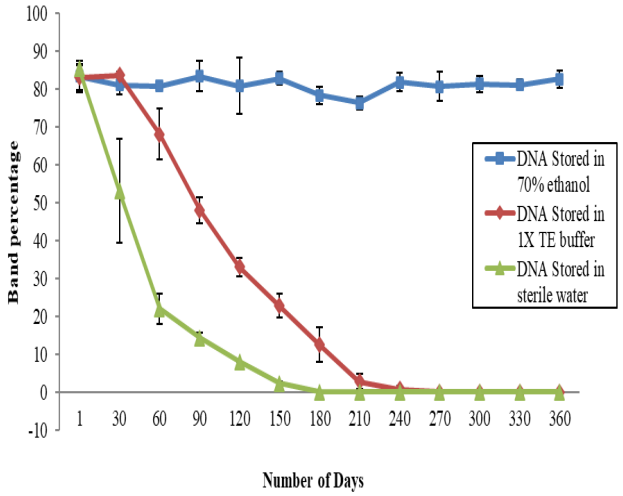

Fig. 4. *Effect of different DNA storing solutions on band percentage of extracted DNA*

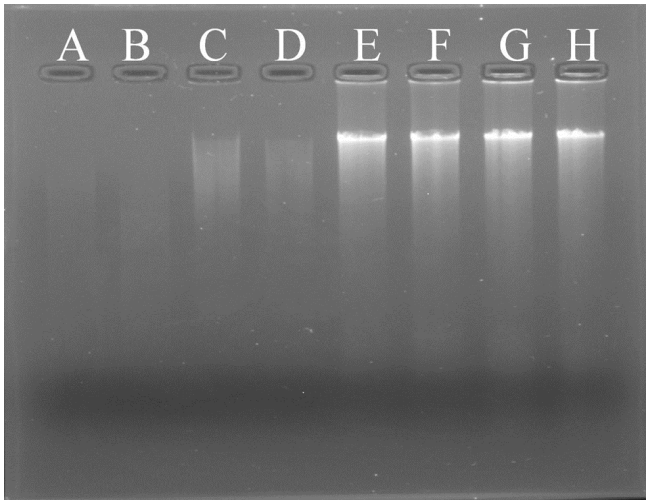

Fig. 5. *0.8% Agarose gel showed the effect of different DNA storing solutions on DNA band integrity after a year (before RNase treatment); A & B – DNA samples stored in sterile water showed more degradation; C & D - DNA samples stored in 1X TE buffer also showed DNA degradation; E, F, G & H - DNA samples stored in 70% ethanol showed intact bands with less degradation.*

monly used in many conventional DNA extraction methods (Ginwal and Maurya, 2010; Fatima *et al*., 2018; Semagn, 2014). Liquid nitrogen was used to pulverize the leaf tissues to avoid oxidation of phenolic compounds and enzymatic reactions. In this method, the leaf tissues were ground with CTAB extraction buffer containing BME and EDTA, which act as antioxidants and inhibit enzymatic reactions. Phenol was used to digest the hard plant tissues (i.e., wood, bark, seed, etc.). However, leaf tissues were easily digested with chloroform using the present method. PVP and ascorbic acid are commonly used to precipitate the phenolic compounds during DNA extraction and prevent secondary metabolite oxidation (Ginwal and Maurya, 2010). In the present method, PVP (1 – 4 %) and ascorbic acid (5 to 20 mM) failed to precipitate the phenolic compounds and resulted in brown color DNA pellets. PVP is also co-precipitated with DNA pellets, making it very hard to dissolve in TE buffer. Alzate-Marin *et al*. (2009) reported low DNA quality (A260/280 - 1.0 to 1.3) in the leaf samples of some tropical tree species in Brazil using their modified CTAB method with PVP. Therefore, PVP and ascorbic acid were avoided using the present method. The present DNA extraction was superior to other methods due to the absence of costly chemicals like liquid nitrogen, phenol and Proteinase-K. After a year storage period with 70% ethanol, the present method resulted in high DNA quality, quantity and yield compared with other methods (Aboul-Maaty and Oraby, 2019; Ginwal and Maurya, 2010; Fatima *et al.,* 2018; Semagn, 2014; Alzate-Marin *et al*., 2009). The extraction and long-term storage methods were standardized for the leaves of tropical tree species belonging to different plant families. Therefore, the present methods are versatile and can be used for other plant species.

Conclusion

High quality DNA extraction is an important step in the

Table 3. ANOVA between DNA quality, quantity, and yield values based on different storage

ANOVA		Sum of squares	Degrees of freedom	Mean square	F-value	Significance
DNA quantity (ng/µl)	Between ethanol, TE buffer, and sterile water	2.659	2	1.329	160.329	0.000
	Within ethanol, TE buffer, and sterile water	0.224	27	0.008		
	Total	2.882	29			
DNA quality (A260/280)	Between ethanol, TE buffer, and sterile water	813.254	2	406.627	0.011	0.989
	Within ethanol, TE buffer, and sterile water s	1037398.554	27	38422.169		
	Total	1038211.808	29			
Total yield (µg/100 mg of leaf)	Between ethanol, TE buffer, and sterile water	32.530	2	16.265	0.011	0.989
	Within ethanol, TE buffer, and sterile water	41495.942	27	1536.887		
	Total	41528.472	29			

