# QUALITY AND QUANTITY ASSESSMENT OF GENOMIC DNA IN NEOLAMARICA CADAMBA (ROXB.)

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**Abstract:** *Neolamarckiacadamba*(Roxb.) under family Rubiaceae is a fast growing, medium to large and deciduous forest tree species. *N. cadamba*is a multipurpose function and utility forest tree. The present work is to better understand the genetic diversity and genetic structure of *N. cadamba*. The purity of*Neolamarckiacadamba* varied from 1.60 to 2.04. The range was estimated qualitatively from the ratio between the reading of absorbance at 260 and 280 nm ($OD_{260/280}$) in UV-Vis Spectrophotometer. Quantity of DNA with modified CTAB based method (Doyle & Doyle (1987)) was varied from 1100 to 4740 µg/ml. In present investigation we encountered difficulties from the stage of cell lyses to DNA separation in the supernatant and subsequent reactions when following the procedures described by Doyle & Doyle (1987), Murray and Thompson (1980) and Dellaporta*et al.*, (1983). Modified CTAB based method got clean DNA. DNA from CTAB based methods showed RNA contamination and smear on gel electrophoresis, which means DNA is not really pure. Random primer did not show any amplified band. Because of the DNA isolated by a mentioned method was sticky, viscous and color which inhibited the activity of Taq DNA polymerase enzymes.

**Keywords:***NeolamarcikaCadamba,* Genetic Diversity and Structure, CTAB Method, Taq Polymerase.

**Introduction:** *Neolamarciacadamba* is an evergreen tropical tree native to South and South-east Asia. The genus name honors French naturalist Jean-Baptiste Lamarck. The tree is a medium to large size deciduous tree attaining a height of 20-40m and a girth of about 2-2.5m with clean cylindrical branches and rounded crown. It is frequently found all over the India on the slopes of evergreen forest up to 500m. It is also frequently cultivated for ornament and as a shade tree in plantations throughout the country. It is also found nearer to temple and river. Leaves coriaceous, entire margin, elliptical-oblong or ovate, pulvinate base, with acute apex. Flowers are scented, small, yellow or orange colored with globose heads. Fruit are fleshy, orange, globose, and yellow when ripe. Seeds are small and muriculate. Bark is dark brown, roughish with longitudinal fissures peeling off in thin scales. *Neolamarckiacadamba* is an important medicinal plant. It is used in traditional ayurvedic medicine for the treatment of fever, uterine disease, skin disease dysentery and diabetes. In Ayurvedic medicine the plant is used for uterine complaint, disease, leprosy and for improvement of semen quality and also cure diabetes mellitus, diarrhea, inflammation, heamoptysis, cough, vomiting, wounds, ulcers and debility (Jeyalalitha et al 2015). In traditional system of medicine warm aqueous extract of *N. cadamba* leaves have been used to alleviate the pain, swelling and for cleansing and better wound healing (Chandrashekar and prasanna 2009).

The availability of information on the genetic variation within populations and the differentiation between populations plays a significant role in the formulation of appropriate management strategies for conservation of genetic resources. These markers have been widely used to assess genetic diversity and population structure and require comparatively small amount of DNA (Wolfe *et al.*, 1998, Esselman*et al.*, 1999). Each ISSR primer is composed of not only 2-3 repeats complementary to

microsatellite region of the genome, but also 1-3 additional arbitrary nucleotides at the 5' or 3' end. The latter serve as anchors against reverting strand slip page during amplification (Gupta *et al.,* 1994). Further, the anchoring nucleotides facilitate attachment of primers to specific inter microsatellite regions, resulting in diverse banding patterns from identical repeat sequences with varying anchoring nucleotide(s) (Wolfe *et al.,* 1998). In contrast to other molecular markers, the target sequences for ISSR primers are abundant throughout the eukaryotic genome and evolve rapidly. ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology (Reddy *et al*., 2002). It involves PCR amplification of regions between adjacent, inversely oriented microsatellites using a single simple sequence repeat primer. The use of the tandem repeat motifs of di-, tri-, or tetra nucleotides that are abundant in all eukaryotic genomes produces a high number of polymorphic fragments, especially in plants (Hamada *et al*., 1982). Since it is simple, fast, cost effective, highly discriminate and highly reliable, it is widely applied in plant genetic analysis. The main aims of this articleare (i)Isolation of genomic DNA from *Neolamarckiacadamba*Roxb. and (ii ) Quantification of genomic DNA by using UV-Vis Spectrophotometer and Gel electrophoresis.

***Method:*** **Plant Sample Collection**: Fresh leaves of *Neolamarciacadamba*Roxb. were randomly collected from campus of Indira Priyadarshini college, Chhindwara (M. P.). The leaves were washed with double distilled water. The washed leaves were dried on tissue paper and used for isolation of total genomic DNA.

