Research Paper

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Genomic Dna Isolation From *Plumbago* L. Species Growing In India

Botany

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ABSTRACT

Plumbago L. is a genus belonging to family plumbaginaceae consists of twelve species of which, three species P. zeylanica L., P. auriculata Lam., and P. rosea L. occur mostly in warm temperate to tropical regions of the India. The genus Plumbago is highly medicinal and has high amount of phenolic content i.e. plumbagin or 2-4 napthoquinone. The present study aims at standardization of DNA isolation protocol from leaves of all the three species i.e. P. zeylanica, P. auriculata, and P. rosea. The DNA isolation was performed by CTAB method without liquid nitrogen and some modifications. The DNA was purified and quantified comparatively in all the three species. The purification absorbance values obtained were 1.76, 1.82, and 1.86 for P. rosea, P. zeylanica and P. auriculata respectively, DNA concentration was calculated as 4.40, 4.55 and 4.65 µg/µl. This DNA was compared with the DNA isolated by standard method of Doyle J.J. and Doyle J.L. (1987). The pure isolated DNA is used for further molecular studies and the focus is on genome characterization of the three species using molecular markers RAPD, RFLP and SSR.

INTRODUCTION

Plumbago namely *P. auriculata* (Linn.), *P. rosea* (Linn.), *P. zeylanica* (Lam.) grown throughout India have long been used traditionally in Indian folk medicine to treat inflammatory disorders such as rheumatoid arthritis and skin disorder such as leucoderma ringworm, scabies, leprosy etc. Among these *P. zeylanica* has been widely assigned for its various medicinal properties and is used in formulations of number of ayurvedic compounds (Kirtikar and Basu 1993). Several commercially important compounds have been isolated from these plants including plumbagin. Plumbagin (5-hydroxyl, 2methyl, 1-4 napthoquinone) is an important napthoquinone (Komaraiah *et al.* 2003).

Intact high molecular weight plant DNA is essential for molecular studies and genomic DNA library construction. Previously, tissues have been homogenized in buffers using a blender, mortar and pestle or glass. These methods are limited because of degradation of DNA by DNases and other nucleases (Sharma and Sharma 1980). Molecular techniques helps researcher not only to identify genotypes but also in assessing and exploiting genetic variability through molecular markers RAPD analysis has proved for estimating genetic diversity particularly to assist in the conservation of rare species and plant genetic resources. RAPD analysis in particular has proven to be a rapid and effective mean of genomic mapping and is well suited for the genetic recourses characterization (Williams et al. 1990).

The problems encountered in the isolation and purification of DNA especially from medicinal and aromatic plants (MAPs) include degradation of DNA due to endonuclease. Isolation of highly viscous polysaccharides, inhibitor compounds like polyphenols and other secondary metabolites which directly and indirectly interfere with enzymatic reactions. Moreover contamination of RNA that participate along with DNA causes many problems including suppression of PCR amplification (Pikkart and Villeponteau 1993), interference with DNA amplification involving random primers e.g. RAPD analysis. Different plant taxa often may not permit optimal DNA yields from one isolation protocol. Thus an efficient protocol for isolation of DNA is required. Various protocols for DNA extraction have been successfully applied to many plant species (Doyle and Doyle (1987); Zeigenhagen (1993).

In most cases of DNA isolation with liquid nitrogen is used to flash freeze tissues followed by grinding the frozen tissues. Liquid nitrogen can be difficult to procure in remote locations thus a method without liquid nitrogen not requiring its use would be helpful (Sharma *et al* 2003) and the same has been worked out in the present study.

Materials and Methods:

Plant Material

The three species *P. zeylanica, P. auriculata,* and *P. rosea* were collected from various nurseries, *Plumbago rosea* was collected from department of Kokan Agriculture University, Dapoli, Dist. Ratnagiri Maharashtra, *Plumbago auriculata* from Forest department at Parbhani and *Plumbago zeylanica* from local nursery at Amravati, Maharashtra. These plants were grown at department of Botany. After acclimatization of the plants 1 g of young leaves harvested fresh for DNA isolation.

Solutions

An extraction buffer consisting of 2% CTAB (W/V), 0.5 M Tris HCl (pH 8.0), 0.5 M EDTA (pH 8.0), 5M NaCl, was prepared Ribonuclease A (10mg/ml); Chloroform: Isoamylalcohol (24:1).

DNA isolation protocol

- Freshly harvested young leaf sample (0.4 g) was washed in distilled water and midrib was removed and ground in ice cold mortar and pestle with small amount of (1 to 2 ml) of extraction buffer.
- While grinding .1gm of PVP was added.
- The pulverized leaves were quickly transferred to 3 ml of freshly prepared prewarmed (65 °C) extraction buffer shaken vigorously by inversion and the tubes were kept for incubation at 65° C for 1hour.
- An equal volume of (5ml) of chloroform: Isoamyl (24:1) was added and mixed gently by inversion.
- The mixture was centrifuged at 10000 rpm for 15 min. at 25 to 30 °C.
- Top aqueous layer was transferred to fresh tube and equal volume of cold isopropanol was added to each tube again centrifuged at 10000 rpm for 15 min. at room temperature.
- Liquid was decanted leaving the pellet attached to the wall of tube.
- The pellet was washed with 70% ethanol (4 °C), air dried for 5 min. at room temperature
- Resuspended in TE buffer (2ml) and 5 µl of RNase was added and incubated over night at 37° C (An overnight RNAse treatment helped achieving in proper genomic DNA).

