

Rapid Method for Extraction of Genomic DNA From *Vitex negundo* L.

R. Mary Sujin¹, A. John De Britto^{2*}, R. Mahesh³ and K. Dharmar⁴

^{1,2,3,4} Plant Molecular Biology Research Unit, St. Xavier's College, (Autonomous) Palayamkottai - 627 002, Tamilnadu, India.

*bjohnde@yahoo.co.in (corresponding author). Tel: 0091- 462 4264374, Fax: 0091- 462-2561765.

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ABSTRACT DNA extracted from plants which contains rich amount of polyphenols and or polysaccharides are often problematic when subjected when mature tissues are used for DNA extraction. In order to overcome these problems, we develop the first reliable and efficient method for isolating *vitex negundo* L. genomic DNA that is free from solubilising polysaccharides and polyphenols. This protocol uses NaCl, PVP, mercaptoethanol, SDS and incubation at 68°C for 1 hour. Mild temperature conditions during extraction and precipitation were also recognized as important parameters. The quantity of isolated genomic DNA was confirmed by means of spectrophotometric analysis (260/280=1.6-1.8) routinely yielding 250-500 ng/μl per gram of leaf material and it was proved through RAPD-PCR analysis.

(Keywords: DNA extraction, RAPD-PCR analysis, *Vitex negundo* L.)

INTRODUCTION

Vitex negundo L. (Local Name-Nochi) belongs to the family Verbenaceae. It is a small tree credited with innumerable medicinal activities like anti-cancer, analgesic, anti-inflammatory, anticonvulsant, antioxidant, bronchial relaxant, hepatoprotective, etc. The ethanolic extract of leaves has been found safe as LD50 dose (by oral route) of it was recorded in non-toxic dose range [1].

The roots, fruits, flowers, leaves and bark of *V. negundo* species are used for medicinal purposes like foot and mouth diseases, impaction with fever [2]. Juice extracted from 100gm leaves is consumed to cure Dyspnoea (Shwasam mutt) [3]. Leaves are smoked in headache. Flowering tips are chewed for curing ulcers. Twigs are used as toothbrush. Leaf paste is applied for curing wounds [4].

Isolation of plant nucleic acids for use in PCR, RFLP, RAPD, AP-PCR, DAF and genomic library construction is one of most important and time-consuming steps. The degree of purity and quantity varies among applications. A good extraction procedure for the isolation of DNA should yield adequate and intact DNA of reasonable purity. Dangerous chemicals should not be used, if possible, and the procedure should be simple, inexpensive and quick [5].

The extraction process involves breaking or digestion away cell walls to release the cellular constituents. This is followed by disruption of the cell membranes to release DNA into the extraction buffer. This is normally achieved by using detergents such as sodium

dodecyl sulphate (SDS) or cetyltrimethylammonium bromide (CTAB). This released DNA should be protected from endogenous nuclease. The initial DNA extracts often contain large amounts of RNA, proteins, polysaccharides, tannins and pigments, which may interfere with the extracted DNA and are difficult to separate. Most proteins are removed by means of denaturation and precipitation from the extract with chloroform and /or phenol. Polysaccharides contaminates are particularly problematic [6]. They can also coprecipitate with DNA after alcohol addition during DNA isolation to form highly viscous solutions [7].

Antioxidants are commonly used to address problems related to phenolics. Examples include the use of β-mecaptoethanol, ascorbic acid, bovine serum albumin (BSA), Sodium azide and polyvinylpyrrolidone (PVP) [8] [9]. Phenol extraction coupled with SDS is also helpful. However SDS tends to produce low DNA yields of plants rich in polyphenolics [10].

The optimised protocol described here is specifically designed to isolate genomic DNA of *Vitex negundo* L. and amplification detecting through RAPD-PCR techniques.

MATERIALS AND METHODS

Plant materials

The leaves sample of *Vitex negundo* L. were used to determine the extracting sufficient amount of DNA. The leaves were collected during the summer season (April). The collected leaves were placed in plastic bag and stored in a deep freezer at -70°C.

