**Bulletin of Environment, Pharmacology and Life Sciences**
Bull. Env. Pharmacol. Life Sci., Vol 8 [2] January 2019 : 101-105

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95


**ORIGINAL ARTICLE** **OPEN ACCESS**

# Extraction Of High Quality Genomic DNA From Mango (*Mangifera indica* Linn.) Genotypes

**A. Himabindu*, D. Srihari, M. Rajasekhar, V. Sudhavani, P. Subbarammamma and K. Uma Krishna**
Horticultural Research Station, Dr. Y.S.R Horticultural University, Venkataramannagudem, West Godavari, Andhra Pradesh – 534101
Email: himabindu291188@gmail.com

**ABSTRACT**
*High quality genomic DNA is the basic requirement for genome analysis in plant tissues. DNA extraction in mango (Mangifera indica* Linn*.) is difficult because of the high polyphenols, tannins and polysaccharides present in its leaf tissues. Therefore, a modified protocol is reported here, which is an improvement over the existing CTAB method. It involves using 3.0% CTAB in the initial cell lysis followed by RNase treatment. This protocol not only resulted in higher yield (Mean DNA concentration-900.86 ng/µl) but also superior quality DNA from 34 mango genotypes evident from the purity indices, A 260 /A 280 ranging between 1.61 to 1.80. This new method has been validated for other fruit crops like sapota, guava, litchi and jamun which yields DNA amenable for PCR amplifications without use of any PCR enhancers.*
**Key words:** CTAB method, Genomic DNA, Leaf tissues, Mango and fruit crops

Received 21.09.2018 Revised 19.11.2018 Accepted 20.12.2018

## INTRODUCTION

Mango (*Mangifera indica* L.) is an important member of the family Anacardiaceae in order Sapindales and is the most important fruit crop in India having a great cultural, socio-economic and religious significance since ancient times. It is said to be originated in the Indo-Burma (Myanmar) region [1]. By virtue of its excellent flavor, delicious taste, attractive color, fruit quality with richness in vitamins and minerals, accessibility to common man, liking by the masses, mango has been assigned the status of the 'King of the fruits' in the tropical world and it is the 'National Fruit of India'.

Random amplified polymorphic DNA (RAPD) marker system has been attempted for assessing the genetic diversity and understanding the phylogenetic relationships among mango genotypes. For any molecular studies, the prerequisite is the superior quality genomic DNA without any contamination of RNA, proteins and metabolites. In perennial fruits trees, leaf tissues are rich in polysaccharides, polyphenols and tannins as reported in apple [2], mango [3], litchi [4] and sapota [5 and 6]. Thus, in tissues with higher levels of polysaccharides and polyphenols, genomic DNA extraction becomes very difficult. For plant genomic DNA isolation the common method is CTAB (Cetyl trimethyl ammonium bromide), method [7]. Many variants of CTAB method have been developed for different perennial crops, *viz.,* mango [3] and litchi [4]. Though, these protocols resulted in good quality DNA, overtime there was oxidation of phenolic compounds that further inhibited PCR reactions. The disturbances due to excess phenols being co-isolated with DNA has been highlighted earlier by different workers [8]. We report here a protocol for extraction of DNA which not only resulted in higher yield but also superior quality DNA over already established protocols.

## MATERIAL AND METHODS

A well- planned germplasm collection survey based on diversity richness was conducted in coastal districts of Andhra Pradesh which includes Horticultural Research Station and private owned mango orchards. Random sampling strategy was followed for collection of samples. Three plants in each cultivar were taken as sample size. The experimental material consists of 34 indigenous mango cultivars and variants within them obtained from the coastal districts of Andhra Pradesh and was conducted during 2013 and 2014 at Horticultural Research Station, Venkataramannagudem.

