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Method for the Isolation of Genomic DNA from Medicinal Plants Producing Large Amount of Secondary Metabolites

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---------- **Abstract** ----------
The isolation of genomic DNA from various plant samples is the crucial and most important initial step. Isolation of genomic DNA is very difficult especially from those plant materials that produce a large amount of secondary metabolites. Many times even if genomic DNA gets isolated, either the concentration is very low or further downstream processes does not work due to the presence of impurities (either in the form of complex reagents used during isolation or the alkaloids, proteins, carbohydrates and other secondary metabolites) those have not been removed completely during isolation. Hence, a simple and rapid method to get a good quality and quantity of genomic DNA from the medicinal plants is needed. Various genomic DNA isolation methods like Dellaporta et al (1983), CTAB (Murray and Thompson, 1980), HiPurA plant genomic DNA isolation and purification miniprep spin kit and Khanuja et al (1999) were tried and Khanuja et al (1999) method is found to be the well suited to most of the medicinal plants, however DNA isolation was not achieved in a satisfied way from the following medicinal plants such as Azadirachta indica A.Juss.(Meliaceae), Ricinus communis L.(Euphorbiaceae), Butea monosperma(Lam.)Taub. Var. Monosperma, Vigna radiata(L.), Desmodium gangeticum (L.)DC (Fabaceae), Xanthium indicum Koen. (Asteraceae), and Cucumis melo L Var. magrestis (Cucurbitaceae). The present paper deals with extraction and isolation of pure genomic DNA samples from these medicinal plants by modification of Khanuja et al (1999) method and further downstream processes like PCR amplification.

KEY WORDS: Genomic DNA isolation, medicinal plants, secondary metabolites, PCR amplification, Restriction digestion assay, RAPD analysis.

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1. Introduction

The extraction of the genomic DNA from any plant sample (leaves) needs the basic requirement that is first to break the cell to release all cellular constituents like DNA, RNA, polysaccharides, carbohydrates, enzymes, etc. The cell membrane can be ruptured by detergents like SDS or CTAB. EDTA is used as a chelator of most metal ions requires for cellular nucleases activity. Most proteins are removed by chloroform extraction while the polysaccharides are removed by salt (NaCl, KCl or NaAc) together with detergent (Murray and Thompson, 1980; Paterson et al, 1993). The RNA can be degraded by Rnase enzyme. Various genomic DNA isolation methods like Dellaporta et al (1983), CTAB (Murray and Thompson, 1980), HiPurA genomic DNA isolation kits and Khanuja et al (1999) methods were tried and Khanuja et al (1999) method is the well suited to most of the medicinal plants. Further improvement of Khanuja et al (1999) method was developed by modification of few steps in the genomic DNA isolation protocol to get not only the good quantity and quality of the genomic DNA which is used for further downstream processes like Polymerase Chain Reaction etc.
The age of the leaf also affects the quality of extracted DNA (Moreira and Oliveira, 2011). The young leaves yield a good quality and quantity of DNA with fewer impurities (secondary metabolites etc.). It was found that the young, frozen leaves yield good quality of DNA.

2. Materials And Methods

All the existing genomic DNA isolation methods (Dellaporta, CTAB, HiPurA gDNA isolation kits and Khanuja) have been tried for 65 medicinal plants, however, the results for selected seven medicinal plants obtained from the modified Khanuja method are discussed here as follows.

3. Plant Samples For Isolation Of DNA

Azadirachta indica A.Juss.(Meliaceae), *Ricinus communis* L.(Euphorbiaceae), *Butea monosperma*(Lam.)Taub. Var. Monosperma, *Vigna radiate* (L.), *Desmodium gangeticum* (L.)DC (Fabaceae), *Xanthium indicum* Koen. (Asteraceae), and *Cucumis melo* L Var. magrestis (Cucurbitaceae), collected from the RTMNU Campus were selected for current study. Only the young leaves (1g) of the plants were collected, sterilised them with 70% ethanol, washed with double distilled water and kept them at -20^{0}C for 30 minutes before grinding. The DNA extraction buffer (Suman et al, 1999) was kept at 65^{0}C in water bath for half an hour and added immediately to finely grinded leaves preparation (1ml). Mixed with pipette and the solution was kept at 65^{0}C water bath for 30 mins. Allowed to cool at room temperature and added equal amount of chloroform: Isoamyl alcohol (24:1), mixed by inversion and centrifuged at 12000rpm / 10 mins. Collected the aqueous layer in fresh tube, added equal amount of Chloroform: Isoamyl alcohol mixed by inversion and centrifuged at 12000rpm /10 mins. Collected the aqueous layer in fresh tube, DNA was precipitated by adding 1/5th volume of ice cold isopropanol and 0.6 volume of sodium acetate (pH 5.2). Mixed well and kept at -20^{0}C for 30 mins, centrifuged at 12000rpm/10mins and discarded the supernatant. The pellet was washed with 80% ethanol twice. Air dried the pellet completely and dissolved in 500µl TE buffer. 3µl Rnase was added and incubated at 37^{0}C for 30 mins and extracted with equal amount of chloroform: Isoamyl alcohol. Transferred the aqueous layer in fresh tube and added 2 volumes of ice cold ethanol, centrifuged 10000rpm/10mins and washed the pellet with 80% ethanol. Dried and dissolved in 100µl TE.