**Genomic DNA Extraction:** The genomic DNA were extracted by modified Doyle and Doyle (1987) method. About 250 mg fresh leaveswere gently ground into paste with 1 ml CTAB extraction buffer (preheated at 60$^{O}$C for atleast about 15 min.). Equal volume (approx. 1ml) of resultant paste was distributed into 2ml Eppendorf tube and then incubated in water bath at 60$^{O}$C for 1 hour. Samples were retrieved from water bath after incubation and allowed to return to room temperature for 5-10 minutes. An equal volume of Chloroform :Isoamyl alcohol (24 : 1) was added for extraction and mixed gently by inversion for at least 1 minutes. Samples were centrifuged at 12,000 rpm for 3 minutes at room temperature for phase separation then transferred the upper clear aqueous layer to another 2ml tube than again added 500 µl chloroform-isoamyl alcohol (24:1) and centrifuged at 12,000 rpm for 10 min. Again aqueous phase was transferred to new 1.5 ml eppendorf tube along with 0.75 volume chilled isopropanol to precipitate the DNA.

Samples were mixed gently by continuous inversion and kept at room temperature for overnight. The samples were then centrifuged at 12000 rpm for 3 minutes at room temperature. Pellet was taken and washed in 70% ethanol. Air dried until ethanol evaporated completely from sample (this was done by inverting the tubes on tissue paper). The pellet was dissolved in 50µl TE buffer and treated with 3µl RNAse. The samples were incubated for 30 minutes at 37$^{O}$C. Finally resuspended DNA was stored at 4$^{O}$C for further studies.

**Quantification of Genomic DNA:** Quantification of genomic DNA is performed to determine the optimal concentration of DNA in a mixture as well as their purity**.** DNA is quantified by the using of UV-Vis spectrophotometer (Systronics, India) and gel electrophoresis (Takara, japan).

**UV-Vis spectrophotometer:** The genomic DNA extracted was measured by using a UV – VIS spectrophotometer at 260nm and 280nm.The purity of DNA was determine by calculating the ratio of absorbance at 260nm to 280nm.
The Quantity of DNA was determined by the following formula:

$$\text{Quantity of DNA} = OD_{260} \times 50\mu l/ml \times \text{dilution factor}$$

**Gel Electrophoresis:** The extracted DNA were loaded and run on gel electrophoresis unit. Firstly electrophoresis unit were assembled and agarose gel (1%) were prepared using 1X TBE buffer and double distilled water, homogenizing the gel in an micro-oven until it become transparent, then EtBr (0.5µg/µl)were added into the gel and poured the solution into the gel casting tray. After solidification, small amount of gel tank buffer were added and removed the comb from gel casting tray. Added gel

tank buffer (325ml) in electrophoresis unit and gel casting tray were kept. Prepared the DNA sample by mixing 4 µl DNA and 6 µl diluted gel loading dye and finally the DNA sample were loaded into the 1% agarose gel wells and ran the sample at 50V for 40 to 45 min. The DNA bands were viewed under UV transilluminator and subsequently photographed.

***DNA Amplification and Visualization:***

**Setting of PCR Reaction:** The PCR reaction was carried out in a sterile 0.2ml thin walled PCR tubes obtained from AxivaSichem biotech Delhi.Template (genomic) DNA amplification of *Neolamarciacadamba* was carried out in a 50 µl reaction volume containing 2x PCR assay buffer, 200 µM each of dNTPs, 1ul Taq DNA polymerase, 1µM random primer and 2µl genomic DNA in Takara PCR Thermo Cycler (TAKARA Bio INC. japan). ISSR profile consisted of an initial denaturation at 94°C for 3 min followed by 30 cycles each of denaturation for 30 sec. at 94°C, annealing for 30sec at 50°C and extension for 1 min at 72°C followed by a final extension step for 10 min (Zietkiewicz et. al. 1994).At the end of the run, the PCR, tubes were taken out and 1/10$^{th}$ volume (here 2.5 µl) of 10x loading dye (containing 0.025% Bromophenol blue and 40% Sucrose) was added into the PCR product. The amplification products were stored at 4°C till electrophoretic separation.

**Agarose Gel Electrophoresis:** The amplification products for ISSR were separated on 2% agarose gel containing 0.5 µg ml$^{-1}$ ethidium bromide using 1.0x TBE buffer (pH 8.0). Separation was carried out by applying constant voltage of 50 V for 1 h. DNA fragments were visualized under UV transilluminator and subsequently photographed.