**Extraction of plant DNA**

DNA was extracted by CTAB (Cetyl Trimethyl Ammonium Bromide) method [7] with major modifications as detailed below:

a. Healthy young leaves (5 g) were collected from new flush and brought to laboratory on ice. The leaves were surface sterilized with 70 per cent ethyl alcohol and cut into pieces and immediately used for further processing.

b. Approximately 0.5 g of fresh leaf sample was weighed and homogenized completely with liquid nitrogen using mortar and pestle.

c. Powdered leaf material was transferred to eppendorf tubes and suspended in a pre-heated ($65^0$C) 1000 µ1 of extraction buffer (containing 3% CTAB, 100 mM Tris HCl, 20 mM EDTA, 1.4M NaCl, 3% PVP (Polyvinyl Pyrrollidone)s and 2% β-mercapto ethanol.

d. The contents were mixed by vortexing for few minutes by using Tarsons tube spin rotator and the reaction mixture was incubated for 45 minutes at $65^0$C in water bath.

e. The tubes were centrifuged at 14,000 rpm for 10 minutes at $16^0$C by using Eppendorf centrifuge 5430R.

f. Supernatant was collected in a separate tube to which 900 µl Chloroform: Isoamyl alcohol (24:1) mixture was added and centrifuged at 14,000 rpm for 10 minutes at $4^0$C.

g. To the supernatant, 0.6 volume of ice cold isopropanol was added and centrifuged at 14,000 rpm for 10 min at $4^0$C.

h. Pellet was collected and added with 150 µl of TE buffer (10 mM Tris- HCl and 1mM EDTA of $p^H$ 8.0) to dissolve the pellet.

i. RNAase (10 mg/ml) at 1mg/ml was added and incubated for 1h at $37^0$C.

j. To this 150 µl Phenol and 150 µl Chloroform: Isoamyl alcohol (24:1) was added and centrifuged at 14,000 rpm for 10 minutes at $4^0$C.

k. To the supernatant collected equal volume of Chloroform: Isoamyl alcohol (24:1) was added and centrifuged at 14,000 rpm for 10 minutes at $4^0$C.

l. Supernatant was collected in a separate tube to which equal volume of 95 per cent ethanol and 1/3$^{rd}$ volume of sodium acetate (pH 5.2) was added and left at -$20^0$C overnight for precipitation of DNA.

m. After incubation, the tubes were spun at 14,000 rpm for 10-15 minutes. Later, the pellet obtained was washed with 70 per cent ethanol (100 µl) and centrifuged at 14,000 rpm for 5 minutes.

n. The pellet obtained was air dried and dissolved in TE buffer and stored at -$40^0$C for further use.

The concentration and quality of DNA was estimated by using Helma Nano Drop spectrophotometer at 260 nm and verified by running sample on 0.8 per cent agarose along with 330 bp lamda marker by using Major science gel electrophoresis.

**PCR amplification using RAPD:** In order to confirm the usability of DNA for downstream applications PCR was performed using random primers of OPA series, with components comprising of 50ng of template DNA, 1 unit of Taq polymerase (Genei Pvt. Ltd., Bangalore, India), $MgCl_2$ - 2.5 mM; dNTPs (Genei)- 0.2 mM; 10 p mole of Random decamer primer (Operon, USA), Buffer (Genei) – 10X in a reaction volume of 25 µl in the RAPD programme, *viz.*, $94^0$C for 3 min, 39 cycles of $92^0$C for 1min, $36^0$C for 1 min, $72^0$C for 1.30 min, followed by$72^0$C for 10 min and held at $4^0$C till end. The PCR products were analyzed on 1.4% agarose gel and documented.

**RESULTS AND DISCUSSION**

Genetic diversity, evolutionary relationships, biochemical and metabolic networks, genome sequencing, numerical taxonomy and molecular systematics are dependent on good quality DNA as a base or starting material. For such molecular studies, the first and foremost requirement is a superior quality DNA which depends on DNA extraction procedures. Extraction of DNA by CTAB method yielded good quality DNA in mango, but the yield was low and slowly oxidation of phenolic compounds occurred which inhibited PCR reactions. The isolation of genomic DNA from plants containing polysaccharides, phenolics, terpenoids, tannins and metabolites is cumbersome because these compounds binds with DNA and get co-extracted with DNA [9 and 4]. Polysaccharides interfere with biological enzymes such as polymerases, restriction endonucleases and ligases [9]. We report here the modifications made in CTAB method [7] for isolation of superior quality genomic DNA from mango. A wide range of antioxidants are used during DNA extractions to reduce the phenolic contaminations which include: β –mercaptoethanol and polyvinyl pyrrolidone (PVP). Use of PVP (3.0%V/V) and 3.0% CTAB (instead of 2% as in classical CTAB method), phenolic and polysaccharide contaminations in DNA were reduced in the mango samples in the present study. PVP forms complex hydrogen bond with latex lactone, lactucin and other phenolics and co precipitates with cell debris upon lysis. When the extract is centrifuged in presence of chloroform, the PVP complex accumulated at the interface between the organic and the aqueous phase [2 and 9]. CTAB binds the fructans and other polysaccharides and forms complex that are removed during subsequent chloroform

