



Efficient genomic DNA Extraction from leaves of some economically important fruit species of Sri Lanka

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Abstract

Genomic DNA extraction is an important aspect of plant molecular biological research. The objective of the study was to recommend the cheap and efficient genomic DNA extraction method for some economically important fruit species of Sri Lanka. The modified plant genomic DNA extraction methods explained by Doyle *et al.*, and Cheng *et al.*, and the DNeasy plant extraction kit (Qiagen) method were applied with eight different fruit species such as *Mangifera indica* (Mango), *Anacardium excelsum* (Cashew nut), *Syzygium jambos* (Rose apple), *Punica granatum* (Pomaganrate), *Averrhoa carambola* (Star fruit), *Spondias dulcis* (Ambarella), *Carica papaya* (Papaya) and *Annona muricata* (Annona). Based on the quantity of the extracted genomic DNA tested by measuring the absorbance at 260 nm using Nanodrop® ND-1000 spectrophotometer, quality 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, the method explained by Cheng *et al* and the Genomic DNA extraction kit yielded good quality DNA with satisfactory concentration for all the fruit species tested. Therefore the modified method of Cheng *et al*, 1987 could be recommended for the efficient and cost effective DNA extraction from fruit species instead of the commercially available expensive and chemically hazardous DNeasy plant kit method.

Keywords: Genomic DNA Extraction, Nanodrop, TBE buffer, quality and quantity, agarose gel.

Introduction

Extraction of plant DNA in a relatively purified form is very important in further studies of plants which based on the molecular biological studies, i.e. PCR, sequencing, etc (Sunil kumar et al., 2012). DNA isolation from plant tissues/leaves is usually compromised by excessive contamination of secondary metabolites, polysaccharides and polyphenols which impede the extraction of high quality intact genomic nucleic acids (Sunil kumar et al.,2012; Hosseinpour et al., 2013). If these contaminants are not removed, it will affect further subsequent assays such as PCR (Tamari et al.,2013). Polysaccharides inhibit the activity of restriction enzyme and Taq DNA polymerase (Sunil kumar et al.,2012).The presence of polysaccharides in a DNA

sample, form a highly viscous solution through the co-precipitation with extracted DNA(Anil kumar et al.,2013).The oxidized form of polyphenols bind with DNA covalently and give a brown colour and it is not suitable for further molecular studies (Sunil kumar et al.,2012).

Extraction of DNA with higher quality and quantity yield has lead to the development and introduction of new protocols, however the fundamentals of the extraction is similar (Tiwari et al., 2012). Many tree species require highly complex protocol than other annual plants (Shepherd et al., 2002), because it is difficult to obtain DNA from trees than others (Anil kumar et al., 2013). Also a single isolation protocol is

not successful for different plant species for getting high DNA yield (Padmalatha and Prasad, 2006). As well as DNA quality and quantity are vary among the species of same genera and in different sources of tissues in same tree (Shepherd et al., 2002). Sometime different isolation protocols are required even in closely related plants (Ranganathan Kapilan, 2015). Many different methods were suggested for isolating genomic DNA from plants (Anil kumar et al., 2013). Original hexadecyl trimethyl ammonium bromide (CTAB) based method, described by Doyle and Doyle in 1987 was important for the development of the majority of DNA extraction methods (Adam Healey et al., 2014). The purification method which based on CTAB work best for a variety of different plant tissues (Michiels et al., 2003). Disruption of plasma membrane and the nuclear membrane is occurred because of the protein digestion and by the action of ionic detergents (Tamari et al., 2013; Tiwari et al., 2012). Higher concentration of Cetyl Trimethyl Ammonium Bromide (CTAB) is important for the removal of polysaccharides. EDTA prevents the degradation of DNA by chelating the Mg^{2+} which is important for enzymes to DNA degradation (Tiwari et al., 2012). Contaminants are separated in the organic phase and the nucleic acids are separated in the aqueous phase by using chloroform-isoamyl alcohol mixture (Tamari et al., 2013). Initial grinding of frozen plant tissue with liquid nitrogen ($-196^{\circ}C$) which would freeze the tissue to become fragile and make it to be a fine powder that increases the surface area of extraction. The ultimate aim of this step is to access the nuclear material without degradation (Sunil kumar et al., 2012).

