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ORIGINAL ARTICLE

Molecular studies relating Genomic DNA isolation from *Elaeocarpus ganitrus* (Roxb.) callus and leaf tissue

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ABSTRACT
Elaeocarpus ganitrus is a woody plant species and synthesizes large number of polysaccharides, phenolic compounds compromising high quality genomic DNA. Elaeocarpus ganitrus (*E. ganitrus*) *well known as Rudraksh. E. ganitrus is a species from family Elaeocarpaceae. To overcome the difficulty in genomic DNA extrction from this woody plant species, the present study deals with the rapid and reliable isolation method of genomic DNA by CTAB method, successfully using leaf tissue and from the in-vitro grown callus. The approach may be useful for the molecular studies of E. ganitrus and may be easily utilized for rapid DNA extraction from other species of the genus Elaeocarpus.*
Key words: *E. ganitrus*, genomic DNA, CTAB, molecular biology

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INTRODUCTION

Molecular biology studies required rapid isolation of nucleic acids. The protocols available in the research papers are usually developed for herbaceous plant species. However, these protocols do not work satisfactorily in other taxa which comprise high amount of phenolics and polysaccharides. Some molecular biological research studies performed previously by the researchers which are as followed.

DNA extracted from the leaves of several plant species by some modification in Lin *et al* SDS method of DNA extraction. DNA band was estimated using 1D agarose gel electrophoresis and visualization was done under UV trans-illuminator [1]. Original CTAB methodology was used for DNA extraction from immature leaves of *E. ganitrus*. The DNA purification was carried out using phenol: chloroform: isoamyl alcohol and by using ice-cold isopropanol, the DNA was precipitated. And for other species of *Elaeocarpus* i.e., *E. serratus*, the original method of CTAB was modified by using poly-vinyl-pyrrolidone (PVP) and β-mercaptoethanol during the initial grinding step [2].

In *Prunus persica* (L.) Batsch, isolation of gene encoding S28 cytoplasmic ribosomal protein and its molecular characterization was reported [3]. DNA isolation was reported successfully in which younger leaves and older leaves were used, out of which younger leaves shown good results as compared to older leaves and further PCR results obtained from DNA of younger leaves which were successful and shown bands for RAPD markers [4]. DNA was isolated from *Leguminosae* tree leaves and from the bark tissue taken from Atlantic Forest and Cerrado. The quantity of DNA isolated from the leaf samples were higher as compared to bark sample, with same purity. The obtained RNA may be useful for several genomic studies [5]. An inexpensive protocol for DNA isolation from mature leaves of *Acer*, *Fraxinus*, *Prunus* and *Quercus* was developed [6]. A protocol was developed for RNA extraction from macadamia, avocado and mango tissues that are difficult to work with. The extracted RNA was successfully used to produce cDNA for real time quantitative PCR for generating good quality RNA-seq libraries. For fast DNA extraction from different tropical and sub-tropical tree species the protocol can easily be converted. The method developed can be used for fast DNA and RNA extracion from the recalcitrant tree species [7]. Successful amplification of polymorphic noncoding region of chloroplast DNA using PCR was performed. For the

work various samples of oak wood, which includes recent and more ancient samples from various oak species of around 600 years old. To obtain results, adaptation of DNA isolation and amplification procedures was necessary. The polymorphisms useful in distinguishing geographical origination of samples could scored by sequencing. The polymorphisms cover one substitution and two microsatellite kind polymorphisms, because of variable A-T repeats number. In two different laboratories independently same results were achieved [8].The average yielding of mDNA for the samples like leaf, root, stem, bulk and rhizospheric soils obtained through Spin Column Based methodology (SCB) and Indirect SDS based methodology (ISB). For 16SrRNA and ITS sequence, successive PCR amplification was observed consistently for ISB, SCB and mDNA extracted from kit, confirmed highly pure mDNA extraction using these methods. More than 96% quantitative PCR efficiency, partial restriction digestion and construction of metagenomic libraries which has confirmed that two methods developed and implemented are highly efficient. The methods enhanced metagenomic DNA extraction efficiency from various *Quercus brantii* (Lindl.) tissues [9]. Using DNA extraction methodology described by Lefort *et al* DNA isolation was performed from callus initiated from the seeds of *Salvia* L. *spp.* [10].

Using various tissues, including callus developed from the leaves of *Jatropa curcus*, simple and high quality genomic DNA extracted. The DNA extracted found fit for restriction digestion, ligation and PCR (Polymerase Chain Reaction) amplification. For developing molecular markers and studying genetic diversity, it was used for DNA fingerprinting techniques, AFLP (Amplified Fragment Length Polymorphism) and RFLP (Restriction Fragment Length Polymorphism) [11]. Genomic DNA isolation and amplification from callus cultures using *Carpobrotus* (Aizoaceae) species was performed. No genetic variation associated with conditions of callus cultures was observed. When the traditional DNA extraction methodology do not work, the proposed technique is suitable for genomic polymorphic studies in succulent and other plants [12].