Reagents and chemicals

- An extraction buffer consisting of 2% SDS, 800 mM NaCl, 25mM Tris/HCl(pH 8.0), 25mM EDTA 2% PVP and 0.8% β -mecaptoethanol was prepared.
- Chlorform-isoamylalcohol (24:1)
- Wash solution: 15mM potassium acetate, 75% ethanol
- TE buffer: 10mM Tris-HCl (pH 8), 1mM EDTA (pH 8)
- 0.5V of isopropanol

DNA extraction protocol

- Plant tissues (200 mg leaf) were quickly frozen in liquid nitrogen, powdered with motor and pestle and transferred into 1000 μ l of extraction buffer.
- The extract was slightly mixed and incubated in a waterbath at 68°C for 1 hour. Then 15mM potassium acetate was added followed by 30 minutes incubation on ice and centrifugation (12000rpm, 10 minutes, 4°C).
- The supernatant was transferred into a new tube, mixed 0.5 vol isopropanol by inverting the tubes three times and incubated on ice for 10 minutes.
- After another centrifugation (12000rpm, 10 minutes, 4°C) the supernatant was discarded completely.
- The pellet was dried under vaccum, dissolved in 20 μ l 1 \times TE and extracted once with 500 μ l of Chlorform-isoamylalcohol (24:1).
- After centrifugation (12000rpm, 10 minutes, 4°C) the aqueous phase was transferred and nucleic acids were precipitated in 0.5V of isopropanol(20°C, 15 minutes).
- During this time the tubes were gently inverted at least ten times. Finally the tubes centrifuged again (12000rpm, 10 minutes, 4°C)
- The pellet was washed in 75% ethanol, dried under vaccum and dissolved in 20 μ l 1 \times TE.
- Store the DNA at -2°C for long-term storage. If needed, treat the DNA solution with RNase before use.

DNA obtained with this technique constantly gives a 260/280 absorbance ratio of 1.6-1.8 indicating high quality intact DNA [11]. All of these DNA were used for RAPD-PCR analysis using the method [12]. Arbitrary

decamer primes successfully amplified DNA fragments from *Vitex negundo* L.

RESULTS AND DISCUSSION

Medicinal plants have large amounts of secondary metabolites. These secondary metabolites make hindrance in DNA isolation and isolated DNA is not suitable for PCR amplification and restriction digestion. We followed the protocol described by [13]. Midiprep method for the isolation of DNA from plants with a high content of polyphenolics.

DNA extracted, however was very viscous and could not be resolved on the agarose gel. The other problems encountered were low DNA yields and poor PCR amplification as well as restriction digestion. We increased the concentration of PVP, β -mercaptoethanol and NaCl and incubation temperature from 65°C to 70°C temperature. These modifications helped in the extraction of DNA of high purity and high quantity. We also eliminated the use of phenol for the purification of extracted DNA, which makes our method less hazardous. We also used 2 μ l of RNase per 20 μ l DNA samples for removal of RNA. The DNA yields and resolution on agarose gel however, were much higher and better from both fresh and dried root samples of medicinal plants with the use of our protocol.

Sufficient amount of highly purified clean genomic DNA was obtained when the optimised protocol was described by us.

All of these DNAs were used for RAPD-PCR analysis. Arbitrary decamer primers successfully amplified DNA fragments from 5 accessions of *Vitex negundo* L. The DNA sample was extracted using this procedure and assayed for RAPD-PCR using the primer OPX-17(CAGACAAGCC) by using a TGradient thermal cycler. Each PCR 25 μ l reaction mixture consisted of 4.0 μ l dNTPs, 0.3 μ l MgCl₂, 2.5 μ l PCR Buffer, 2.4 μ l Primer, 0.5 μ l Taq polymerase, 13.3 μ l Sterile water, 2.0 μ l template DNA. After the initial denaturation 94°C for 5min, 35 PCR cycles were performed with 35 sec at 93°C, 55 sec at 53°C and 45 sec at 72°C,. The DNA extracts as well as the amplification products were run in 2% agarose gels repectively, stained with ethidium bromide and visualized under UV light.

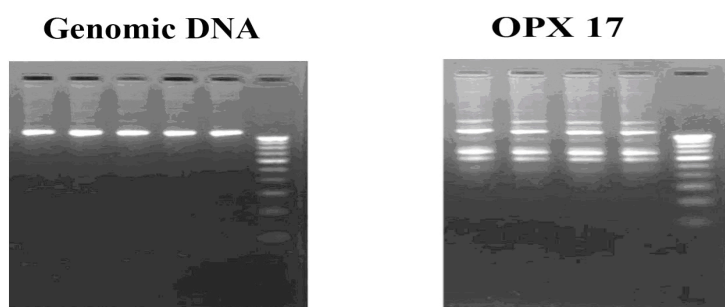
Fig1. shows the results of PCR amplification using the primer OPX 17 of *Vitex negundo* L. This DNA preparation method yielded a prdominance of high molecular wieght DNA reliably produced PCR amplification products. Considering all factors involved, this

method appears to be the most efficient, reliable and labor-effective DNA isolation procedure from plants containing a high content of polyphenolics.