Plant research at molecular level is important for assessing the diversity of plants and for improving the medicinal and economical value of the traits through breeding (Anil kumar et al., 2013). Developing DNA marker/finger prints of economically and industrially important plants is useful for making a molecular database and for analyze the information systematically. The advantages of using DNA isolation kits are they are fast, simple, involves minimal handling by extracting DNA with sufficient quality (Sunil kumar et al., 2012). Although highly purified DNA is yielded by the usage of kits, they are some serious disadvantages too. They are very expensive and scientists from developing countries and from not very equipped laboratories would not afford to purchase these kinds of expensive kits for their routine need of genomic DNA extraction (Adam Healey et al., 2014). The chemicals used in the kits are mostly toxic, hazardous and may lead to diseases to

human in the long run (Cheng et al., 2003, Kapilan, 2015). There have been thoughts and attempts to eliminate the use of hazardous chemicals and expensive kits and equipments for the future practice of genomic DNA extraction. However, owing to the practical difficulties and methodological needs, it is essential to optimize the conditions to maximize the yield and purity of DNA obtained from different kinds of samples using diverse methods. A simplified method, demonstrated in the present study, for the extraction of genomic DNA from plants that reduces unnecessary steps virtually eliminates the contamination of DNA and also substantially conserves the time duration of the analysis that would be useful to the researchers as well as to the population-based research community. Therefore, the objective of this study was to examine the quality, quantity and amplifiable capacity of genomic DNA extracted from eight different economically important fruit species by modified plant genomic DNA extraction methods explained by Doyle et al. 1987, Cheng et al. 2003, and the DNeasy plant extraction kit (Qiagen) methods and to recommend the cheap and efficient genomic DNA extraction method for these fruit species.

Materials and Methods

Plants material

Fresh young leaves (2nd and 3rd fully expanded leaves from top) from eight different fruit species of *Mangifera indica* (Mango), *Anacardium excelsum* (Cashew nut), *Syzygium jambos* (Rose apple), *Punica granatum* (Pomagrante), *Averrhoa carambola* (Star fruit), *Spondias dulcis* (Ambarella), *Carica papaya* (Papaya) and *Annona muricata* (Annona) were collected from different area of Northern Province, Jaffna District Sri Lanka and brought to the laboratory in ice box and stored at $-20^{\circ}C$ freezer. Leaves were ground using sterile mortar and pestle until they became fine powder. Time to time the addition of liquid nitrogen facilitated the grinding process. Resulted powder was stored in a sterile falcon tube at $-20^{\circ}C$ until use.

Extraction method

Genomic DNA extraction methods explained by Cheng et al., 2003, Doyle and Doyle, 1997 and the DNeasy plant extraction kit (supplied by Qiagen) method were used. There were three replicates for each fruit species for each method and experiments were repeated.

Quantitative analysis

Genomic DNA from the leaf samples were quantified by measuring the absorbance at 260 nm using Nanodrop® ND-1000 spectrophotometer.

Qualitative analysis

The ratio (A₂₆₀/A₂₈₀ nm) was calculated using Nanodrop® ND-1000 spectrophotometer to determine the purity of the DNA sample to find out whether it was contaminated with protein or not.

DNA integrity

To check the amplifiable quality and yield analysis 16 tubes (Contain 2 replicate samples of each species) were selected which contained high amount of DNA pellet. By adding a mixture of 5µl DNA and 5µl Bromophenol Blue to the separate wells, electrophoresis was done in 1% agarose gel for 40 minutes with 50V current and with 0.5X TBE buffer. The gel was stained with Ethidium Bromide and the bands were visualized under UV light. Each DNA sample was graded, according to the electrophoretic migration of sample DNA compared with a known molecular weight marker.

