Plant Archives Vol. 26, Supplement 2, 2026 pp. 1134-1137 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-2.132

DNA EXTRACTION PROTOCOL FOR LOCAL MANGO GERMPLASM (*Mangifera indica* L.) IN MANIPUR, NORTH EAST INDIA

S.R. Singh[1*], Kh. Priya Devi[1], T. Deem Singh[1], Rahul Senjam[1] and Ng. Umakanta[2]

[1]Department of Horticulture, AICRP-Fruits, Imphal Center, College of Agriculture, CAU, Imphal, Manipur, India

[2]ICAR complex for North East Hilly Region, Manipur Centre, India

*Corresponding author E-mail: romensenjam@yahoo.com

(Date of Receiving : 26-02-2026; Date of Revision : 09-04-2026; Date of Acceptance : 03-05-2026)

ABSTRACT

Investigation was carried to recommend efficient time and genomic DNA extraction protocol for the local mango germplasm found in valley region of Manipur. Modified DNA extraction CTAB method was applied and the quality of genomic DNA was determined by the ratio of A260/A280 and the amplifiable quality of DNA determined by the horizontal agarose gel electrophoresis using 1% agarose in TBE buffer at constant voltage of 60V. Modified CTAB methods yielded good quality DNA having average OD (260/280) ratio of 1.692 having concentration of DNA 289.37 (µg/µl) using young copper colour stage of leaves. Observation revealed that young copper colour stage of leaves with modified CTAB method could be recommended for the efficient DNA extraction method for local mango genotypes in the future.

Keywords: Mango, young leaves, DNA, CTAB method.

Introduction

Mango (*Mangifera indica* L.) popularly known as the "King of the fruits" in India, having chromosome (2n = 40) is a member of the Anacardiaceae family and a popular fruit crop in Manipur locally known as "*Heinou*". This tasty fruit has anti-oxidant, anti-diabetic, gastroprotective, cardiotonic, antiviral, and anti-inflammatory qualities (Shah *et al*., 2010). Additionally, it is fairly rich in other nutrients, minerals, and pro-vitamin A (4800-16800 I.U.). At present, India produces 23.14 million tons of mangoes annually on 2.41 million hectares of land (Anon, 2024). Mangoes from India are exported to the United Arab Emirates, the United Kingdom, Saudi Arabia, Yemen, Kuwait, Qatar, the United States, Nepal, Bangladesh, China, and Germany. India exports about 27,872.77 MT of fresh mango fruit, worth Rs. 3.27 billion. Asia's share in global mango production is nearly 77%, while rest comes from the African and American continents (Gulshan Kumar *et al*., 2023).

The genus Mangifera has 69 species, many of which are tropical trees (Kostermans and Bompard 1993). Due to a long period of domestication, alloploidy, and out crossing, *Mangifera indica* has a high degree of genetic variability and is highly heterozygous (Singh, 1960). The genus Mangifera has historically been categorized using the phenological and morphological traits of flowers, foliage, fruits, and seeds. Nevertheless, a lot of these traits are influenced by the environment, which frequently results in false parallel selection and reduces the accuracy of these techniques for characterizing mango germplasm. Because of its perennial nature, high degree of heterozygosity, single seed per fruit, low fruit set, and inadequate knowledge of the genetics of significant horticultural traits, the mango is regarded as a difficult plant species to improve using traditional breeding techniques (Iyer and Schnell, 2009).

Manipur being a part of Indo-Myanmar region, genetic diversity of local mango germplasm are exists in valley and foothills of Manipur which need evaluation, characterization and released for commercial cultivation. To ensure the preservation of these priceless genetic resources, molecular characterization using DNA markers is crucial to check duplicate germplasm having different local

nomenclature for different communities but similar in fruit morphotypes in Manipur.Several marker systems have been applied to mango, including simple sequence repeats (Ravishankar et al. 2011), inter-simple sequence repeats (Pandit *et al*., 2007), amplified fragments length polymorphism (AFLP) (Bajpai *et al*., 2008). Because of their codominant nature, polymorphic behavior, multiallelic nature, high reproducibility, and extensive genome coverage, SSR markers are gaining more attention among these (Srivastav *et al*., 2021). However, studies on indigenous local mango germplasm evaluation and characterization using molecular markers found in Manipur are lacking at present scenario.

Therefore, the present investigation was aimed to know study the suitable leaves sample collection stage and standardization of DNA extraction protocol for evaluation genetic diversity of local mango germplasm found in valley and foothills of Manipur using molecular markers application.

