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Concurrent Isolation of DNA and RNA in *Myristica fragrans* Houtt.

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ABSTRACT: *Myristica fragrans Houtt or nutmeg is a tropical tree known for its twin spices- nutmeg and mace. Isolation of DNA and RNA from any part of Myristica is pre requisite for any molecular study. In nutmeg, it was found to be tedious and difficult due to the presence of high amounts of polyphenols and secondary metabolites which binds to the nucleic acid and gets co-isolated with them, thereby hindering PCR amplifications. So, attempt was made to standardize a protocol for simultaneous isolation of DNA and RNA free from contamination to minimize the time and resources required to initiate a molecular study of nutmeg.*

Keywords: *Myristica, DNA, RNA, Isolation, Polyphenols*

INTRODUCTION

Myristica fragrans Houtt. is an evergreen tree indigenous to the Moluccas islands, also called spice islands, of Indonesia. The tree is known for its twin spices – nutmeg and mace. Nutmeg is the dried kernel of the tree and mace is the bright red coloured aril covering the seed. Both the spices are of culinary and medicinal value. *Myristica* also has the distinction of being a member of the most archaic existing order of angiosperms.

Isolation of DNA and RNA is the essential pre requisite for understanding any organism at molecular level such as DNA and cDNA library construction, genetic assessment, expression profiling studies and hence need to be isolated efficiently and obtained with purity. Presently literature available on nutmeg genome and molecular biology is very limited. Physio-chemical profiling of nutmeg shows the presence of polysaccharides, lipids, polyphenols and other secondary metabolites (Packiyasothy, 1991). These compounds bind to nucleic acids during their extraction process and are co-isolated along with them. Polyphenols are easily oxidised by the cellular enzymes and the resultant products cause the browning of DNA isolates. Presence of polysacharides makes the nucleic acid isolates viscous and hence it directly or indirectly interferes with the enzymatic reactions. In either case, both DNA and RNA is rendered unsuitable for further molecular analysis (Khanuja *et.al*., 1999). Moreover, RNA is a sensitive molecule *in vitro*. It tends to get easily degraded by RNAse present in the environment. So, it has to be isolated quickly and efficiently for use in molecular studies. (Sambrook *et.al*., 1989). Several protocols and their modifications are available for isolation of total cellular DNA (Murray and Thompson, 1980; Doyle and Doyle, 1990) and RNA from plant tissues (John, 1992). Since the biochemical profiles vary between species, a single universal protocol cannot be designed to isolate DNA and RNA from all sources. Hence, here we modified available DNA and RNA isolation and PCR amplification protocols to suit the profiles of our plant sample *Myristica fragrans* Houtt. So, a protocol has been standardized for concurrent isolation of DNA and RNA of high purity for use in molecular studies in the tropical spice crop *Myristica fragrans*.

MATERIALS AND METHODS

Fully expanded leaves of *Myristica fragrans Houtt*, were collected from field. Extraction buffer was prepared using 3% CTAB, 20mM EDTA, 100mM Tris at pH 8, 2M NaCl. Extraction buffer and 10% CTAB solution were sterilised before use. RNAse free water was strictly used in all solutions and autoclaved for

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double purity. All the chemicals used were of molecular biology grade and from Genei, Bangalore.

1gm of leaf sample was homogenised in liquid nitrogen in a sterile pre-chilled mortar in the presence of 200 mg of Polyvinylpyrrolidone and an equal amount of Sodium metabisulfite and 0.2% β mercaptoethanol. Homogenate was transferred into sterile centrifuge tubes and 7ml of pre warmed 3% CTAB buffer and 5µl of proteinase K was added and gently mixed by inverting the tubes and incubated at 65°C for 30 minutes. Equal volume of chloroform-isoamyl alcohol mixture (24:1) was added and the tubes were centrifuged at 12000 rpm, 4°C for 15 minutes. Supernatant was transferred to a fresh tube and 1/10th volume 10% CTAB with 2M NaCl was added and gently mixed. Again the nucleic acids were purified with equal volume of Chloroform-isoamyl alcohol (24:1) and centrifuged at 12000 rpm, 4°C for 15 minutes. The supernatant was transferred into fresh tubes and an equal volume of isopropanol was added for final precipitation. Tubes were incubated at -20°C for 1 hour to facilitate precipitation of DNA and highly sensitive RNA. The mixture was then pelleted out washed in 70% alcohol. The pellets were air dried and dissolved in 50µl RNAse free water. The vials were stored at -20°C for future use. DNA and RNA could be eluted out from gel to separate them. Together DNA and RNA could also be used for multiplex PCR. The samples were run on 1.8% agarose gels for checking the presence and purity of DNA and RNA

RESULTS AND DISCUSSIONS

The present protocol is a modified version of the protocol given by Doyle and Doyle (1990). The same protocol with RNAse and without RNAse free water can be used for isolation of high molecular weight genomic DNA from Myristica efficiently (Sasidharan, S. *et. al.*, 2013). Presence of polyphenols and polysaccharides in the nutmeg leaf homogenate has been dealt with using PVP which adsorbs and remove these compounds from the homogenate on centrifugation. Also, high ratio of extraction buffer to plant material has been shown to dilute polyphenols and thereby reduce their adverse effects (Couch and Fritz, 1990). Similarly, Polysaccharides render the nucleic acids inactive and insensitive to enzyme treatments by Restriction endonucleases and Taq Polymerases (Demeke and Adams, 1992). Elevated concentrations of CTAB and NaCl were used to effectively precipitate out the polysaccharides (Doyle and Doyle, 1990). The DNA was run on 1.8% agarose to ascertain its purity. (Fig 1)

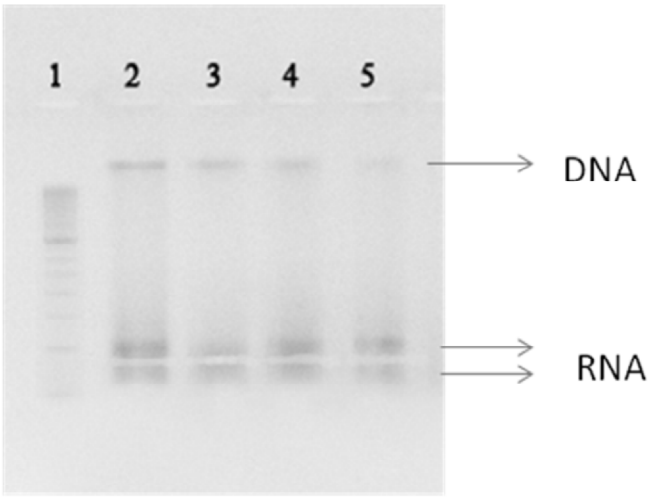

Figure 1: Gel showing DNA and two strands of RNA in lanes 2-5. Lane 1:250 bp Ladder

This protocol has comparatively fewer steps and so exposes RNA and DNA to fewer chemicals. So, RNA could be efficiently isolated for use in expression profiling using micro arrays or cDNA preparation. Since it takes comparatively less time, DNA can be isolated from about 100 samples a day. This protocol is, thus, apt to be used for isolating high quantity of good quality DNA from leaves of *Myristica fragrans* Houtt for amplification, restriction and cloning experiments.

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