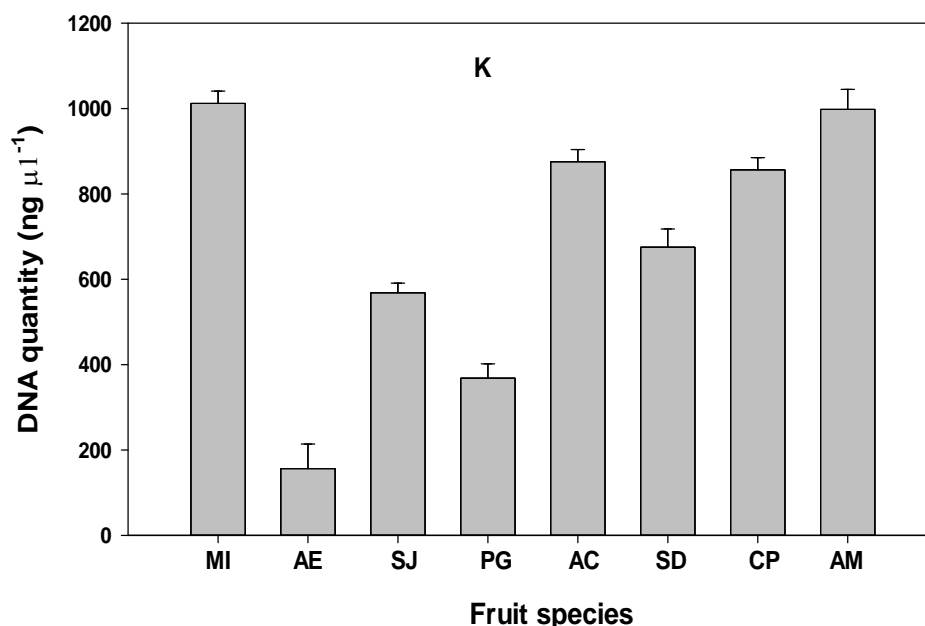
PCR analysis

The genomic DNA extracted from the fruit plants by modified Cheng et method, was adjusted to 10 ngL⁻¹. The amplification reactions were done in a total volume of 20 µL consisting of 10 ng genomic DNA,

2.5 µL of 10X buffer II, 3.75 µL of 10 mM MgCl₂, 0.5 µL of 10 mM deoxynucleotide triphosphates, 0.33 µL of 50 pM forward and reverse PIP2;5 AQP gene specific primers, 0.1 unit (µL) of Taq polymerase and 16.82µL of distilled deionized H₂O. The 10X buffer II, 10 mM MgCl₂ and Taq polymerase enzyme were all purchased from Perkin-Elmer (Foster City, CA). PCR amplifications were performed on an Eppendoff thermocycler with the following amplification conditions. 1 cycle at 94°C for 5 min, 40 cycles at 94°C for 40s, 50°C for 40s, and 72°C for 1.5 min, followed by 1 cycle at 72°C for 5 min. The amplified DNA fragments were subjected to 1% agarose gel electrophoresis (60 V; 90 min) with 0.5X TBE buffer, and visualized under ultraviolet light. The size of the amplified DNA fragments was estimated based on 100 bp DNA ladder from MBI (Amherst, NY).

Results and Discussion

Among the fruit species tested, fresh leaves of *Mangifera indica*, yielded maximum amount of DNA with overall mean of 942 ngµL⁻¹ followed by *Carica papaya* with overall mean of 936 ngµL⁻¹ (Figure 1). The DNA extraction kit method yielded maximum amount of DNA with overall mean of 687 ngµL⁻¹ for eight type of fruits, where as modified Cheng et al. (2003) method yielded maximum amount of DNA with overall mean of 768 ngµL⁻¹ and the modified Doyle and Doyle (1987) method yielded an overall mean of 337 ngµL⁻¹. Amount of DNA yield was higher in the DNA extraction kit method and lower in the modified Doyle and Doyle method.



