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Method for quality DNA isolation from different endangered Indian medicinal plants

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Isolation of better quality DNA from endangered Indian medicinal plants such as *Rauvolfia serpentina* (Sarpagandha), *Rhaphidophora decursiva (*Atukulatiga*), Piper longum* (Pippali, Pippallu*), Entada pursaetha* (Gilateega*),* and *Zanthoxylum Rhesta* (Rhesta manu) is very much needed to further genomic study, because these are having rich source of secondary metabolites like cellulose, polyphenolic compounds, tannins, and other metabolites. The presence of certain metabolites can hamper the DNA isolation. In this study we are describing the finest method for isolation of superior quality DNA from selected medicinal plant materials by changing the composition of extraction buffer.

Key words: Indian medicinal plants, DNA Isolation, CTAB, quantification.

INTRODUCTION

According to IUCN 2014 red list (www.iucnredlist.org), most of Indian medicinal plants are prone to be extinct for their intensive collection and threat factors. Over utilization of medicinal plants from non-managed natural resources has not only resulted in shortage of various plants, but extinction of several species in nature (Sangita et al., 2011). The selected medicinal plants like *R. serpentina* (Sarpagandha), *R. decursiva (*Atukulatiga*), P. longum* (Pippali, Pippallu*), E. pursaetha* (Gilateega) and *Z. Rhesta* (Rhesta manu) are placed in critically endangered category. Most of these endangered medicinal plant species are available in Maredumilli forest area. It Is one of the richest diversity area in the Eastern Ghats located in East Godavari district Andhra Pradesh state, India.

A number of Indian medicinal plant species generate various secondary metabolites like alkaloids, flavanoids, phenols, gummy polysaccharides, terpenes and guinones are used by the various pharmaceutical, cosmetic, and food industries (Ortega-Ramirez, 2014; Pushpam, 2004). Many of these medicinal and aromatic plants are valued natural possessions, unintended development as well as tissue culture growth. In the genetic improvement methodologies, screening and selection of molecular markers is very much essential for choosing parents and selection of progeny. The presence of certain metabolites can hamper getting good quality DNA isolation. Many regular protocols existing for isolation of DNA from plants but they do not work consistently in plant tissues that are rich in polysaccharides and phenolic compounds.

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These impurities can directly disturb the quality as well as the magnitude of nucleic acid isolated. Polysaccharide often interferes with downstream uses, thus making the nucleic acid unstable (Khan, 2004). Since different plants contain different amounts of nucleic acids, a particular genetic material separation method is not likely to be suitable for all plants (Loomis, 1974), plant parts such as seed, seedling, young or old leaves of a particular plant. Therefore, the composition of plant tissues in different species is expected to change significantly and a particular isolation protocol is unlikely to be equally effective for all species (Weishing, 1995). In this study we are slightly changing the composition of extraction buffer for isolation of various plant DNAs.

MATERIALS AND METHODS

Plant material

We collected five different Indian medicinal plant species, that is, *R. serpentine*, *R. Decursiva, P. longum, E. pursaetha* and *Z. Rhesta* from Maredumilli forest area located in East Godavari district, Andhra Pradesh, India. The total plant species were carefully transferred from forest area to the laboratory with properly maintained environmental conditions as well as soil conditions to avoid cross contamination.

Solutions and reagents

Method I (Extraction of buffer composition)

Cetyltrimethylammonium bromide (CTAB), EDTA, TrisHCl, NaCL, Mercaptoethanol (ME), 24:1 (v/v) Chloroform/Isoamylalcohol (CI), Isopropanol, TE buffer, 70% Ethanol, DNase free RNase, mortar and pestle and eppendorfs.

Method II (Extraction of buffer composition)

Sodium Dodecyl Sulphate (SDS), EDTA, TrisHCl, NaCL, 24:1(v/v) Chloroform/Isoamylalcohol (CI), Isopropanol, TE buffer, 70% Ethanol, DNase free RNase, mortar and pestle and eppendorfs (Edwards, 1997).

