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RESEARCH ARTICLE

Optimised DNA isolation protocols for key arid and semi-arid fruit trees to enhance genetic improvement

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Abstract

The study was conducted at Forest College & Research Institute, Mettupalayam, Tamil Nadu to optimise the DNA extraction protocols for important arid and semi-arid fruit trees. Arid and semi-arid fruit trees, including *Emblica officinalis*, *Aegle marmelos*, *Ziziphus mauritiana*, *Buchanania lanzan* and *Carissa grandiflora*, play a crucial role in India's rural economy by providing nutritional security and supporting agroforestry systems. However, the availability of quality planting materials resilient to climate variability remains a big challenge. DNA analysis is a valuable tool for the genetic improvement of long-rotation tree species, aiding in the selection of high-yield and climate-resilient genotypes. Effective DNA extraction is essential for molecular studies but is hindered by the presence of high levels of polysaccharides, polyphenols and tannins. This study aimed to standardise species-specific DNA isolation protocols using three methods cetyltrimethylammonium bromide (CTAB), modified CTAB and sodium dodecyl sulphate (SDS). DNA yield and purity were assessed using a Thermo Scientific NanoDrop™ 1000 Spectrophotometer and agarose gel electrophoresis. The highest DNA yield for *E. officinalis* was obtained using the modified CTAB method (1074 ng/µL), while *A. marmelos*, *B. lanzan* and *C. grandiflora* showed optimal results with the CTAB method, yielding 508 ng/µL, 1106.6 ng/µL and 474 ng/µL, respectively. For *Z. mauritiana*, the SDS method was superior, yielding 1159.6 ng/µL with a purity ratio (A260/A280) of 1.6. These optimised protocols facilitate molecular breeding, stress-resistance screening and genetic enhancement of these vital species.

Keywords: agroforestry; arid and semi-arid regions; CTAB method; DNA isolation; fruit trees; modified CTAB; SDS method

Introduction

Arid and semi-arid regions of India account for approximately 53 % of the country's total cultivable land, spanning the states of Rajasthan, Gujarat, the Northern Plains, Central Highlands and the Deccan Plateau. Fruit trees such as *Emblica officinalis*, *Aegle marmelos*, *Ziziphus mauritiana*, *Buchanania lanzan* and *Carissa grandiflora* are well adapted to these extreme climatic conditions and play a crucial role in the rural economy by providing nutritional security. However, the availability of quality planting materials suitable for these regions remains a significant challenge.

DNA analysis is a powerful tool for the genetic improvement of long-rotation tree species. Recent advancements in molecular breeding and marker-assisted selection (MAS) have accelerated the selection of high-yielding and climate-resilient genotypes (1). Polymerase chain reaction (PCR)-based molecular tools are extensively used for this purpose. However, obtaining high-quality DNA from mature tree species, particularly woody plants, remains a major challenge in plant molecular biology (2–4). Many conventional DNA extraction methods fail to yield high-purity DNA due to the presence of polysaccharides, polyphenolics and tannins, which interfere with DNA extraction and purification. These compounds can degrade DNA or irreversibly bind to it, rendering it unsuitable for downstream molecular applications.

Several DNA extraction protocols have been developed to address these challenges (5–7). However, standard protocols for species such as *E. officinalis*, *A. marmelos*, *Z. mauritiana* and *C. grandiflora* often fail to produce DNA of sufficient quality for PCR and restriction fragment length polymorphism (RFLP) analyses. Studies have shown that high salt concentrations during DNA precipitation help remove residual phenols and polysaccharides (8–10). Molecular characterisation of plants requires high-molecular-weight genomic DNA as a starting material. The quality and quantity of DNA significantly impact the efficiency of PCR-based techniques, including real-time PCR, multiplex PCR and DNA sequencing (11, 12). However, extracting high-purity DNA from trees remains more challenging than from most herbaceous plants, as DNA yield and quality often vary among species and even within tissues of the same plant (13). Impurities such as terpenes, polyphenolics and

polysaccharides, commonly found in perennial foliage, frequently co -extract with DNA, complicating the purification process (14).

DNA extraction remains a critical issue in plant molecular biology, especially for large-scale studies such as genetic diversity assessments and breeding programs (15). Efficient and reliable methods are essential for obtaining DNA of high purity and yield. In this study, we compared DNA extraction methods, including the cetyltrimethylammonium bromide (CTAB) method, modified CTAB method and sodium dodecyl sulphate (SDS) based method to standardise an optimised DNA isolation protocol for fruit trees (16–20). This study specifically focused on obtaining high-quality DNA from young leaves of *E. officinalis*, *A. marmelos*, *Z. mauritiana* and *C. grandiflora* to facilitate downstream molecular applications in tree improvement programs.