A non expensive protocol for DNA isolation from five different tissues of palm species from Atlantic forest of Brazil was proposed [13].Without using liquid nitrogen and phenol a simple fast and faithful DNA isolation procedure was developed. Method better suitable for the plants consists of high polysaccharides and secondary metabolites [14]. A modified, time saving protocol by combining CTAB and plant RNA kit method was proposed to extract RNA from *Neolamarckia cadamba*. The method can be used to obtain RNA from recalcitrant plant material [15].The result was obtained regarding construction of vegetative and generative cDNA libraries of *Tectona grandis* from the RNA pool that were isolated from vegetative and generative bud tissues of *T. grandis* [16].The total RNA of high quality from *Prunus persica* was isolated using fast and effective method [17]. A high quality of RNA was yielded from the Lychee tree. The method may be suitable for isolating RNA from recalcitrant tissue samples [18]. The DNA extraction was performed successfully using fresh tissues of the plants [19].

The present investigation deals with the molecular studies regarding genomic DNA isolation of *Elaeocarpus ganitrus* (Rudraksh) using CTAB methodology with some modifications in the original CTAB protocol proposed by Doyle and Doyle (1990). Here, we have developed a modified method for DNA isolation from *E. ganitrus* leaf and callus tissues and the protocol may facilitate novel insights on woody plant species such as *E. ganitrus* that are too difficult for molecular studies and experimentation.

MATERIAL AND METHODS

Soft leaves and callus were used for the study. Leaves of *Elaeocarpus ganitrus* were collected from approximately 8 years old tree located within Shobhit Institute of Engineering and Technology, Modipuram, Meerut (Fig. 1, 2, 3). For the isolation of DNA, green colored calli were used. Calluses of *E. ganitrus* were developed at Plant tissue culture laboratory, Department of Biotechnology, Shobhit Institute of Engineering and Technology, Modipuram, Meerut.

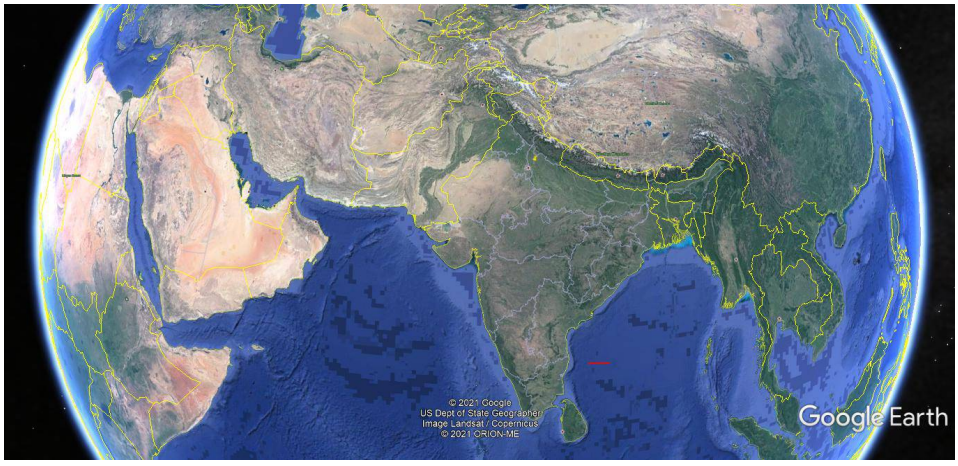

Fig. 1 The pin point location from where leaf samples were collected (29.0712° N, 77.7123° E). The location is marked with a black arrow and the red ruler represents an area of 200 km.

Fig. 2 The satellite image of location (SIET, Meerut) from where the samples were collected.

DNA isolation

For genomic DNA isolation, leaves samples were collected and then ground in liquid nitrogen along with the addition of PVP (poly-vinyl-pyrrolidone), 0.01% using pestle and mortar. The sample was suspended in CTAB (cetyl trimethyl ammonium bromide) extraction buffer. 1ml of CTAB buffer, [consists of 2% (*w/v*) CTAB, 0.1M Tris-HCl (pH 8), 0.2 M EDTA (Ethylenediamine tetra acetic acid) (pH 8), 1M NaCl (Sodium Chloride) and 0.5% β- mercaptoethanol] was added to the freshly ground powdered tissue (~100 mg). The suspension was incubated in water bath for 40 min at 65 □. The mixture was allowed to cool down at room temperature (RT) and then centrifuged at 10,000 g for 1 min. To the supernatant, 1 ml of chloroform: Isoamyl alcohol (24:1) (*v/v*) was added. The solution was gently mixed and centrifuged at 10,000 g for 10 min. To the above layer 0.5 ml of chilled isopropanol was added and incubated at -20 □ for overnight. Then the sample was centrifuged at 10,000 g for 10 min. and supernatant was discarded. 1ml 70% ethanol was added and centrifuged at 10,000 rpm for 1 min. After centrifugation the ethanol was discarded and eppendorf tube containing pellet of DNA was kept for drying in laminar hood. 30 µl of nuclease free water was added to air dried pellet and stored at 4℃ for quantitative and qualitative analysis.