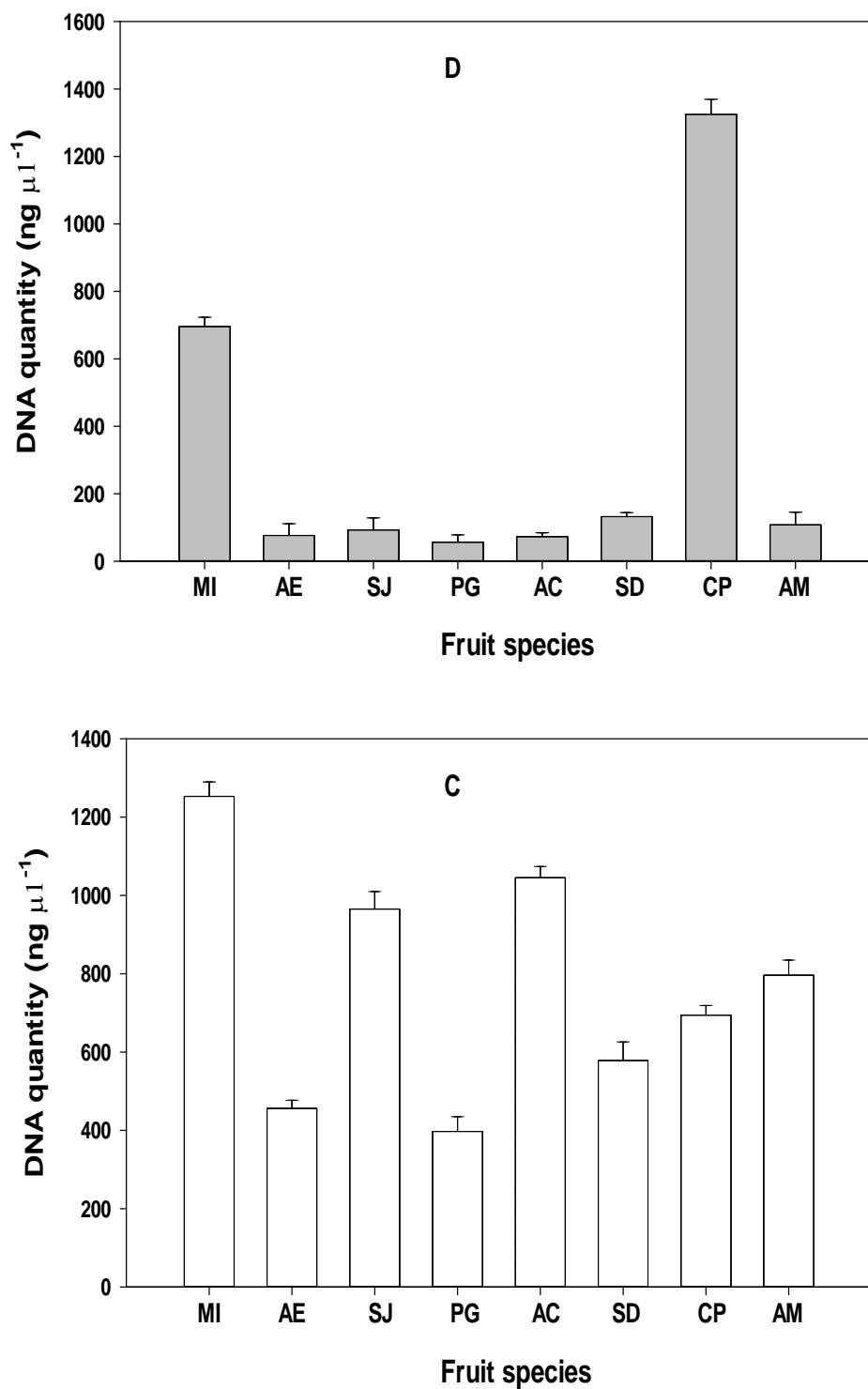
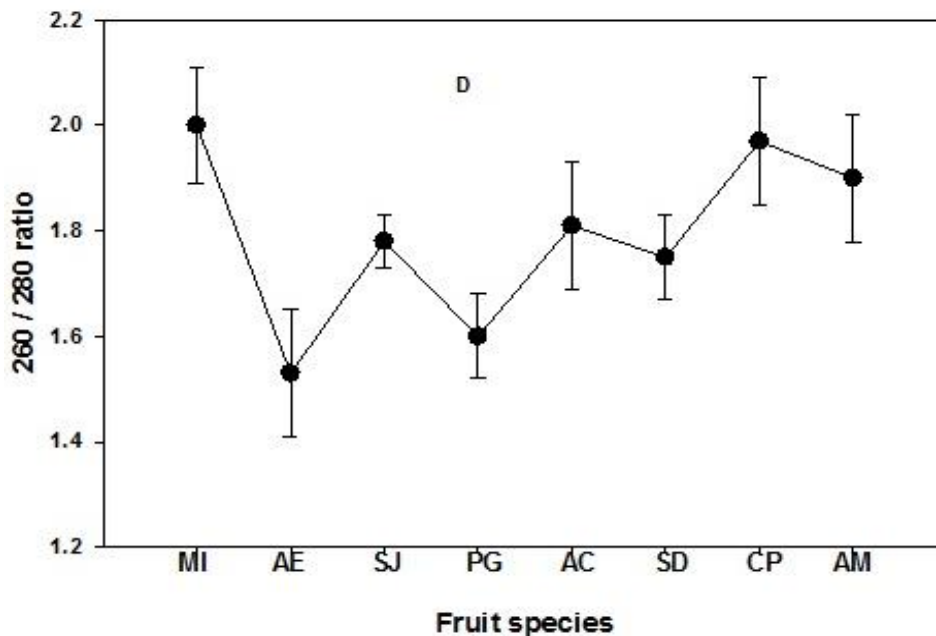
