

EXTRACTION OF HIGH QUALITY DNA FROM *DIPLOKNEMA BUTYRACEA*

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ABSTRACT

Diploknema tree species (MPTs) has a great economic value in respect of fodder, fuel wood, timber and other product. It has also a great medicinal value in Rheumatism, Ulcers, Itching, Hemorrhage, Inflammation of tonsils etc. Having such a great economic and medicinal value *Diploknema* is facing extinction because of relentless anthropogenic pressure. These species are failing to regenerate in spite of reasonable seed production. Very little information exists on the molecular aspects of *Diploknema* which requires high quality DNA. A protocol for extraction of high quality DNA from *Diploknema butyracea* is hereby discussed. [Researcher. 2009;1(3):33-35]. (ISSN: 1553-9865).

INTRODUCTION

Diploknema butyracea also known as Indian butter tree, and locally known as Cheura is a multi purpose tree (MPT). The National Wildlife Development Board (NWDB) has found *D. butyracea* to be useful for block planting and also to be grown in the ravines of hills. The latex yielding plant such as *D. butyracea* suits to different edapho climatic conditions and thus does not compete with the traditional crops. It is a large tree of family Sapotaceae, flowers during cold season and fruit ripens in June-July. It commonly occurs in the sub Himalayan tract between 300-1500m from sea level. In Uttarakhand it occurs abundantly in Pithoragarh district and adjoining areas of Almora, Bageshwar and Nainital District (Negi *et al.*, 1988). Its seed kernel contains saponins. The yield of oil is 42-47% of the weight of seeds. It has consistency of ghee with white colour, pleasant taste and odour. It has a high titer test. The palmitic acid content (56.6%) is the highest yet observed among seed fats. The oil is convenient source of natural oleodipalmitin (62%). The tree produces a durable, hard and strong wood comparable to teak. Bark of the tree is used in the treatment of rheumatism, ulcers, itching, and hemorrhage, inflammation of the tonsils, leprosy and diabetes. The bark contains 17% tannin and is used in tanning, dyeing and as a fish poison. The seeds of *D. butyracea* yield edible oil, known as "Phulwara Butter" which is used in chocolate, soap and candle manufacture. Oil is used as an external ointment to ease rheumatism, paralysis and sprains. Phulwara butter is a valuable preservative for mustard and sweet scented oils. The oil cake contains saponins and act as fertilizer, fish intoxicant, pesticide and detergent. The tree is lopped for fodder and the viability of seed is very low which adversely effects its regeneration.

MATERIALS AND METHOD

Plant material

Biotypes of *Diploknema butyracea* were collected from Berinag, district- Pithoragarh, and Department of Forestry, D.S.B. Campus, Nainital.

DNA Extraction

Total genomic DNA was extracted using CTAB method (Doyle & Doyle, 1987) with some modification. 1 gm freshly harvested leaf whose gel was removed was ground to fine pulp using liquid nitrogen along with 0.1 g PVP. Extraction buffer (pH-8) preheated to 65°C containing 2% CTAB (w/v), 5.0 M NaCl, 0.5 M EDTA and 0.5 M tris HCl were added to the pulp in a centrifuge tube, shaken and incubated for 1 hour at 65 °C in a water bath with intermittent shaking and swirling in every half an hour. To this equal volume of Chloroform:Isoamylalcohol (24:1) was added and mixed by inversion for 30 min and centrifuged at 12,000 rpm for 15 min. Supernatant was transferred to a new tube and was precipitated with equal volumes of cold Isopropanol, and gently mixed to produce fibrous DNA and incubated at -20 °C for 30 min. Samples were centrifuged at 12,000 rpm for 15 min. The pellete was washed with 70% ethanol and kept for drying. After drying, the pellete was dissolved in 3 µl of TE buffer (1 mM EDTA and 10 mM Tris HCL pH-8). To remove contaminating RNA 5 µl of RNAs (10 mg/ml) was added. The tubes were incubated over night at 37 °C. Dissolved DNA was extracted with equal amount of Phenol:Choloroform:Isoamylalcohol (25:24:1.v/v/v) and centrifuged at 8000 rpm for 15 min. then aqueous layer was transferred to a fresh 15 ml tube and equal volumes of chloroform:isoamylalcohol (24:1) was add and centrifuged at 12,000 rpm for 15 min. Finally supernatant was transferred to a fresh tube, equal volume of absolute alcohol and 1/10 volume

of sodium acetate were added and incubated at -20 °C for 30 min and centrifuged at 12,000 rpm for 15 min. The final pellet was dried and resuspended in TE buffer.

RESULT AND DISCUSSION:

The DNA of leaf tissues from two biotypes of *Diploknema* was analyzed and the amount of DNA in mg per gm was calculated by taking absorbance at 260 nm/290 nm. The ratio of absorbance 260 nm/280 nm was found to be in the range of 1.8 to 2.0 and the DNA yield ranged from 0.67µg/ml to 0.86µg/ml. This work shows that the DNA which was isolated by some modifications in the CTAB method was of high quality containing very low contamination of terpenoids and polysaccharides. The chemicals which were used during isolation of DNA by CTAB method increase DNA purity by removing all impurities. Long term chloroform isoamylalcohol treatment removes chlorophyll, pigments and dyes. Overnight treatment of RNase degrades RNA. Other precipitates (detergents, protein, polysaccharides etc.) were removed by additional step of phenol:chloroform:isoamylalcohol (25:24:1,v/v/v) and phenol:chloroform (24:1).

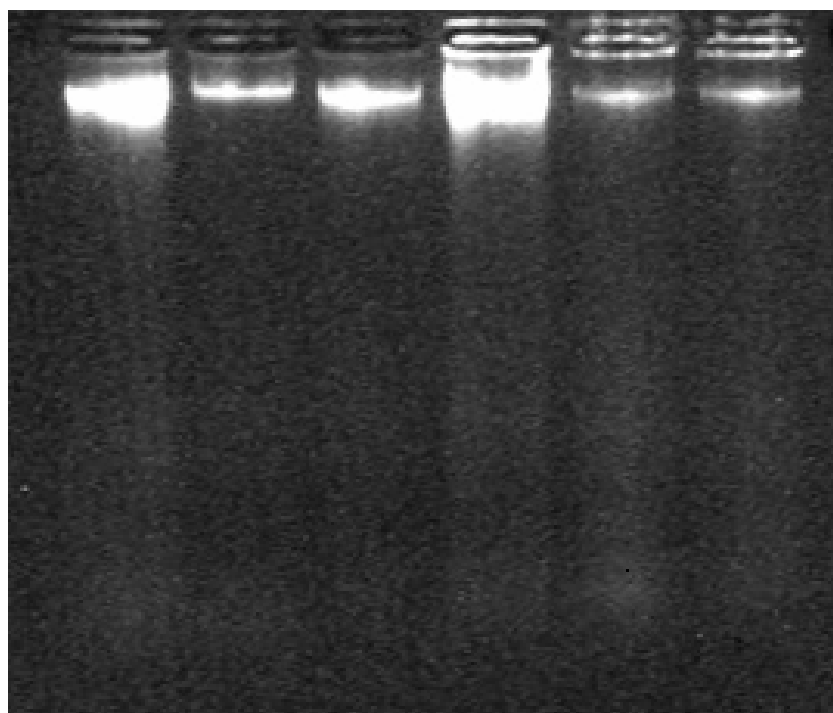


Fig 1. Agarose gel photograph of DNA extracted from leaf tissue of *Diploknema butyracea*

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