Materials and Methods

Plant-based materials: Fresh young mango leaves (copper colour young leave stage) were gathered from valley regions of Manipur *viz*. Imphal East, Imphal West and Kakching district for molecular analysis. Old and mature leaves of mango resulted in poor quality of DNA. Copper colour young leave stage from new flush give quality DNA for mango. They were transported to the lab in an ice cube box and kept in a freezer at -200°C from the selected germplasm. A motor and pestle were used to grind the leaves into a fine powder. The process of grinding the samples was occasionally stimulated by the addition of liquid nitrogen, which aids in the removal of moisture. Before being used for DNA extraction, the resulting powder was kept at -20^0C in a 2 ml micro-centrifuge tube. The reagents use in modified CTAB-DNA extraction protocol for young copper stage of leaves are given in Table 1.

Table 1: Reagents use in DNA extraction and their functions

Sl. No.	Reagents	Functions
1	Liquid nitrogen (-196^{0}C)	For the removal the moisture from the leaf
2	β-merceptaehanol	For the removal of protein from DNA
3	CTAB (Cetyl Trimethyl Ammonium Bromide)	It acts as a strong detergent and helps in rupturing of cell wall
4	Sodium Chloride (NaCl)	Maintains the ionic balance and helps in separation of different dissolved organic debris
5	Tris Cl	It helps in maintaining pH of buffer at 8.0
6	EDTA	Strong chelating agents which deplete Mg ions in solution, inhibit nuclease activity by that we can get nucleic acid
7	Phenol	Removal of histone protein in DNA
8	Chloroform	Removed proteins and help in separation of different particles in liquid and remove carbohydrates
9	PVP	Help to isolate high-quality DNA, RNA, or proteins from plant tissues
10	Absolute alcohol	Precipitate DNA out of the solution, making it visible and collectable as a solid pellet
11	Sodium acetate	Helped in removal of carbohydrates and proteins and also gives stabilization to DNA
12	TE buffer	Helped to dissolved the DNA pellet and maintain pH 8.0
13	Ethanol 70%	It absorbed moisture and salts in DNA pellet and acts on wetting agents and helps in better penetration in TE buffer

Reagents for CTAB methods for modified CTAB method

CTAB buffer (100ml): 3% CTAB (Hexadecyl trimethyl ammonium bromide), 100nM Tris CL, 20nM, 29nM EDTA (pH 8.0), 1.4M NaCl, 3% PVP (Poly vinyl pyrrollidone) and 2% β-merceptaehanol. Mix all the reagents and adjust the pH to 8.0 with HCL and make up to 100 with distilled water.

Procedures:

1. Healthy young leaves were collected from new flush, cut into small pieces completely grind in liquid nitrogen using mortar and pestile. (If needed leaves should clean/sterilized with 70% ethyl alcohol).
2. Take around 0.5g homozinitzed grinded transfer to 2ml tube.

3. Preheated extraction buffer at 65°C
4. Put 1000µl extraction buffer to grinded leaves inside the tube.
5. Put these tubes (leaves sample extraction buffer) inside the water bath at 65°C for 1 hours (shake it every 15 mins interval)
6. The tube was centrifuge at 13500 RPM for mins
7. Supernatant were collected in a separate tube 400µl PCI added and centrifuge at 13500 RPM for 10 mins
8. Collected supernatant 300µl and add sodium acetate one-tenth of supernatant i.e., 30µland add ethanol (diluent) full
9. Keep in -21°C for 2 hours overnight
10. Centrifuge for 10 mins in 13500 RPM
11. Throw the liquid and add 500µl 70% ethanol (diluent) and centrifuge for 7 mins in 7000 RPM
12. Remove the liquid and drying until the alcohol smell has gone
13. Add 100µl TE buffer and keep overnight at 4°C
14. Stored the dissolved DNA at 4°C for immediate use & -20°C for future use.

Estimation of DNA quality

The purity of DNA and RNA is evaluated using the spectrophotometer's ratio of absorbance at 260 and 280 ¹m. For DNA and RNA, a ratio of roughly 1.5 and 2.0, respectively, is considered pure. Protein, phenol, or other pollutants may be strongly absorbed at or near 280 ~m if the ratio is much lower in either scenario. 1% agarose gel electrophoresis was also used to assess the DNA's purity.