Given the importance of high-quality DNA for molecular breeding and genetic studies, this research aims to standardise DNA isolation protocols for key arid and semi-arid fruit trees. By comparing CTAB, modified CTAB and SDS methods, we identify the most effective approach for obtaining high-purity DNA suitable for PCR applications. The optimised protocol will aid in genetic improvement, conservation and stress resilience studies of these species. The objectives of this study are to identify a suitable method for high-purity DNA extraction from fruit tree leaves and to standardise a DNA extraction protocol for young leaves to support molecular breeding studies.

Materials and Methods

Plant material and sample collection

Young leaf samples were collected from existing plantations at the Research Farm of the Forest College & Research Institute, Mettupalayam, Tamil Nadu during 2013. Fresh leaf samples from different populations of *E. officinalis*, *A. marmelos*, *Z. mauritiana*, *B. lanzan* and *C. grandiflora* were collected in the morning when the weather was cool. The samples were transported to the laboratory in an ice box and stored at −20 °C in a deep freezer until further processing.

DNA extraction and quantification

Genomic DNA extraction by CTAB method

Genomic DNA was isolated following the CTAB method with slight modifications (21). Approximately 1 g of plant tissue was ground into a fine powder using liquid nitrogen and transferred to a 50 mL Falcon tube containing 15 mL CTAB extraction buffer. The mixture was incubated at 65 °C for 15 min in a recirculating water bath. Following incubation, the extract was centrifuged at 12000g for 5 min, and the supernatant was transferred to a clean Falcon tube. To this, 250 µL of chloroform:isoamyl alcohol (24:1) was added and mixed by inversion, followed by centrifugation at 13000 rpm for 1 min. The upper aqueous phase containing DNA was carefully transferred to a new microfuge tube. DNA was precipitated by adding 50 µL of 7.5 M ammonium acetate and 500 µL of ice-cold absolute ethanol, followed by gentle inversion. After precipitation, DNA fibers were collected either by pipetting or incubating at −20 °C for 1 hr. The precipitate was washed twice with ice-cold 70 % ethanol, and the DNA was spun down at 13000 rpm for 1 min to form a pellet. The DNA pellet was air-dried and resuspended in 50–400 µL of sterile DNase-free water or TE buffer. RNase A (10 µg/mL) was added to remove RNA contaminants, and the DNA was incubated at 65 °C for 20 min before storage at 4 °C.

Modified CTAB method of DNA extraction

A modified CTAB method was followed with slight alterations to improve DNA quality (21). The extraction steps remained the same as in the standard CTAB method, except for an extended incubation period of 45 min at 65 °C and the addition of 6 % polyvinylpyrrolidone (PVP) to the CTAB buffer. These modifications helped in reducing polysaccharide contamination and improving DNA yield and purity.

DNA extraction by SDS method

The SDS-based protocol was also employed for DNA extraction with slight modifications (20–22). Fresh 200 mg leaf tissue was ground in a mortar and pestle with 1 mL of SDS extraction buffer. The homogenised mixture was incubated at 65 °C for 45 min, followed by centrifugation at 14000g for 5 min at room temperature. The supernatant was carefully transferred to a fresh tube, mixed with an equal volume of 5 M potassium acetate, vortexed and centrifuged again at 14000g for 5 min. The resulting supernatant was extracted with chloroform:isoamyl alcohol (24:1), gently mixed by inversion and centrifuged at 14000g for 5 min. The upper aqueous phase was transferred to a new tube, and DNA was precipitated by adding double the volume of ice-cold isopropanol. The DNA fibers were spooled into a clean tube, washed with 70 % ethanol, air-dried and dissolved in 500 µL of TE buffer. The resuspended DNA was incubated at 65 °C for 10 min, followed by RNase A (10 µg/mL) treatment at 37 °C for 30 min to remove RNA contamination. DNA was re-precipitated using ethanol, centrifuged at 10000g for 5 min, air-dried and finally resuspended in 200 µL TE buffer.

DNA quantification and purity assessment

The concentration and purity of the extracted genomic DNA were assessed using a Thermo Scientific NanoDrop™ 1000 Spectrophotometer by measuring absorbance at 260 nm and 280 nm. The DNA purity was determined using the A260/A280 ratio, where a value of approximately 1.8 indicated high-quality DNA suitable for molecular applications.