extraction [9]. Washing with ethanol was critical for washing impurities as well as recovery of DNA as a lot of degraded nucleic acids that influence A 260 values as this is critical determinant of DNA concentration and yield. With remnants of ethanol, the nucleic acids cannot be fully rehydrated and wash step using washing buffer was essential. On 0.8% agarose gel, a brighter intact band was visualized in all 34 mango samples (Fig. 1) without any RNA or protein contamination. This was further confirmed by UV-spectrophotometric analysis in which the DNA concentration and quality based on purity index (A 260/A 280) values (Table 1) matched with the visual quantization and quality analysis on agarose gel.

**DNA yield and purity assessment**

The DNA was isolated from young healthy leaves of 34 mango cultivars by using the modified CTAB method**.** This protocol resulted in white translucent DNA pellets that easily solubilized in $T_{10}E_1$ (Tris EDTA) buffer. This DNA was homogeneous and not degradable. The quality of DNA isolated was verified by Nano-drop spectrophotometer and also by agarose gel electrophorosis. The results indicated that the isolated DNA was of good in quality and had high molecular weight (Fig. 1).

The yield of genomic DNA ranged from 139.8 to 3816.8 ηg/µl (Table 1). The purity was determined by calculating the ratio of $A_{260}$:$A_{280}$ ratio which varied from 1.61 to 1.80 indicating that the DNA was relatively of high purity and was suitable for PCR amplification.

**Table 1: Yield and absorbance ratio of DNA in different mango cultivars**

| S. No | Name of the cultivar | Quantity (ng/µl) | A260/A280 ratio |
|---|---|---|---|
| 1 | Banganapalli – 1 | 374.1 | 1.76 |
| 2 | Banganapalli – 2 | 1254.0 | 1.80 |
| 3 | Banganapalli – 3 | 900.2 | 1.79 |
| 4 | Banglora – 1 | 736.0 | 1.67 |
| 5 | Banglora – 2 | 820.6 | 1.64 |
| 6 | Baramasi | 856.7 | 1.71 |
| 7 | Cherukurasam | 842.5 | 1.76 |
| 8 | Chinnarasam | 1290.5 | 1.70 |
| 9 | Chinna Suvarnarekha | 470.4 | 1.72 |
| 10 | Elamandala | 208.5 | 1.63 |
| 11 | Hyder | 1007.5 | 1.65 |
| 12 | Imampasand | 721.4 | 1.64 |
| 13 | Jalal | 914.6 | 1.78 |
| 14 | Jehangir | 711.9 | 1.65 |
| 15 | Kolanka Goa | 419.5 | 1.78 |
| 16 | Kottapalli Kobbari | 2163.6 | 1.78 |
| 17 | Kowsuri Pasand | 1846.4 | 1.76 |
| 18 | Nalla Andrews | 1569.3 | 1.75 |
| 19 | Nalla Rasalu | 944.3 | 1.68 |
| 20 | Navaneetam | 2052.5 | 1.73 |
| 21 | Nuzividu Tiyya Mamidi | 952.7 | 1.79 |
| 22 | Nuzividu Rasalu | 664.7 | 1.71 |
| 23 | Panchadara Kalasa | 663.6 | 1.64 |
| 24 | Panduruvari Mamidi | 3816.8 | 1.67 |
| 25 | Paparao Goa | 716.6 | 1.74 |
| 26 | Peddarasam | 411.4 | 1.70 |
| 27 | Panukula Mamidi | 291.5 | 1.73 |
| 28 | Royal Special | 508.4 | 1.74 |
| 29 | Rajamanu | 436.6 | 1.61 |
| 30 | Sora Mamidi | 361.5 | 1.79 |
| 31 | Suvarnarekha | 139.8 | 1.75 |
| 32 | Tella Gulabi | 594.9 | 1.73 |
| 33 | Tella Rasalu | 427.7 | 1.63 |
| 34 | Raja Mamidi | 538.7 | 1.78 |