DNA Extraction protocol

Method 1

Weigh about (0.5 to 1.0 g) of plant material (leaves, seeds, roots, stem and flowers) and wash with 30% ethanol for 5 min, then suspend in distilled water. Cut the leaf into small pieces with sterile scissors. Grind it in a clean mortar and pestle. Add CTAB buffer and grind for some more time gently. Pour the mix into centrifuge tubes and incubate in a water bath at 60°C for 1 h. After slight cooling, centrifuge the mixture at 2000 rpm for 20 min to remove the cell debris. Supernatant is taken. An equal volume of chloroform: Isoamylalcohol (24:1) was added and mixed properly by inversion and centrifuged at 8000 rpm for 10 min to separate the phases (long term mixing of samples in Chloroform: Isoamylalcohol approximately for 30 min, will help in removal of pigments and formation of brownish colour in DNA sample can be omitted). The supernatant was carefully decanted and transferred to a new tube and was precipitated with equal volume of chilled isopropanol, gently mixed and centrifuged at 8000 rpm for 10 min. The pellet was washed with 70% ethanol; air dried and resuspended in 30 µl of TE buffer and 2 µl of RNase was added and incubated at 55°C for 30 min.

Method II

The tissue is macerated in the motor and pestle. Extraction buffer was added and the contents were transferred to an eppendorf tube. The extracts are vortexed for 5 sec and left at room temperature for 2 min. The sample is centrifuged at 13,000 rpm for 3 min and supernatant is transferred to a fresh eppendorf tube. To this supernatant, add equal volumes of isopropanol, vortexed and left at room temperature for 2 min. The mixture is again centrifuged at 13,000 rpm for 5 min, and the supernatant is discarded. To the pellet add 70% ethanol and centrifuge at 2000 rpm for 5 min to precipitate the DNA, then the supernatant is discarded and the pellet is air dried to remove traces of ethanol. The air dried pellet is resuspended in 100 µl of TE buffer and 2 µl of RNase was added and the contents are vortexed for 5 sec and incubated at 55°C for overnight and stored at -20°C.

Quantification of DNA

Two methods were carried out for DNA quantification:

(a) UV spectrophotometric method: The OD values of the isolated DNA were measured at 260 and 280 nm wavelength to calculate the quality and quantity of DNA samples.
(b) Gel electrophoresis: The DNA samples along with loading dye were run through 0.8% agarose gel at 50 to 100 volts power supply. The gel was visualized using Gel doc and the results were documented.

RESULTS AND DISCUSSION

In any molecular biology work, the quality of DNA is more important than its quantity. But isolation of quality DNA from different medicinal plants is very difficult because of the occurrence of polysaccharides and other metabolites. In this work, we have applied two methods cited here as Method I (General CTAB method) and Method II (Modified CTAB method). Five types of plant materials (seeds, young leaf, stem, roots and flowers) from five different Indian medicinal plant species as mentioned above were used. The basic variation between the two methods is method I (CTAB) was used and in the other it was excluded. In method I also, we have not added SDS component, but in method II, SDS was added in place of CTAB. By changing this modification we have successfully extracted DNA isolation in selected Indian medicinal plant species materials (Table 1). After successful completion of DNA isolation, we have gone for quantity and quality analysis of isolated DNA by using spectrophotometric analysis as well as gel electrophoresis.

Table 1. DNA quantification analysis (Method I).

Name of plant species	Part of plant	Optical density at 260/280	Total DNA(µg/g of tissue)
Rauvolfia serpentina	Leaves	2.02	52.52
	Flowers	1.72	44.72
	Roots	0.7	18.2
	Stem	1.3	33.8
Piper longum	Leaves	2.50	65
	Seeds	No DNA	No DNA
Rhaphidophora decursiva	Leaves	1.4	36.4
	Stem	1.02	26.52
	Roots	0.75	19.5
Entada pursaetha	Leaves	1.0	13
	Seeds	No DNA	No DNA
Zanthoxylum rhesta	Leaves	2.2	28.6
	Stem	1.02	13.26
	Root	1.72	22.36

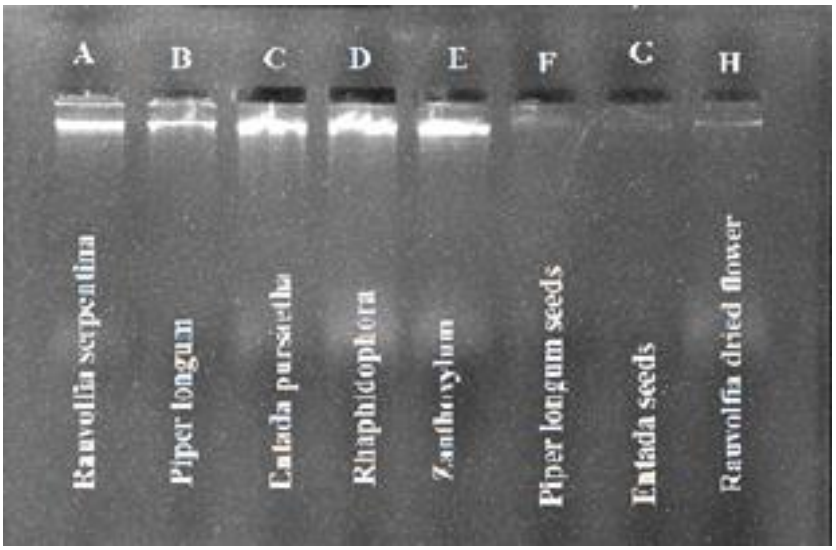

Figure 1. 1% agarose gel showing DNA product in five plant species.