Results

In this study, three DNA extraction protocols were assessed for their suitability, and DNA yield and purity ratio (A260/A280) were estimated using the Thermo Scientific NanoDrop™ 1000 Spectrophotometer. The DNA yield ranged from 0 ng/µL to 1159.6 ng/µL and the purity ratio varied from 0.8 to 1.8 across different methods. The modified CTAB method yielded DNA concentrations ranging from nil to 1074 ng/µL, with purity ratios between 1.5 and 1.8 (Table 1). The CTAB method produced DNA concentrations between 110.0 ng/µL and 1106.6 ng/µL, with a purity ratio ranging from 1.4 to 1.8 (Table 1). The SDS method yielded DNA concentrations ranging from 13.2 ng/µL to 1159.6 ng/µL, with purity ratios varying between 0.8 and 1.8 (Table 1).

Comparison of DNA yield and purity across different extraction protocols

Among the tested species, the modified CTAB method provided the highest DNA yield for *E. officinalis* (1074 ng/µL), while the lowest concentration (110 ng/µL) was observed using the CTAB method. The SDS method resulted in lower DNA quality for this species. For *A. marmelos*, *B. lanzan* and *C. grandiflora*, the CTAB method yielded

Table 1. Concentration of DNA (ng/µL) and purity was given in absorbance ratios at 260 nm/280 nm by using the Thermo Scientific NanoDrop™ 1000 Spectrophotometer

Species	CTAB method		Modified CTAB method		SDS method	
	DNA (ng/µL)	Ratio	DNA (ng/µL)	Ratio	DNA (ng/µL)	Ratio
Emblica officinalis	110.0	1.5	1074.0	1.6	498.0	0.8
Aegle marmelos	508.0	1.8	–	–	70.7	1.5
Zizyphus mauritiana	154.2	1.4	322.3	1.7	1159.6	1.6
Buchanania lanzan	1106.6	1.8	115.5	1.5	315.1	1.4
Carissa grandiflora	474.0	1.7	558.7	1.8	13.2	1.4

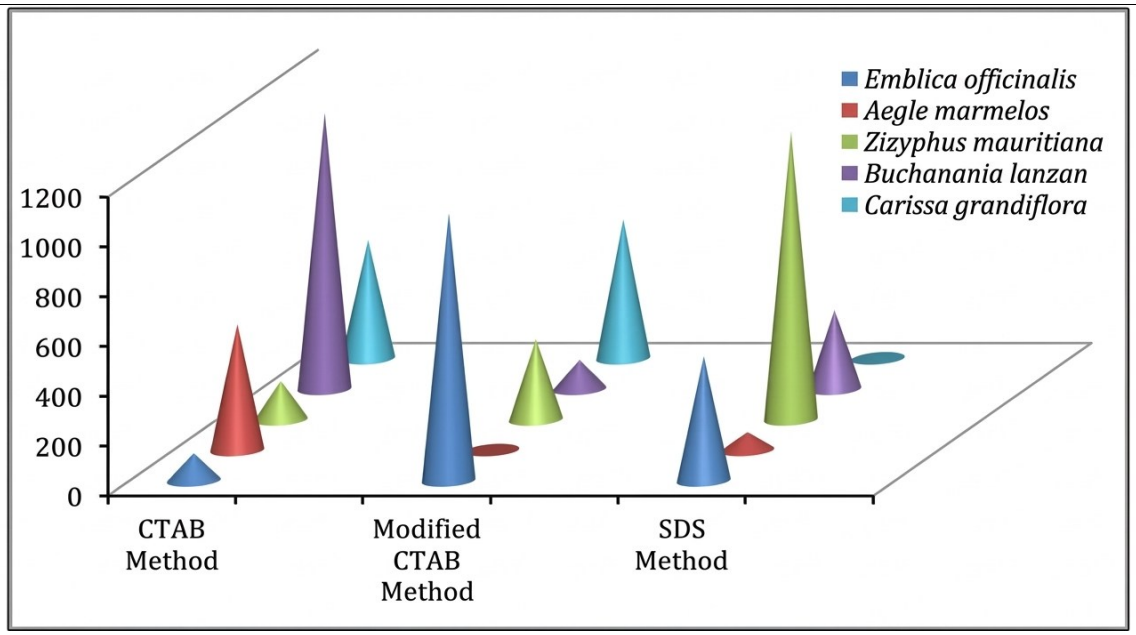

Fig. 1. Yield of DNA from different methods using the Thermo Scientific NanoDrop™ 1000 Spectrophotometer.

the highest concentrations at 508 ng/µL, 1106.6 ng/µL and 474 ng/µL, respectively. In contrast, the SDS method was more suitable for *Z. mauritiana*, producing the highest DNA concentration (1159.6 ng/µL) and acceptable purity. A comparative analysis of purity ratios obtained using the NanoDrop method is represented in Fig. 1, highlighting differences among the three extraction methods.