**Results:** Recent technological developments have facilitated the assessment of polymorphisms among genotypes at the DNA level, surpassing old techniques like morphological differences or biochemical variations. With the help of molecular markers, fingerprinting of genotypes, genetic fidelity, molecular mapping, marker assisted selection of traits etc. has become possible now. ISSR (Inter-Simple Sequence Repeats) are dominant markers and detect polymorphisms in microsatellite and inter-microsatellite loci and do not require prior information of DNA sequences (Zietkiewicz*et al.,* 1994). These markers have been widely used to assess genetic diversity and population structure and require comparatively small amount of DNA (Wolfe *et al.*, 1998, Esselman*et al.*, 1999). The purity of*Neolamarckiacadamba* varied from 1.60 to 2.04 **(Table 1 and Fig.1).** The best quality is between 1.8-2.0 (Sambrook*et al.*, 1989). The range was estimated qualitatively from the ratio between the reading of absorbency at 260 and 280 nm ($OD_{260/280}$) in UV-Vis Spectrophotometer. Quantity of DNA with modified CTAB based method (Doyle & Doyle (1987)) was varied from 1100 to 4740 µg/ml**(Table-1 and 2).** In present investigation we encountered difficulties from the stage of cell lyses to DNA separation in the supernatant and subsequent reactions when following the procedures described by Doyle & Doyle (1987), Murray and Thompson (1980) and Dellaporta*et al*., (1983).

**Table 1:**Quantity and Quality of Genomic DNA in *NeolamarckiaCadamba*Roxb.

| Samples | $OD_{260}$ | $OD_{280}$ | Quantity($\mu g/ml$) | Quality ratio ($A_{260}/A_{280}$) |
|---|---|---|---|---|
| K-1 | 0.110 | 0.058 | 1100 | 1.89 |
| K-2 | 0.304 | 0.190 | 3040 | 1.6 |
| K-3 | 0.474 | 0.294 | 4740 | 1.61 |
| K-4 | 0.412 | 0.201 | 4120 | 2.04 |

**Table 2:** Visualization of genomic DNA band on Agarose Gel.

| No. of wells | Young leaves | Mature leaves | Type of DNA |
|---|---|---|---|
| 1 | Intense Band | ..... | Slightly yellow |
| 2 | Very Intense Band | ..... | Slightly yellow |
| 3 | ..... | Very very Faint | Intense brown |
| 4 | ..... | Very faint | Brown |
| 5 | ..... | Faint | Brown |
| 6 | DNA Ladder | | |

Modified CTAB based method (Doyle & Doyle (1987) got clean DNA. According to Croy*et al.*, (1993), most of the plants cells had very tough cell wall and may need vigorous method to break the cell. The excessive force makes the degradation of very high molecular weight molecules through the shearing. While the DNA from CTAB based methods showed RNA contamination and smear on gel electrophoresis, which means DNA is not really pure**. (Table 1 and Figure 1).**The DNA contamination can be protein and RNA. The amount of RNase A and protein removable were suggested to accumulate to get pure DNA. There are difficult to get plant DNA free from contaminating proteins and polysaccharides. Different methods need for different plants that contain diverse secondary compounds that interfere with the extraction (Croy*et al.*, 1993). Spectrophotometer measures the intensity of absorbance of DNA solution at 260nm wavelength, and also indicates the presence of protein contaminants but it does not tell the condition of the DNA which is degraded or not (Semagn*et al.*, 2006). Random primer did not show any amplified band. Because of the DNA isolated by a mentioned method was sticky, viscous and color which inhibited the activity of Taq DNA polymerase enzymes.

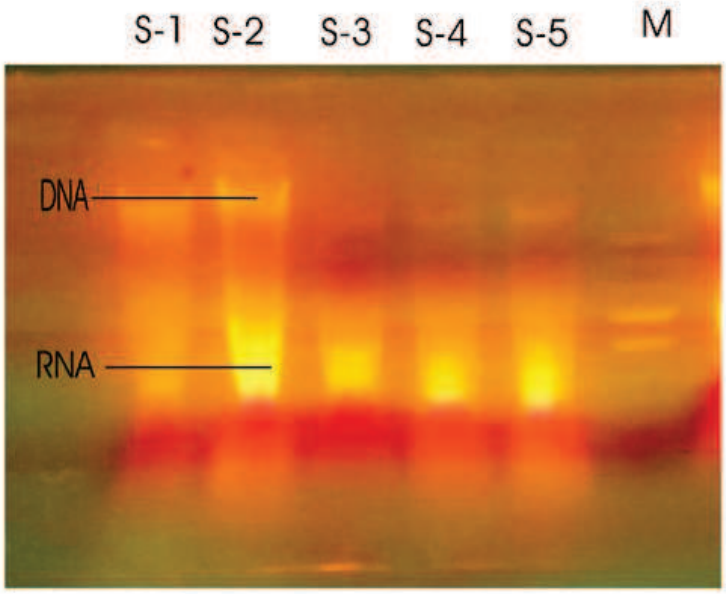


Fig: Genomic DNA extract by modified Doyle - Doyle Method (1987) before RNAse treatments; lane S-1 & S-2 = young leaves, lane S-3, S-4 & S-5= mature leaves, M= 100 bp DNA ladder

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