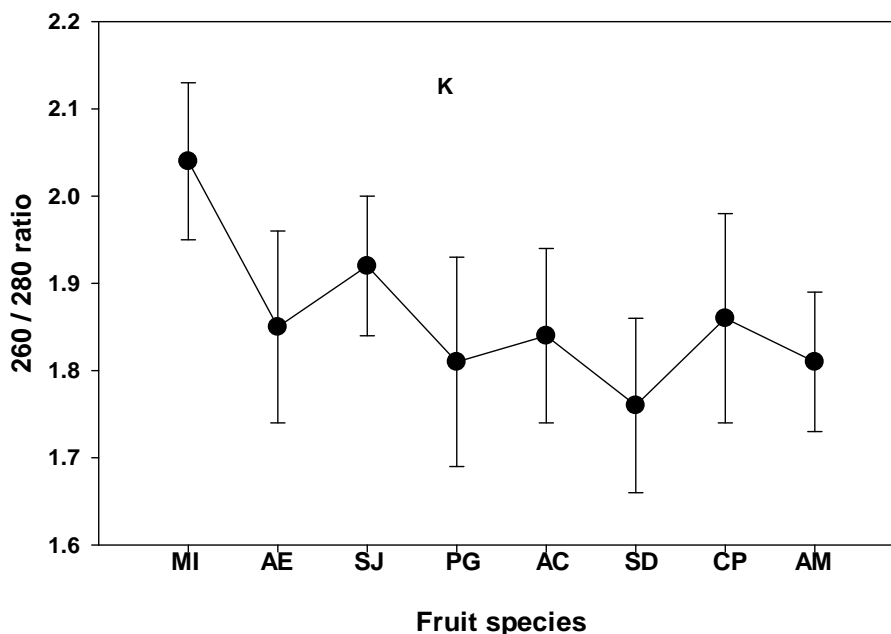