Genomic DNA quantification by spectrophotometer and agarose gel electrophoresis

Spectrophotometric readings at A260/A280 nm indicated potential contamination from proteins, polysaccharides, secondary metabolites or RNA (Table 1 and Fig. 1). To further assess DNA quality and the extent of shearing, genomic DNA was analysed using agarose gel electrophoresis (Fig. 2). The presence of smearing on the gel suggested contamination from polysaccharides, proteins and other secondary metabolites. The intensity of DNA bands corresponded to DNA concentration, confirming that the extracted DNA was sufficient for PCR applications, although the presence of polyphenols and polysaccharides may interfere with amplification. DNA isolated using the SDS, CTAB and modified CTAB methods showed varying degrees of shearing, as evident from the genomic DNA smears in Fig. 2.

Overall, while all protocols yielded DNA, the Modified CTAB method was the most effective for *E. officinalis*, whereas the CTAB method was superior for *A. marmelos*, *B. lanzan* and *C. grandiflora*. The SDS method proved to be the most suitable for *Z. mauritiana*, producing both high concentration and acceptable purity of DNA.

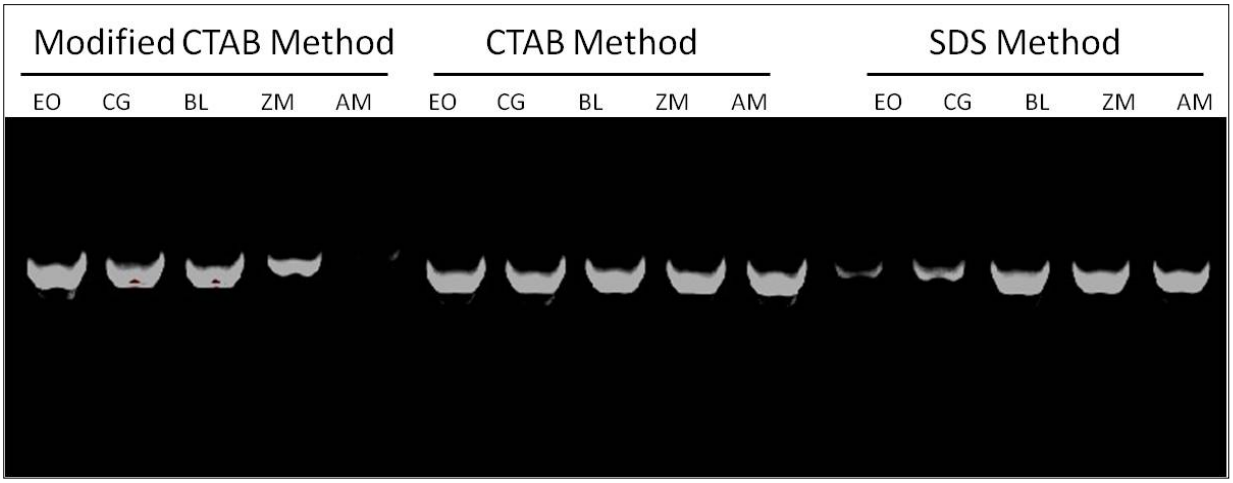

Fig. 2. Analysis of DNA quality in agarose gel electrophoresis. Genomic DNA was extracted by three different protocols in five semi-arid fruit plants and examined by agarose gel electrophoresis. EO: *Emblica officinalis*; AM: *Aegle marmelos*; ZM: *Zizyphus mauritiana*; BL: *Buchanania lanzan*; CG: *Carissa grandiflora*.

Discussion

The extraction of intact, high-molecular-weight DNA with sufficient purity is essential for genomic studies. DNA extraction typically involves two major steps: cell lysis and solubilisation, followed by the removal of contaminants, including proteins, RNA, polysaccharides and secondary metabolites. In this study, three DNA extraction methods such as CTAB, modified CTAB and SDS protocols were evaluated for their efficiency in isolating high-quality, PCR-compatible DNA from arid and semi-arid fruit tree species. PCR-based genomic studies and genetic diversity analyses require DNA of high purity, free from phenolics, polysaccharides and protein contaminants. Arid tree species often contain high levels of secondary metabolites, including polyphenols and polysaccharides, which can interfere with DNA extraction and purification (23). Standard genomic DNA extraction methods often result in DNA contaminated with proteins and polyphenols, making them unsuitable for downstream applications (24, 25). CTAB- and PVP-based protocols have been widely used to improve DNA purity in tree species (26). In this study, DNA was successfully extracted from *E. officinalis*, *A. marmelos*, *Z. mauritiana*, *B. lanzan* and *C. grandiflora* using these protocols, and the obtained DNA was of relatively high purity. Several factors contribute to DNA shearing and degradation during extraction, with endonuclease activity being a major challenge (27). In this study, increasing β-mercaptoethanol concentration to 1 % in the CTAB method helped maintain a non-oxidative environment, reducing endonuclease activity and preventing DNA degradation (28). However hazardous nature of β-mercaptoethanol is limit its practical application (29). The CTAB method, though effective in extracting DNA, resulted in higher polysaccharide contamination, whereas Walbot's method retained DNA purity with an A260/A280 ratio close to 1.8.