Qualitative and quantitative analysis of isolated genomic DNA

For quantification of DNA Nanodrop spectrophotometer was used. 260/280 reading were taken to analyze the purity of isolated genomic DNA from callus and leaf tissue. Nuclease free water was used to set the readings at zero. Further, genomic DNA samples were analyzed by Nanodrop. For agarose gel electrophoresis, 0.8% Agarose (0.8 g in 100 ml) was taken in 1X TAE buffer and heated and cooled down to room temperature for solidification. Just before solidifying, 0.001% Ethidium bromide (EtBr) was added to the gelling solution, mixed properly and poured in a casting tray. The DNA samples 2 µl was taken along with the loading dye 4 µl λ *Hind* III DNA marker was taken to know the size of DNA. The gel

was run at 40 volts for 60 min. After the gel running was over, the gel was kept under UV transilluminator for analysing the DNA bands on gel.

RESULTS AND DISCUSSION

The genomic DNA by applying CTAB method was isolated successfully from the leaves and from the callus of *E.ganitrus* (Fig. 3). Sumanarathne *et al*., 2020 applied original and modified CTAB method for DNA isolation from *E.ganitrus* and *E.serratus*, they have also used PVP in the initial stage of grinding . In other study, for DNA extraction of *E.ganitrus* and other plant species, SDS based DNA extraction methodology of Lin *et al* was applied by some modification in the steps and chloroform; isoamyl alcohol (24:1) was used by Khasdeo *et al*., 2014. From mature leaves of *Acer*, *Fraxinus*, *Prunus* and *Quercus* DNA isolation was performed by Lefort and Douglas, 1999.

In our study it was observed that the DNA isolated from same amount of callus showed less DNA concentration as compared to leaf tissue. The DNA yield is shown in Table 1. The 260/280 readings of all the samples were in the range of 1.8-2.1, which shows that the isolated genomic DNA was of good quality (Table 1, Fig. 4).

Table 1. Nanodrop readings observed for the isolated gDNA

S. no.	Plant sample	DNA yield (ng/µl)	260/280
1	Callus 1	99.36	1.92
2	Callus 2	89.23	2.01
3	Callus 3	69.23	1.98
4	Leaf tissue 1	150.1	1.87
5	Leaf tissue 2	301.56	1.99
6	Leaf tissue 3	120.25	1.86

A B C

Fig. 3: A-*E. ganitrus* tree, B- 15-20 d old leaves used for the study, C-Callus developed from leaf tissue .

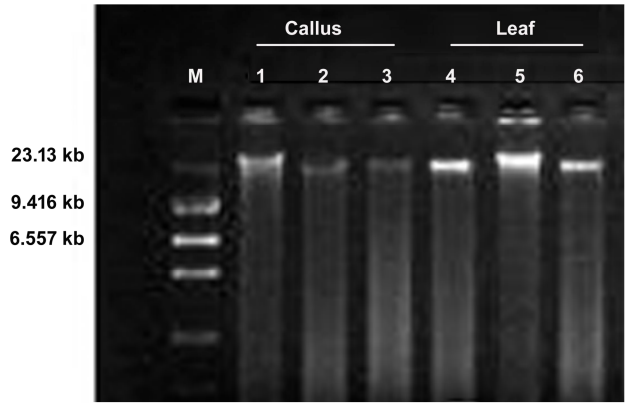

Fig. 4 Analysis of genomic DNA isolated from *E.ganitrus* callus and leaf tissue on agarose gel. M is λ *Hind*III DNA marker. Lane 1-3 represents gDNA from callus, Lane 4-6 represents gDNA from leaf tissue.

CONCLUSION

Here we have shown a simple and fast method for DNA isolation from leaf and callus tissue of *E.ganitrus*. Till date very less studies are being carried on molecular biology aspect of this tree species, so it is

necessary to take initiative for more research in the field of molecular biology specially the work related to the nucleic acids. Our method of DNA isolation may be utilized for species of the Genus *Elaeocarpus* and other woody plant species.

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