Amount and purity of DNA

The yield of DNA per gram of leaf tissue extracted was measured using a UV-VIS spectrophotometer (Cintra 5, GBC Scientific, and Australia) at λ260 nm. The purity of DNA was determined by calculating the ratio of absorbance at λ260 nm to that of λ280 nm. DNA concentration and purity was also determined by running the samples on 0.8% agarose gel based on the intensities of band along with the Lambda DNA marker (used to determine the concentration). The nucleic acid concentration was calculated following (Sambrook *et al.* 1989).

DNA Quantification by Specrophotometric method
The quantification of DNA was done by recording its absorbance at λ260 nm to λ280 nm using a UV- VIS Spectrophotometer in the following steps:

i. 200 µl TE buffer was taken in a cuvette and spectrophotometer was calibrated at λ260 nm to λ280 nm wavelengths.5 µl of DNA mixed in 1500 µl of TE buffer properly and the absorbance (A) at λ260 nm to that of λ280 nm was recorded.
ii. DNA concentration was calculated by employing the following formula:

$$\text{Amount of DNA } (\mu g / \mu l) = \frac{A_{260} \times 50 \times \text{dilution factor}}{1000}$$

iii. Quality of DNA was judged from the ratio of values recorded at λ260 nm and λ280 nm.

DNA Quantification by Agarose gel method
0.8 % agarose gel was prepared (.5µg/ml of ethidium bromide was added).The gel was submerged in running buffer, 5µl gel loading dye and 20µl DNA Sample was loaded to the wells 50V was applied to electrophoresis apparatus till the dye migrated one third of the distance in the gel DNA can be visualized using a UV transilluminator and quantified in comparison with standards

Results:
It was observed that DNA extraction from young leaves of *Plumbago* species was improved by modifying some steps in original CTAB method of Doyle (1987). DNA can be isolated without using liquid nitrogen. DNA was isolated from three species of *Plumbago* (Fig.1) which was of high quality low polysaccharides. The quality and quantity of the DNA was checked by running on 0.8% agarose gel in electrophoretic unit and viewed under

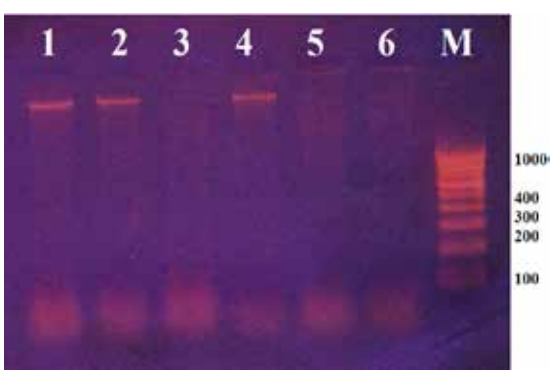

Fig 1: Genome DNA isolated from leaves of different *Plumbago* species at room temperature
M Markers,
Modified Method:
1 *Plumbago rosea,*
3 *Plumbago zeylanica,*
5 *Plumbago auriculata*
Standard Method:
2 *Plumbago rosea,*
4 *Plumbago zeylanica,*
6 *Plumbago auriculata.*
UV transilluminator with ethidium bromide staining.

In the present study selective precipitants (Extraction buffer, PVP etc) were added to remove the contaminants one by one and yield pure DNA, modification in centrifugation speed and time was also found effective in the yield of DNA. The extracted DNA was of high quality as it ranging from 1.76 to 1.96 in all the species of *Plumbago* and the obtained yield ranges from 4.40 - 4.65 µg/µl (Table 1). We can't found any change between standard and modified method.

Table 1: DNA obtained from different species of *Plumbago* L.

Species	OD λ260/λ280 modified method	OD λ260/λ280 standard method	DNA Concentration By modified method		DNA Concentration By standard method	
			µg/µL	µg/gm	µg/µL	µg/gm
Plumbago rosea	1.76	1.83	4.40	440	4.57	457
Plumbago zeylanica	1.82	1.94	4.55	455	4.85	485
Plumbago auriculata	1.86	1.96	4.65	465	4.90	490

Discussion:
Various types of plant materials and protocols for DNA isolation was tested to obtain excellent quality of DNA for PCR reactions. In the present study DNA was isolated by CTAB method without using liquid nitrogen and the results were good when compared to the Doyle method.

We successfully isolated DNA from Plumbago species. The RNA free DNA produced was from 44mg/gm, 45mg/gm, and 46mg/gm of fresh leaves of *Plumbago rosea*, *Plumbago zeylanica and Plumbago auriculata* respectively.

Molecular studies in medicinal and aromatic plant are rare in comparison with other cultivated plants due to presence of large amount of secondary metabolites and extracted oils which inhibit DNA isolation and ultimately PCR reactions (Khanuja *et al.* 1999).

The aim of the present study was to develop an efficient protocol for DNA and optimize PCR reaction further. The present optimized protocol for DNA isolation can serve as an efficient tool for the further molecular studies.

Conclusion:
We conclude that DNA can be easily extracted from leaves tissue without liquid nitrogen in good quality and quantity from *Plumbago* leaves.

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