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OPTIMIZATION OF AN IMPROVED DNA EXTRACTION TECHNIQUE IN *Melia composita*

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Abstract: The most important primary step in any molecular biological application is extraction of high quality DNA in sufficient quantity. Biochemical composition of each tree species is unique which makes it impossible to develop a universal protocol. An attempt was made to develop an improved DNA extraction technique in *Melia composita*. In this study, a DNA extraction protocol is optimized for the species which does not rely on liquid nitrogen. The standardized protocol yielded a maximum concentration of 851 ng/µl DNA and showed excellent absorbance of 1.853 in A260/280 indicating high quality of DNA.
Keywords: DNA; *Melia composita*; CTAB; liquid nitrogen.

Introduction

Melia composita is a fast growing multipurpose tree species belonging to the family Meliaceae. The species is naturally found throughout the hilly tracts of north east India. Despite the multipurpose uses, potential of this indigenous species is yet to be fully tapped. Any tree improvement work requires information on genetic diversity present in the populations. No systematic study has been carried out so far to assess the genetic diversity of the species using molecular tools in north east India. And for any molecular diversity study, the most important primary step is extraction of high quality DNA in sufficient quantity. Since, biochemical composition of plant species varies from one another it is quite impossible to develop a single isolation protocol that suits every species. It has been found that even closely related species require different protocol (Rawat *et al.,* 2016). Another bottleneck in most of the DNA extraction protocol is use of liquid nitrogen for grinding plant samples to break down the cell walls. Procurement and storage of liquid nitrogen may be difficult and expensive for many laboratories for transportation and handling. Thus, a method not requiring use of liquid nitrogen that results in DNA of reasonable purity would be helpful to the researchers (Dhakshanamoorthy *et al.,* 2013). Although protocols are available for DNA isolation in *M. composita* and other closely related members of meliaceae family, all these protocols rely on liquid nitrogen. (Rawat *et al.,* 2016, Johar *et al.,* 2015, Rind *et al.,* 2016).

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Therefore, a protocol is required in this species which yield good quality DNA in sufficient amount without using liquid nitrogen. Here, we describe an optimization of a DNA extraction protocol that does not require liquid nitrogen.

Materials and methods:

Plant Sample: Young leaves were collected for DNA isolation from plantation at the campus of Rain Forest Research Institute, Jorhat and stored in −80◦C until use.

Extraction Methods: Though different protocols for isolating DNA without using liquid nitrogen are available, their suitability for *M. composita* has not been assessed. Therefore, five DNA extraction protocols reported in different tree species that does not rely on liquid nitrogen were tested along with a modified CTAB protocol. The protocols reported by Ferdous *et al.,* (2012), Dhakshanamoorthy *et al.,* (2013), Agbagwa *et al.,* (2012), Agrawal *et al.,* (2016), Sahu *et al.,* (2012) were initially assessed. And based on the results of exisiting protocol a modified CTAB prorocol has been optimized which is described below:

i. Preheat the suspension buffer containing (0.5M sucrose, 120mM Tris-HCl, 1.7M NaCl, 50mM EDTA di-sodium salt) and extraction buffers containing (2% CTAB, 100mM Tris-HCl, 1.7M NaCl, 20mM EDTA di-sodium salt) in water bath at 60°C for 60 minutes.

ii. 100mg of (-80°C stored) sample grind in presence of 25mg PVP in pre chilled mortar pestle.

iii. Transfer the sample in to 2ml microcentrifuge tube.

iv. Add 500µl of suspension buffer and 50µl of β mercaptoethanol.

v. Invert and mix gently and incubate the tubes in 65°C for 60 minutes.

vi. Centrifuge the tubes in 10,000 RPM for 10 minutes in room temperature.

vii. Transfer supernatants to another microcentrifuge tube.

viii. Add 500µl of extraction buffer into the microcentrifuge tube and incubate the tubes in 65°C for 30 minutes.

ix. Add double volume of chloroform: isoamyl alcohol (24:1) and mix by inverting 15 - 20 times.

x. Centrifuge the tubes in 12,000 RPM for 10 minutes in room temperature. Carefully collect the aqueous phase and transfer to a new tube.

xi. Add 20 µl of RNase (20 mg/ml) to the tubes and incubate at 37˚C for an hour. 20 µl Proteinase K (20 mg/ml) treatments also give for the same time.

xii. Add equal volume of chloroform: isoamyl alcohol (24:1) and mix by inverting 15 - 20 times.

xiii. Centrifuge the tubes in 12,000 RPM for 10 minutes in room temperature and supernatant collect to a new tube.

xiv. Add 1/4th volume of 5M NaCl and equal volume of chilled isopropanol. Incubate the tubes in -20° c for overnight.

xv. Centrifuge the tubes in 12,000 RPM for 10 minutes. Then remove the supernatants from the tube and wash pellet with 70% ethanol 3 times. Air dry the pellets for 1-2 hours, then dissolve in TE buffer and store in -20° C.

Quantitative and Qualitative Analysis of extracted DNA: The quality and quantity of extracted DNA was checked using Myspec micro-volume spectrophotometer measuring wavelength absorbance ratio 260/280nm and 260/230nm.Further quality and yield assessments of the extracted DNA was also checked on 0.8% (w/v) agarose gel electrophoresis.