The DNA yield (ηg/µl) and the OD ratio (260/280) for all the cultivars collected are evident in Table 1. The highest content of DNA *i.e.,* 3816.8 ηg/µl was obtained in Pandurivari Mamidi whereas, the lowest DNA content (139.8 ηg/µl) was observed in Suvarnarekha.

The purity of DNA was determined by OD 260/280 ratio and the ratio of 1.60 or less indicated protein contamination while values exceeding the ratio of 1.80 indicated the presence of RNA [10]. The purity of DNA of mango cultivars used in the present study ranged from 1.61 to 1.80 indicated that the DNA obtained from this protocol was free of contaminants.

**PCR amplification:** To ensure that whether the isolated DNA is appropriate for further downstream processing, the DNA was amplified using RAPD primers. Twenty primers were screened, of which 15 primers produced prominent amplicons of reproducible nature in all cultivars. A typical agarose gel profile generated by primer OPA series of primers in 34 mango genotypes is shown in fig. 2. Thus, this protocol has high quality DNA amenable for PCR amplification without any additional use of PCR enhancer like PVP or BSA. The protocol described here is an improvement over traditional CTAB method, as high quality genomic DNA with higher yield and purity indices is obtained in all mango samples by extracting with higher salt concentration of CTAB followed by RNase treatment. This protocol was also validated with other fruit crops like sapota, guava and jamun.

