Genomic DNA Extraction From Fresh Core Wood of *Cedrus deodara* (Roxb.) G. Don for Thermo Cycler (PCR) Study

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Abstract- For DNA fingerprint study traditionally plant leaves/needles are used as a source of DNA. However; sampling of leaves from tall tree species can be quite difficult and expensive. We developed alternative method of DNA extraction from core wood of Cedrus deodara (Roxb.) G.Don tree from Uttarakhand forest. We used 2% PVP for removal of phenolics compound and apply high concentration of sodium chloride to removes polysaccharides. The yield of extracted DNA was ranged from 80 to 230 ng/500 mg weight of the tissue with absorbance ratio (A_{260}/A_{280}) ranged from 1.71 to 1.98. DNA extracted by modified CTAB protocol gives positive amplification with PCR using inter simple sequence repeat (ISSR) markers.

Keywords: CTAB, ISSR, Thermo cycler, Cedrus deodara (Roxb.) G.Don core wood, PVP.

I. INTRODUCTION

Cedrus deodara (family: Pinaceae) is a species of Cedar native to the western Himalayas in North-Central India. It occurs at 1200-3050 m altitudes where mean annual temperature is 12–17 ºC, and mean annual rainfall is 200-1800 mm (Tewari, 1994). *Cedrus deodara* prefers loam (predominantly sandy) soils with high levels of organic carbon, low phosphorus and high potash contents. It avoids stiff, badly drained soil, and its growth is stunted on rocky shallow soil. The best growth is attained on deep, fairly porous, fertile soil in cool situations. It is a large evergreen coniferous tree reaching 40–50 m tall, exceptionally 60 m, with a trunk up to 3 m diameter. It has a conical crown with level branches and drooping branchlets. *Cedrus deodara* is an economically important forest tree species, known mainly for its high valued timber. The hard and durable wood obtained from it, it is white to light yellowish brown in colour, with a characteristic odour and oily feel. It is strongest coniferous wood and weight for weight, about as strong as teak. Its average weight is 560 kg/m^3. The deodar wood is highly valuable and extensively used for building, railway sleepers, carriage and railway wagon work and other purposes for which durability is required. It is used in house building, beams, floorboards, door and window frames, furniture and general carpentry. It also produces quality plywoods, Essential oils extracted from Deodar have been reported to have medicinal properties. It is used as antiseptics and against tuberculosis. Its oil and extracts are also used as insecticides and herbal remedies against many animal diseases in India. The herbal pesticide Pesto ban is a liquid concentrate of three Indian medicinal plants including *Cedrus deodara*. It is also a potent molluscicide (Orwa *et al.*,2009).

Traditionally, molecular studies of plant species have used leaf tissues as the source of DNA. The existing protocols Doyle and Doyle, 1990 and Stange *et al*. (1998) are inefficient for extracting DNA from *C. deodara* wood tissues. Therefore, there was a need to optimize the protocols for DNA extraction from wood tissue to yield high concentrations of good quality DNA fit for polymerase chain reaction (PCR) applications. Recently, this species has been used for molecular analysis, such as genetic variation analysis (Seysis *et al*., 2003) and gene mapping (Lombard and Deloureme, 2001, Kole *et al*., 2002). In this paper we reported a modified DNA extraction protocol; it could produce relatively high quality DNA for molecular markers.

II. MATERIAL AND METHODS

Plant material

Genomic DNA was extracted from fresh core wood of *C. deodara* at the Division of Genetics and Tree Propagation, Forest Research Institute, Dehradun. The detailed modified protocol is given below:

Solutions and Reagents

- Cetyl-trimethylammonium bromide (CTAB) extraction buffer consisting of 100 mM Tris-HCl (pH-8.0), 20 mM ethylenediaminetetraacetic acid (EDTA) (pH-8.0), 1.4 mM NaCl, 3% CTAB, 2% polyvinylpyrollidone (PVP), 5 mM Ascorbic acid and 0.2% β-mercaptoethanol.

- 5 M Amonium acetate solution (v/v).

- Chloroform: isoamyl alcohol (24:1 v/v).
- 76% ethanol, 96 % ethanol (v/v).
- Isopropanol (-20ºC).
- TE Buffer consisting of (Tris HCl 10Mm, EDTA 1Mm, P^H 8.0)
- Wash buffer (998µl of 96%ethanol and 2µl of ammonium acetate solution).