The different protocols are described earlier for isolation of genomic DNA from medicinal plants such as protocol for isolation of genomic DNA from dry and fresh roots of

medicinal plants suitable for RAPD and restriction digestion [14], DNA protocols for plants [15], DNA isolation methods for medicinal and aromatic plants [16] and optimization of DNA isolation and PCR protocol for RAPD analysis of selected medicinal and aromatic plants of conservation concern from Peninsular India [17].

Figure 1: Showing the Genomic DNA and PCR- amplification product using RAPD OPX- 17 primer of *Vitex negundo* L.



This method is a rapid, reliable and cost efficient method for the isolation of the PCR-quality DNA from the *Vitex negundo* L. The DNA solutions are stable at 4°C for at least two months and can be kept frozen at -20°C for longer storage. We anticipate that adoption of this protocol among the vitex species will speed genotyping, the mapping of mutants, transgenic studies and other PCR based applications.

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REFERENCES

1. Vishal Tandon R. (2005). Medicinal uses and biological activities of *Vitex negundo*. *Natural Product Radiance*. 4:162-165.
2. Ambika Nag, Praveen Galav and Kalewa, S.S. (2007). Indigenous animal health care practices from Udaipur District, Rajasthan. *Indian Journal of Traditional Knowledge*. 6(4): 583-588.
3. Arun Vijayan, Liju, V.B. Reena John, J.V. Parthipan, B. and Renuka, C. (2007). Traditional remedies of Kani tribes of Kottoor Reserve forest, Agasthyavanam, Thiruvananthapuram, Kerala. *Indian Journal of Traditional Knowledge*. 6(4): 589-594.
4. Saroj Verma and Chauhan, N.S. (2007). Indigenous medicinal plants knowledge of Kumhar forest division district Solan. *Indian Journal of Traditional Knowledge*. 6 (3):494-497.
5. Daneshwar Puchooa and Sher Ullah Shahnnoo Sulliman Khoyratty (2004). Genomic DNA extraction from *Victoria amazonica*. *Plant Molecular Biology Reporter*. 22: 195a-195j.
6. Scott, K.D. and Playford, J. (1996). DNA extraction technique for PCR in rain forest plant species. *Biotechnology*.s 20:974-978.

7. Do, N. and Adams, R.P.(1991). A simple technique of removing plant polysaccharides contaminants from DNA. *Biotechniques*. 10:162-166.
8. Dawson, C.R. and Magee, R.J. (1995). Plant tyrosinase (polyphenol oxidase) In: Colowick SP and Kaplan NO (eds). *Methods in Enzymology*. 2: 817-827.
9. Clark, M.S. (1997). In: Plant Molecular Biology-A laboratory Manual. Springer-Verlog Berlin Heidelberg, New York. 305-328.
10. Rezaian, M.A. and Krake, L.R. (1987). Nucleic acid extraction and virus detection in grape vine. *J Virol Meth*. 17:277-285.
11. Sambrook, J. and Russel (2000). *Molecular Cloning: A laboratory manual 3rd edn*. Cold Spring Harbor Laboratory Press. New York: 78-85.
12. Williams, J.G.K. Kubelik, A.R. Livak, K.J. and Rafalski, J.A. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*. 18: 6531 - 6535.
13. Uta Pich and Ingo Schubert (1993). Midiprep method for isolation of DNA from plants with a high content of polyphenolics. *Nucleic Acids Research*. 21(14): 3328.
14. Salim Khan, M., Irfan Qureshi, Kamaluddin, Tanweer Alam and Abdin, M. Z. (2007). Protocol for isolation of genomic DNA from dry and fresh roots of medicinal plants suitable for RAPD and restriction digestion. *African Journal of Biotechnology*. 6 (3):175-178.
15. Doyle, J. J. (1991). DNA protocols for plants. in: G. Hewitt, A. W. B.Johnson, and J. P. W. Young (eds.), *Molecular Techniques in Taxonomy*. NATOASI Series H, Cell Biology. 57:283-293.
16. Anna Maria Pirttilä, Merja Hirsikorpi, Terttu Kämäräinen, Laura Jaakola and Anja Hohtola (2001). DNA Isolation Methods for Medicinal and Aromatic Plants. *Plant Molecular Biology Reporter* 19: 273a–f.
17. Padmalatha, K. and Prasad, M.N.V. (2006). Optimization of DNA isolation and PCR protocol for RAPD analysis of selected medicinal and aromatic plants of conservation concern from Peninsular India. *African Journal of Biotechnology*. 5 (3):230-234.