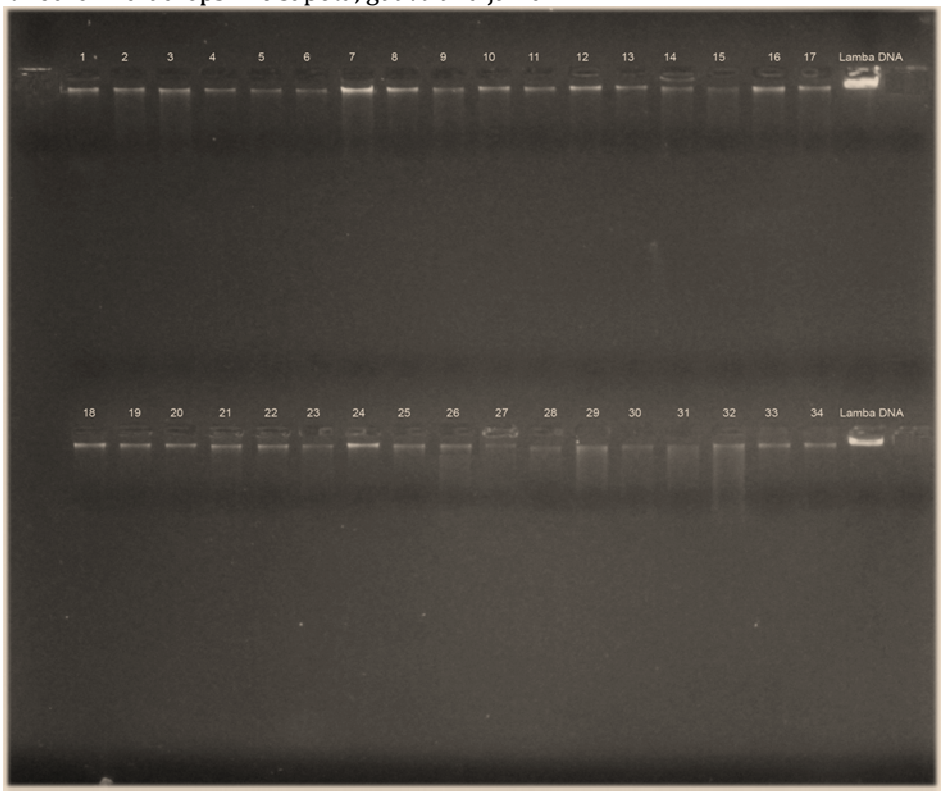
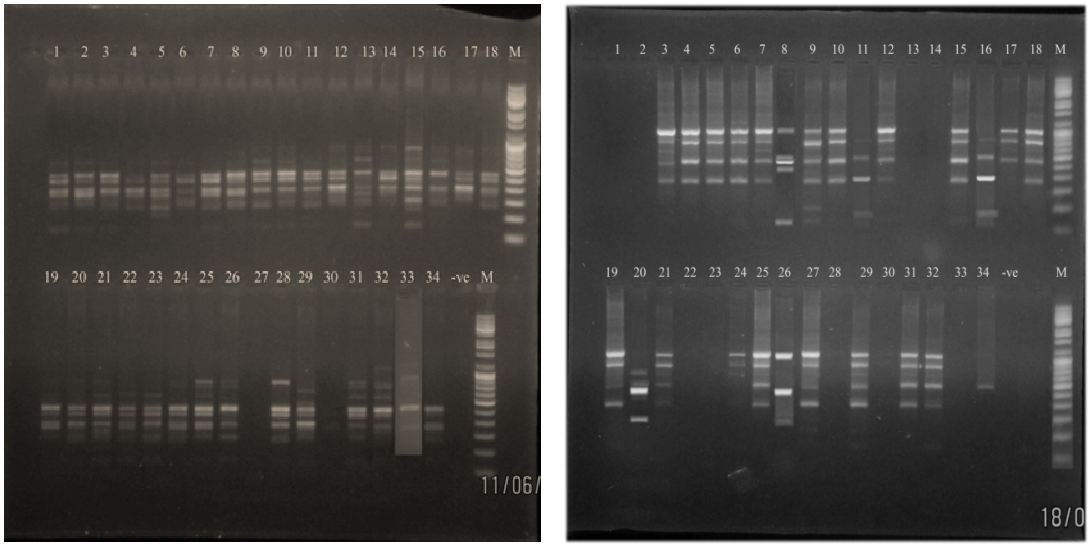



**Fig.1: Gel profile of high quality genomic DNA of mango cultivars**

| | | | | |
|---|---|---|---|---|
| **Lane 1 : Banganapalli- 1** | **Lane 13: Jalal** | | **Lane 25: Paparao Goa** | |
| **Lane 2 : Banganapalli- 2** | **Lane 14: Jehangir** | | **Lane 26: Peddarasam** | |
| **Lane 3 : Banganapalli- 3** | **Lane 15: Kolanka Goa** | | **Lane 27: Panukula Mamidi** | |
| **Lane 4 : Banglora- 1** | **Lane 16: Kottapalli Kobbari** | | **Lane 28: Royal special** | |
| **Lane 5 : Banglora- 2** | **Lane 17: Kowsuri Pasand** | | **Lane 29: Rajamanu** | |
| **Lane 6 : Baramasi**<br>**Lane 7 : Cherukurasam** | **Lane 18: Nalla Andrews**<br>**Lane 19: Nalla Rasalu** | | **Lane 30: Sora Mamidi**<br>**Lane 31: Suvarnarekha** | |
| **Lane 8 : Chinnnarasam** | **Lane 20: Navaneetham** | | **Lane 32: Tella Gulabi** | |
| **Lane 9 : Chinna Suvarnarekha** | **Lane 21: Nuzividu Tiyya Mamidi** | | **Lane 33: Tella Rasalu** | |
| **Lane 10: Elamandala** | **Lane 22: Nuzividu Rasalu** | | **Lane 34: Rajamamidi** | |
| **Lane 11: Hyder** | **Lane 23: Panchadara Kalasa** | | | |
| **Lane 12: Imam Pasand** | **Lane 24: Pandurivari Mamidi** | | | |