Equipment

- Water bath
- Centrifuge
- 2-ml and 1.5-ml micro centrifuge Eppendorf tube.

Procedure

One ml of CTAB buffer with 3 µl β–mercaptoethanol was added in a 2 ml eppendorf tube and incubated in water bath at 65°C for 10 minutes. Sliced and chopped wood (0.5g) were grinded to a fine powder in a mortal pestle in the presence of liquid nitrogen and transferred to pre incubated extraction buffer and incubated 65°C for 1.5 hours. After incubation the samples were allowed to cool down to room temperature and then 500 µl of Chloroform: isoamyl alcohol in the ratio of 24:1 was added to the sample and centrifuged at 6,000 rpm for 6 minute at 4°C. The upper aqueous phase was pipette out and transferred to a fresh micro centrifuge tube (1.5ml). An equal volume of cold isopropanol was added to the supernatant, mixed well and incubated overnight at -20°C. DNA was pelleted by centrifuging at 10,000 rpm for 15 min at 4°C. Pellet was washed with 998 µl of 96 % alcohol and 2 µl of 10 mM ammonium acetate. The pellet was again washed with 500 µl of 76% alcohol and centrifuged at 10,000 rpm for 15 minute, vacuum dried resuspended in 100 µl of T.E buffer and stored at 4°C. The yield of the extracted DNA was quantified by Biophotometer at A260/A280 nm wavelength and the purity of DNA was checked by running the samples on 0.8% agarose gel (Figure1).

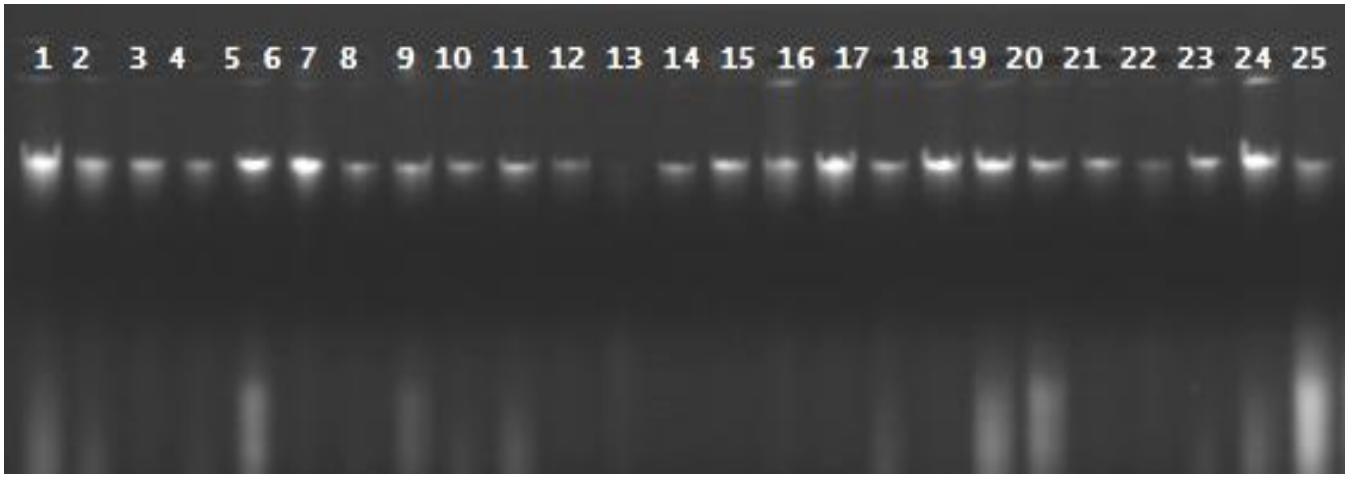

Figure 1. Lane 1-25, showing extracted genomic DNA from core wood of *C. deodara* using modified protocol at 0.8% agarose gel.

ISSR analysis

ISSR primer (University of British Columbia, UBC-825 Sequence: 5'ACACACACACACACACT-3' (Hong *et al*., 2007), was used for the amplification. PCR amplification was carried out in a 20 µl reaction mixture (Table.1). The PCR amplification program consisted of: one cycle at 94°C for 5 min; 40 cycles each at 94°C for 30 sec, 52.6°C for 30 sec and 72°C for 1 min followed by a final extension at 72°C for 10 min. Amplification products were visualized on 2% agarose gel (Figure 2).