Results

Five different DNA extraction methods reported by Ferdous *et al.,* (2012), Dhakshanamoorthy *et al.,* (2013), Agbagwa *et al.,* (2012), Agrawal *et al.,* (2016) and Sahu *et al.* (2012) were assessed. The protocol of Ferdous *et al.* (2012) yielded DNA concentration of 631.797ng/µl. The 260/280nm value was 2.132 which indicates high RNA contamination and in 260/230nm it was 1.809. Electrophoresis in 0.8% agarose gel also revealed shearing bands (Fig. 1, Lane: 1-2) with high contamination of RNA. Therefore, this protocol was not considered to be an effective one. The protocol of Dhakshanamoorthy *et al*. (2013) resulted low amount of genomic DNA concentration of 236.328 ng/µl (Fig. 1, Lane: 3-4). The absorbance at 260/280nm was 1.952 and 260/230nm was 1.517 which indicates contamination of some substances such as carbohydrates, peptides, phenols etc. Agbagwa *et al*. (2012) protocol was tested which was a time consuming protocol and used phenol in chloroform: isoamyl alcohol extraction step. In this protocol, the concentration of genomic DNA was 210.499 ng/µl. The absorbance at wavelength ratio 260/280nm was 1.676 which indicate sub standard quality DNA and in 260/230nm it was 2.450 (Fig. 1, Lane: 5-6). Another protocol from Agrawal *et al,* (2016) has also been tested which yielded very low amount of genomic DNA. The concentration was 13.982ng/µl. The 260/280nm was 2.007 and in 260/230nm it was 0.321. No DNA band was noticed in the 0.8% agarose gel (Fig. 1, Lane: 7-8) and the protocol was not found suitable. Lastly, protocol described by Sahu *et al.* (2012) showed DNA concentration of 142.438ng/µl. The absorbance showed in the wavelength ratio 260/280nm was 1.862 and in 260/230nm 1.995. The results of this method

showed a reasonable quality DNA in the Spectrophotometric results. However as the yield was low which was revealed in gel (Fig. 1, Lane: 5).

Among the five reported protocol the protocol reported by Sahu *et al* (2012) yielded good quality DNA however, the quantity of DNA was low. Based on the results protocol of Sahu et al was further modified for optimization. The DNA isolated using the optimized protocol resulted high DNA yield of 851.464ng/µl and showed excellent absorbance of 1.853 in A260/280 indicating high quality (Fig. 1, Lane: 9-12).

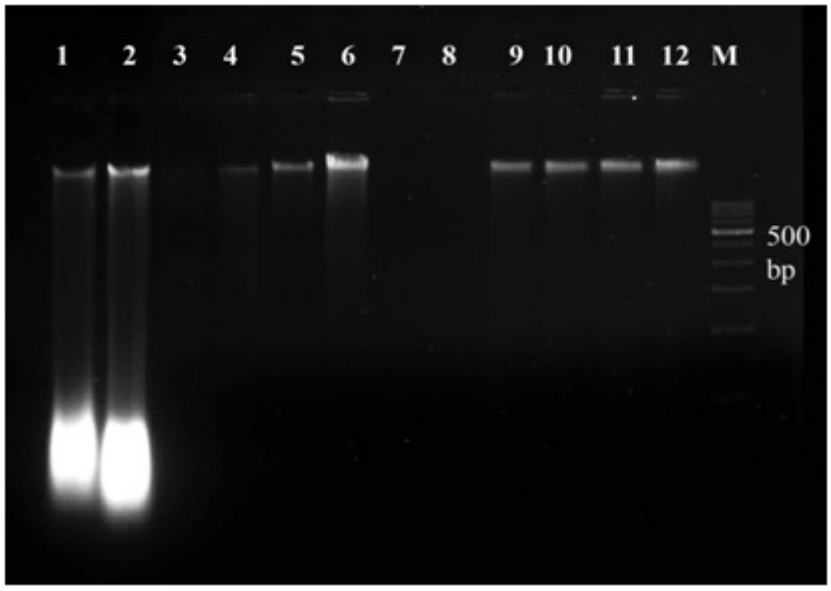

Figure 1: Genomic DNA isolated from *M. composita* leaves (Lane 1 to 12) resolved under 0.8% agarose

Discussion

The different reported protocols tested in this experiment yielded either insufficient or poor quality DNA as evident by 260/280nm and 260/230nm ratios. Further, visualization under gel doc system also revealed sheared and light band in some protocols. The presence of dark brown colour pellet were observed in some of the protocol indicating contamination of polyphenols. The method described by Sahu *et al.* (2012) yielded a good quality DNA; however the DNA concentration was too low. Further, the procedure was also time consuming.

The concentration of NaCl, higher than 0.5M is known to precipitate polysaccharides during DNA extraction and addition of PVP prevents interaction of phenolic compounds with DNA. Moreover, β-Mercaptoethanol is an antioxidant that precipitates polyphenolics and tannins. Changing the concentration of NaCl from 0.5M to 1.7M with higher concentration of PVP

and β-mercaptoethanol in extraction buffers helped improve in yield and quality of DNA. Further, 5 M NaCl was added along with chilled isoporpanol as described by Rawat *et. al.,* (2016) for precipitation of the DNA pellet.

Again, a short time consuming protocol is always desirable. Therefore, some steps in the protocol described by Sahu *et al* were shortened by adding RNase and Proteinase K step directly after first chloroform: isoamylalcohol extraction step.

Conclusion

In this study, a short time consuming, inexpensive DNA extraction protocol was standardized for *M. composita*, which will help in the extraction of high quality and quantity DNA from leaves containing high content of polyphenolics and secondary metabolites. Extracted DNA yielded high concentration as well as quality DNA, which may be useful for further molecular studies in *M. composita.*

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