**OPA-02** **OPA-03**

**Fig.2 DNA amplification among the 34 mango genotypes**

**Lane 1 : Banganapalli- 1**
**Lane 2 : Banganapalli- 2**
**Lane 3 : Banganapalli- 3**
**Lane 4 : Banglora- 1**
**Lane 5 : Banglora- 2**
**Lane 6 : Baramasi**
**Lane 7 : Cherukurasam**
**Lane 8 : Chinnnarasam**
**Lane 9 : Chinna Suvarnarekha**
**Lane 10: Elamandala**
**Lane 11: Hyder**
**Lane 12: Imam Pasand**
**Lane 13: Jalal**
**Lane 14: Jehangir**
**Lane 15: Kolanka Goa**
**Lane 16: Kottapalli Kobbari**
**Lane 17: Kowsuri Pasand**
**Lane 18: Nalla Andrews**
**Lane 19: Nalla Rasalu**
**Lane 20: Navaneetham**
**Lane 21: Nuzividu Tiyya Mamidi**
**Lane 22: Nuzividu Rasalu**
**Lane 23: Panchadara Kalasa**
**Lane 24: Pandurivari Mamidi**
**Lane 25: Paparao Goa**
**Lane 26: Peddarasam**
**Lane 27: Panukula Mamidi**
**Lane 28: Royal Special**
**Lane 29: Rajamanu**
**Lane 30: Sora Mamidi**
**Lane 31: Suvarnarekha**
**Lane 32: Tella Gulabi**
**Lane 33: Tella Rasalu**
**Lane 34: Rajamamidi**
**Lane -ve: Control**
**Lane M: Medium Range DNA Ruler**

## REFERENCES


1. De-Candolle, A. D. E. 1904. Origin of Cultivated Plants. Kegan Paul, London
2. Kim, C. S., Lee, C.H., Shin, J. S., Chung, Y. S., and Hyung, N. I. (1997). A simple and rapid method for isolation of high quality genomic DNA from fruit trees and conifers using PVP. *Nucleic Acids Research. 25(5) :* 1085-1086.
3. Kit, Y. S. and Chandran, S. (2010). A simple, rapid and efficient method of isolating DNA from Chokanan mango (*Mangifera indica* L.). *African Journal of Biotechnology*. *9(36) :* 5805- 5808.
4. Puchooa, D. (2004). A simple, rapid and efficient method for extraction of genomic DNA from lychee (*Litchi chinensis* Sonn.). *African Journal of Biotechnology. 3(4):* 253-255.
5. Kumar, M., Gupta, M., Shrivastava, D., Prasad, M., Prasad, U. S. and Sarin, N. B. (2006).Genetic relatedness among Indian Sapota accessions (*Manilkara achras* (Mill.) Fosberg.) by RAPD markers. *International Journal of Agricultural Research. 1(4):* 390-400.
6. Suhasini, J., Kanamadi, V. C., Swamy, G. S. K., Shirol, A.M. and Chavan, M. (2012). Performance of sapota (*Manilkara achras* (Mill.). Fosberg) varieties and hybrids under Ghataprabha command area. *Karnataka Journal of* Agricultural *Sciences. 25(4):* 548- 551.
7. Murray, M. and Thompson, W. (1980). The isolation of high weight plant DNA. *Nucleic Acids Research*. 8:4321-4325.
8. Manchado, P. S., Roux, E. L., Guerneve, C. L., Lozano, Y. and Cheynier, V. (2000). Phenolic composition of Litchi fruit pericarp. *Journal of Agricultural Food and Chemistry*. *48(12):* 5995-6002.
9. Michiels, A., W. Van den Ende, M. Tucker, L. V. Riet and A.V.Laere (2003). Extraction of high- quality genomic DNA from latex-containing plants. *Analytical Bio-chemistry. 315:* 85-89.
10. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.



**CITATION OF THIS ARTICLE**

A. Himabindu, D. Srihari, M. Rajasekhar, V. Sudhavani, P. Subbarammamma and K. Uma Krishna. Extraction Of High Quality Genomic DNA From Mango (*Mangifera indica* Linn.) Genotypes. Bull. Env. Pharmacol. Life Sci., Vol 8 [2] January 2019: 101-105