Results and Discussions

Leave selection for DNA extraction for mango is necessary. Old and mature leaves result in poor quality and copper stage of new flush stage is the correct stage for extraction quality of DNA in mango. The highest quality DNA was generated using CTAB methods, with a mean absorbance ratio (A260:280) of 1.5. Gel runs of samples from local mango germplasm samples using the DNA extraction method showed that there were several time-consuming extraction steps and variations in the total amount of time required to finish the process. Modified CTAB displays a clear ring on the gel from the local mango sample in addition to DNA extraction methods. The purity of the DNA was evaluated using its absorbance at 260 and 280 nm. According to Ranganathan Kapilan (2015), A260:A280 ratios greater than 1.8 indicate the extraction of very good quality genomic DNA, values less than 1.8 indicate protein contamination of the genomic DNA, and values greater than 2.0 indicate the presence of acetone or alcohol in the DNA preparation. The extracted DNA quality varied significantly depending on the plant species and DNA extraction methods (Smith *et al*., 2011).

Table 2: DNA quantification for local mango germplasm using spectrophotometer

Germplasm of local mango germplasm	µg/µl	A260/A280
P_1	282.43	1.695
P_2	286.12	1.686
P_3	319.22	1.701
P_4	264.01	1.550
P_5	267.41	1.650
P_6	285.95	1.736
P_7	277.26	1.886
P_8	363.75	1.820
P_9	244.70	1.513
P_{10}	303.21	1.683
Mean	**289.37**	**1.692**

Fig. 1 : Young mango leaves

Fig. 2 : Mature mango leaves

Fig. 3 : DNA gel electrophoresis band for local mango germplasm

Acknowledgement:
We are obliged to the ICAR-IIHR, AICRP-Fruits, Bangalore, Karnataka for providing financial support during the research work.

References

Anonymous (2024). Department of Agriculture & Farmers welfare, Govt. of India (Source, https,//agriwelfare.gov. in.)

Bajpai A., Srivastava N., Rajan S. and Chandra R. (2008). Genetic diversity and discrimination of mango accessions using RAPD and ISSR markers. *Indian J. Hort*., **65**(4), 377-382.

Gulshan Kumar, Manish Srivastav, H.S. Sreekanth, Chavlesh Kumar, Jai Prakash, Sanjay K. Singh and Vinod (2023). SSR assisted identification of mango (Mangifera indica L.) hybrids and development of DNA barcodes. *Indian J. Genet. Plant Breed*., **83**(3), 437-445

Iyer C. P. A. and Schnell R. J. (2009). Classical Breeding and Genetics. In, R. E. Litz (ed.), The Mango, Botany, Production and Uses, Vol. II. *CAB International*. pp67-96.

Kostermans A. J. G. H. and Bompard J. M. (1993). The Mangoes, Their Botany, Nomenclature, Horticulture and Utilisation. Academic Press, London.

.

Pandit S. S., Mitra S., Giri A.P., Pujari K.H., Patil B.P., Jambhale N.D. and Gupta V.S. (2007). Genetic diversity analysis of mango cultivars using inter simple sequence repeat markers. *Curr. Sci*., **93**, 1135–1141.

Ranganathan, K. (2015). Efficient DNA extraction technique from leaf tissues of some important tropical plant species. *Journal of Progressive Research in Biology*., **1**(1), 29-32.

Ravishankar, K. V., Mani, B. H., Anand, L. and Dinesh, M. R. (2011). Development of new microsatellite markers from mango (*Mangifera indica* L.) and cross-species amplification. *Am. J. Bot*., **98**, 96-99

Shah, K. A., Patel, M. B., Patel, R. J. and Parmar, P. K. (2010). *Mangifera indica* (Mango). *Pharmacogn Rev*., **4**(7), 42.

Singh, R. N. (1960). The Mango, Botany, Cultivation and Utilisation, 1st ed. Leonard Hill, London

Smith, B, Li, N, Andersen, A.S., Slotved, H.C., Krogfelt, K.A. (2011). Optimising bacterial DNA extraction from faecal samples, *Comparison of three Microbiology Journal*., **5**,14-17.

Srivastav, M., Singh, S. K., Prakash, J., Singh, R., Sharma, N., Ramchandra, S. and Singh, N.K. (2021). New hyper-variable SSRs for diversity analysis in mango (*Mangifera indica* L.). *Indian J. Genet. Plant Breed*., **81**(1), 119-126.