The ratio of the optical density (OD) in UV spectrophotometer at 260 to 280 nm ranged from 0.7 to 2.5 and it's interesting to note that no DNA was found from the seed samples of the two species. It was assumed that the CTAB extraction buffer failed to disrupt the cell walls of the compact seed tissue to release DNA. The ratio of the DNA isolated from seeds and stems was poor in quality, which was also reflected on the gel (Figure 1). After failure of quality DNA from some materials of plant species, we have just modified isolation of DNA protocol, that is, we detached Cetyltrimethylammonium bromide (CTAB) and Mercaptoethanol components. In place of these two components, we added sodium dodecyl sulfate as

Table 2. DNA quantification analysis (Method II).

Name of plant species	Part of plant	Optical density at 260/280	Total DNA(µg/g of tissue)
Rauvolfia serpentina	Leaves	2.5	65
	Flowers	1.9	49.4
	Roots	0.9	23.4
	Stem	1.4	36.4
Piper longum	Leaves	2.0	52
	Seeds	0.7	15.2
Rhaphidophora decursiva	Leaves	1.8	46.8
	Stem	1.2	31.2
	Roots	1.6	41.6
Entada pursaetha	Leaves	1.4	33.8
	Seeds	1.1	22.4
Zanthoxylum rhesta	Leaves	2.4	31.2
	Stem	1.43	18.59
	Root	1.90	24.7

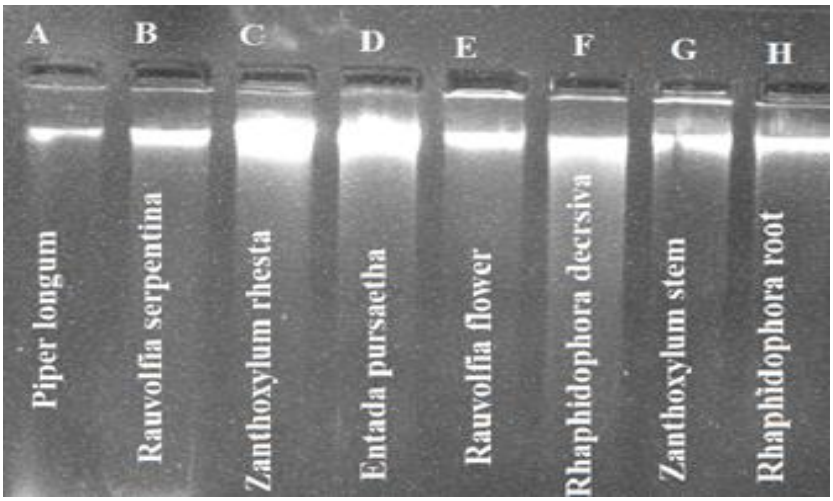

Figure 2. 1% agarose gel showing DNA product in five plant species.

incubation component because it is a strong anionic detergent; it removes the negative ions from the proteins and destroys its conformation. Because of loss of conformation, the protein loses its structure. The proteins from the cell membrane get damaged and the cell gets broken. In Method II, all the parts of selected Indian medicinal plants seem good in quality; besides the quality analysis is also positive result from method II (Table 2). The ratio of OD 260 and 280 nm ranged from 0.9 to 2.5, indicating a wide range of quality. These values were very close to the ratio 1:8 indicating that it was pure DNA. The quality and quantity of the isolated DNA was also reflected on agarose gel (Figure 2).

Conclusion

Proteins associated with the isolated DNA could not be

removed with modified CTAB method. The presence of such proteins may have arrested the movement rate decreasing the quality of the isolated DNA. The yield of DNA ranged from 13.0 to 52.5µg/g using method-I. The quantity of DNA is also dependent on the amount of grinded tissue harvested and collection of the supernatant. The yield of DNA ranged from 18 to 65µg/g using method-II. From the study, it may be concluded that Method-II is suitable for isolating DNA in selected Indian medicinal plants and it's effective for all samples.

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Conflicts of interest

Authors have none to declare.

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