Table 1. Concentrations of reaction components for ISSR.

S. No	Stock	Reaction	Final concentration
1	Taq Buffer	2.00µl	1x
2	$MgCl_2$	1.40µl	1.75mM
3	dNTPs (2.5mM)	1.60µl	0.2mM
4	Primer (20µM)	0.40µl	0.4µM
5	Taq DNA Polymerase(5U)	0.12µl	0.6U
6	Template DNA	1.00µl	15ng/µl
7	Total volume	20.00µl	

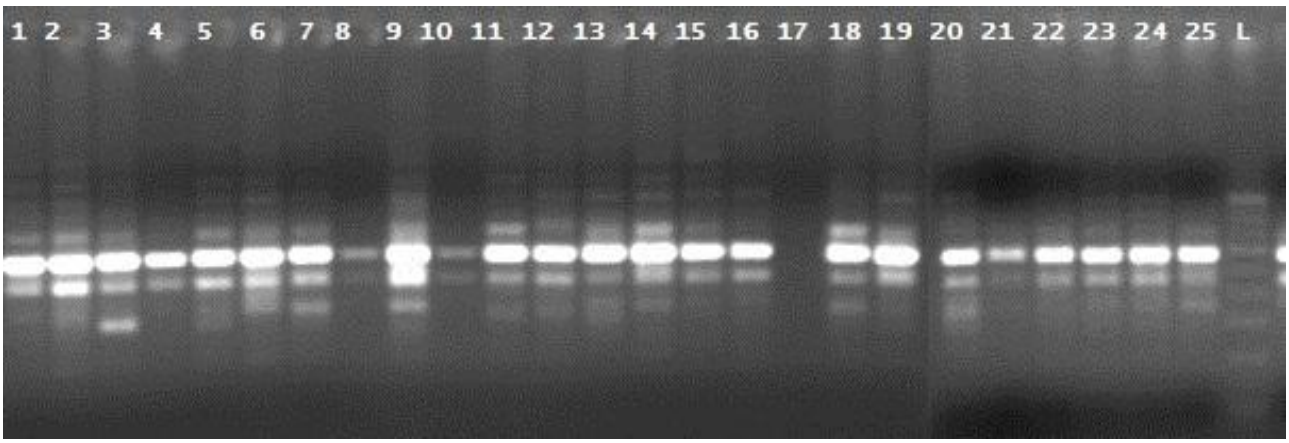

Figure 2. Nuclear ISSR pattern obtained from DNA extracted from modified protocol. 25 different DNA samples (Lanes 1-25) amplified using prime UBC-825. Lane L100bp Ladder .

III. RESULT AND DISCUSSION

DNA extraction was very successful from core wood of twenty five individuals of Cedrus deodara (Roxb.) G. Don. The DNA extracted exhibited high quality and the DNA quantity was also high with an A260/A280 ratio(1.71-1.98), which is within the optimal sample range (Sambrook et al., 1989). The fatty acid and proteins were the main components of the fresh wood of C. deodara. For fatty acid has lower density and nonpolar characteristic, it could easily be distinguished from the aqueous phase when it involved into centrifugation. The phenol and chloroform were frequently used for protein removing in custom DNA extraction method; it needed only need a short centrifugation to separate DNA from all the other contaminants in our present protocol, for the most of proteins removed in the insoluble precipitate. The 1M Nacl was added into extraction buffer to remove the polysaccharides by increasing their solubility in ethanol (Fange et al., 1992). In order to remove polyphenols from the freshcore wood, the PVP was added to the extraction buffer according to the result of Maliyakal (1992). Good amplification result of ISSR molecular markers also verified the good quality of these twenty five DNA samples.

IV. CONCLUSION

All living things have DNA. The major problems encountered in isolation of pure DNA molecules are degradation of high molecular weight nucleic acids by mechanical damage or by hydrolytic action of nucleases, contamination with RNA, proteins and polysaccharides. This modified protocol provides good quality of DNA.

V. FUTURE SCOPES

The results indicate the usefulness of the protocol for the molecular marker investigations of *C. deodara*, the method of DNA extraction from core wood of *C. deodara* is extremely useful for molecular studies involving mature tall trees, where collection of needles is difficult.

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