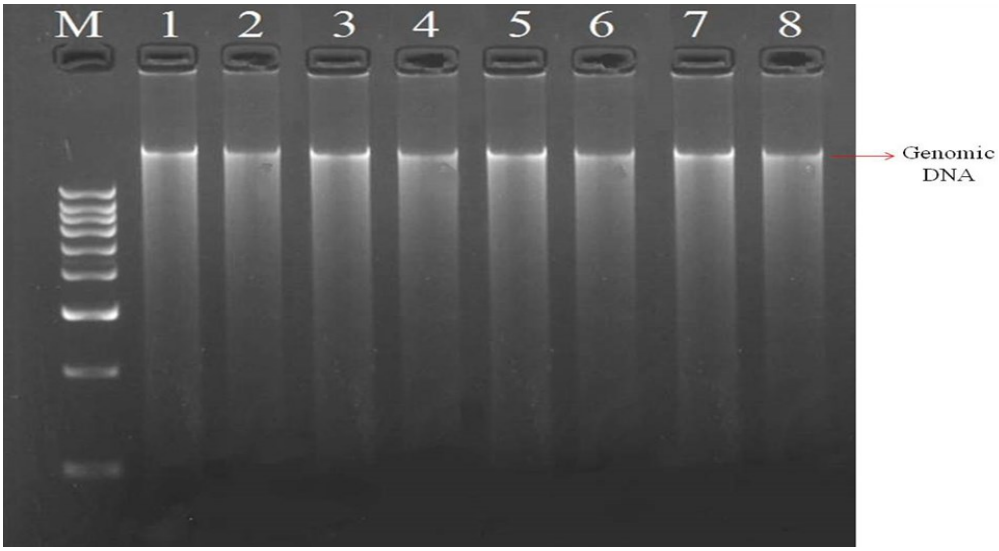

Fig. 6. *Gel image of one-year-old DNA extracted from the selected tropical trees in 0.8 % agarose gel showing good integrity and less degradation after RNase treatmen; M – 1 kb DNA ladder, 1 – Dalbergia sissoides, 2 – Dalbergia latifolia, 3 – Dysoxylum malabaricum, 4 – Myristica malabarica, 5 – Kingiodendron pinnatum, 6 – Mesua ferrea, 7 – Tectona grandis and 8 – Cullenia exarillata.*

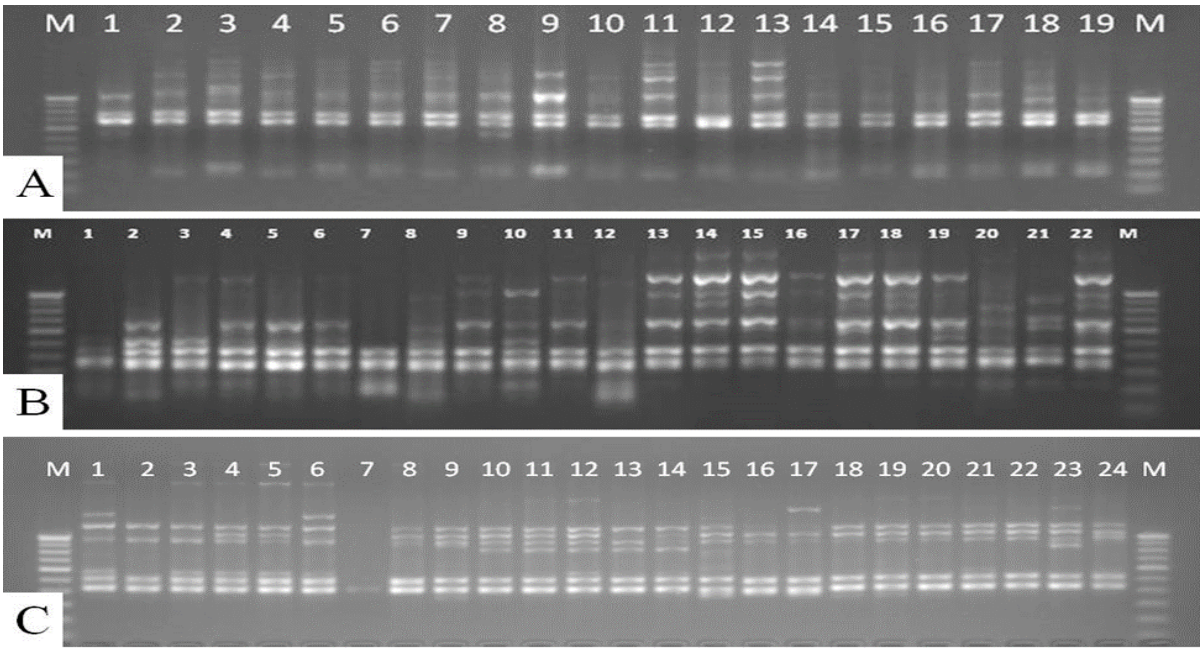

Fig. 7. *Banding profile of one-year-old DNA samples in 1.2 % agarose gel. M – 100 bp DNA ladder; A - Dalbergia sissoides and Dalbergia latifolia samples with RAPD marker; B – Teak samples with ISSR marker; C – Mesua ferrea samples with ISSR marker.*

genetic analysis of plant species. The present method was standardized to isolate the high-quality DNA (Mean A260/280 = 1.90 ± 0.05) from the leaves of tropical tree species viz., *C. exarillata*, *D. latifolia*, *D. sissoides*, *K. pinnatum*, *D. malabaricum*, *M. ferrea*, *M. malabarica* and *T. grandis*. The average DNA quantity is 452.3 ± 410.2 ng/µl as well as the average DNA yield is 90.5 ± 82 µg per 100 mg of leaf sample. The extracted DNA samples stored with 70% ethanol at room temperature for one year showed good integrity with less degradation and did not affect the PCR amplification with RAPD and ISSR markers. The present DNA isolation and long-term storage protocols can be used to extract and store the genomic DNA from the leaves of other tree species.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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