4. PCR Amplification:

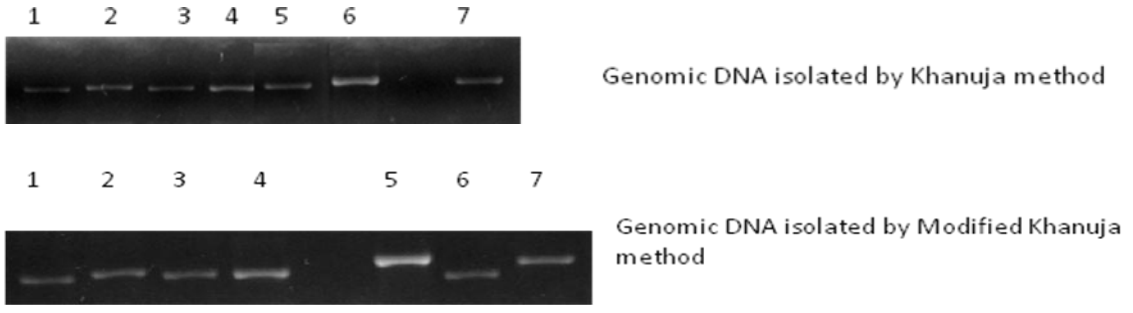
The polymerase chain reaction was carried out in 50µl reaction, containing 50ng of DNA template, 5µl 10X buffer, 3µl 25mM MgCl2, 4µl 10mM dNTPs, 10pmoles of primers and 3 units/µl of Taq polymerase (Eppendorff). The universal ITS primers were used (Sigma) with Forward (5'-GGAAGGAGAAGTCGTAACAAGG-3') and Reverse (5'-TCCTCCGCTTATTGATATGC-3').

Results and discussions

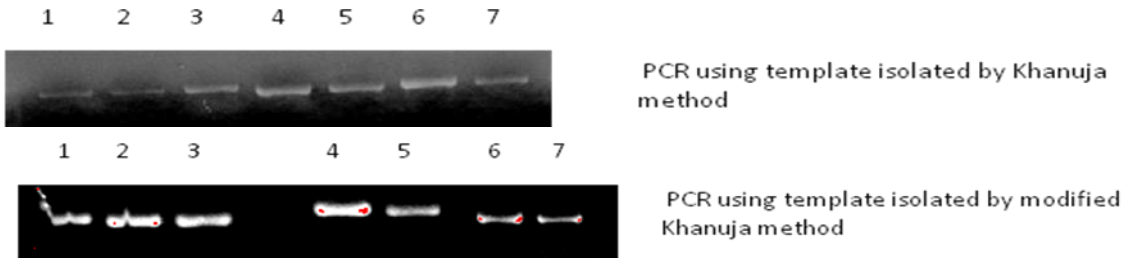
The genomic DNA was isolated by current protocol, which is a modification of Khanuja et al (1999). These DNA samples were measured in UV spectrophotometer (Sican 2301) at 230, 260 and 280 nm, respectively and the values of 260/230 and 260/280 ratios for respective plants were Azadirachta indica A.Juss.(2.00 and 3.27), Ricinus communis L.(2.38 and 2.18), Butea monosperma(Lam.)Taub. Var. Monosperma (2.68 and 1.80), Vigna radiata(L.) (2.22 and 3.10), Desmodium gangeticum (L.)DC (3.0 and 2.16), Xanthium indicum Koen. (1.83 and 2.06), and Cucumis melo L Var. Magrestis (2..2 and 3.30), respectively.

Genomic DNA was loaded on 1% Agarose gel stained with Ethidium bromide (Fig.a and b).

1 – Azadirachta indica A.Juss.
2 – Butea monosperma(Lam.)Taub. Var. Monosperma
3 – Ricinus communis L.
4 – Desmodium gangeticum (L.)DC
5 – Vigna radiata(L.)
6 – Xanthium indicum Koen.
7 – Cucumis melo L Var. Magrestis



PCR amplification of ITS region loaded on 2% Agarose gel: The PCR products of seven plant samples following genomic DNA extraction methods by Khanuja *et al* (1999) and current protocol developed by us are shown in following fig.



From the foregoing results, it is clear that the PCR product amplified and bands obtained were of good quantity indicate the DNA extraction done by using sodium acetate for DNA precipitation instead of Sodium chloride and use of TE buffer instead of high salt TE buffer with other modifications in isolation steps gives better results in medicinal plants.

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