Plants with high polysaccharide and secondary metabolite content require optimised extraction methods to prevent contamination (30). Certain polysaccharides interfere with RAPD and PCR-based reactions, affecting downstream molecular applications. Tree species generally require more complex DNA extraction techniques than herbaceous plants due to their higher metabolite content (14). The findings of this study align with previous reports suggesting that increasing sucrose concentration in Walbot's method enhances nuclear content recovery during grinding (14). The CTAB method remains a reliable approach, suitable for both fresh and dried plant material, while higher CTAB and sodium chloride concentrations in the extraction buffer help reduce polysaccharide contamination (31). In this study, the use of liquid nitrogen facilitated tissue grinding, thereby improving DNA recovery from *E. officinalis*, *A. marmelos*, *Z. mauritiana*, *B. lanzan* and *C. grandiflora*.

DNA quantification and purity assessment

The purity of extracted DNA was assessed using the A260/A280 absorbance ratio, a method originally described to detect protein contamination in nucleic acid samples (32). The A260/A280 ratio of ~1.8 is considered indicative of pure DNA, whereas a ratio of ~2.0 suggests pure RNA. The DNA extracted using different methods in this study exhibited A260/A280 ratios ranging from 1.8 to 2.0, confirming high purity. However, DNA obtained via the CTAB method showed greater variability, with ratios between 0.8 and 1.8, suggesting contamination with polyphenols and polysaccharides.

Among the tested species, the modified CTAB method yielded the highest DNA concentration for *E. officinalis* (1074 ng/µL), although some polysaccharide contamination was observed. The CTAB method produced the lowest yield (110 ng/µL) for this species, while the SDS method resulted in lower DNA quality. For *A. marmelos*, *B. lanzan* and *C. grandiflora*, the CTAB method was the most effective, yielding 508 ng/µL, 1106.6 ng/µL and 474 ng/µL, respectively. In contrast, the SDS method proved to be the most suitable for *Z. mauritiana*, yielding 1159.6 ng/µL, though some phenolic and secondary metabolite contamination was detected. These findings suggest that the selection of an appropriate DNA extraction method depends on the plant species and the nature of its secondary metabolites. The modified CTAB method was more effective for *E. officinalis*, while the CTAB method provided better results for *A. marmelos*, *B. lanzan* and *C. grandiflora*. The SDS method was best suited for *Z. mauritiana*, highlighting the need for species-specific optimisations in DNA extraction protocols for tree species in arid and semi-arid environments.

Conclusion

Genetic improvement of tree species has traditionally relied on selection based on morphological traits. Modern PCR-based techniques can accelerate this process, but their success depends on the availability of high-quality genomic DNA. This study aimed to standardise an efficient DNA extraction method for arid and semi-arid agroforestry fruit trees, including *E. officinalis*, *A. marmelos*, *Z. mauritiana*, *B. lanzan* and *C. grandiflora*. The findings indicate that the modified CTAB method was the most effective for extracting high-purity and high-yield genomic DNA from *E. officinalis*, with a maximum concentration of 1074 ng/µL, whereas the standard CTAB method yielded a lower concentration of 110 ng/µL, and the SDS method resulted in lower DNA quality. For *A. marmelos*, *B. lanzan* and *C. grandiflora*, the CTAB method provided the highest DNA concentrations of 508 ng/µL, 1106.6 ng/µL and 474 ng/µL, respectively. The SDS method was most suitable for *Z. mauritiana*, yielding 1159.6 ng/µL with good purity. These findings establish species-specific DNA extraction protocols that ensure high-quality DNA for molecular studies, including marker-assisted selection and genetic diversity assessments. The optimised methods will facilitate future genomic research and conservation efforts in tree improvement programs for arid and semi-arid fruit tree species.

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Authors' contributions

MK and VRB conducted the experiment and drafted the manuscript. RR prepared the manuscript and provided valuable insights. SB and PM edited and assisted in the preparation of the manuscript. PK contributed to the laboratory analysis. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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