Figure 1 K, D and C: Quantity mean of DNA extracted from fruit species using different methods. Bars are marked with the first letters of the generic and specific name of the plant species. Graphs are marked with the letters corresponding to the methods K – Kit method C -Cheng et al.,2003 D - Doyle and Doyle, 1987.

Among the different extraction methods tested, the extraction kit method and Cheng et al. 2003 yielded DNA of highest quality with the mean absorbance ratio (A260:A280) of 1.84, and 1.85 respectively (Figure 2). Though the modified Doyle and Doyle method resulted in good quality of DNA over all with the absorbance ratio of 1.81, this method did yield satisfactory quality of DNA for *Anacardium excelsum* (1.52) and *Punica granatum* (1.61) species. However, this method, except for *Anacardium excelsum* and *Punica granatum*, yielded genomic DNA with satisfactory quality, for the other fruit species tested with the absorbance ratio between 1.8 and 2.0.

Among the fruit species tested, fresh young leaves of *Mangifera indica* and *Carica papaya* consistently yielded DNA with high purity ratio (A260:A280 1.8) with all the three methods investigated (Figure 2). The quantity of genomic DNA extracted by all the methods were comparatively lower in *Anacardium excelsum* and *Punica granatum* and the reason for this may be due to the small size of the young leaves and the internal morphological and physiological properties of these leaves and adaptability of the extraction methods.



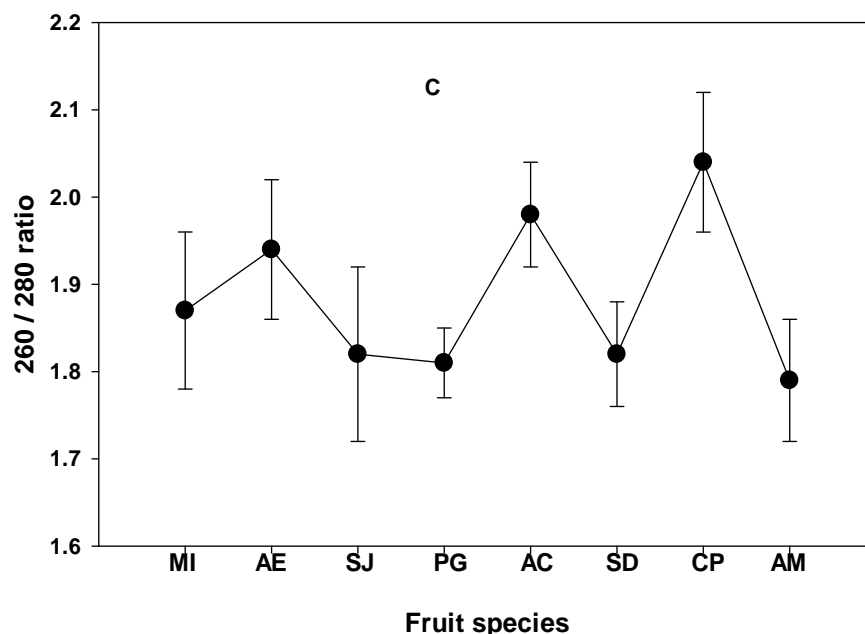


Figure 2 K, D, C: Quality mean of DNA extracted from the fruit species using different methods. Dots are marked with the first letters of the generic and specific name of the selected fruit species. Graphs are marked with the letters corresponding to the methods K – Kit method C -Cheng et al., 2003 D - Doyle and Doyle, 1987.

Gel running of samples from all the fruit species using all the three methods showed considerable amount of amplifiable quality DNA except *Anacardium excelsum* and *Punica granatum* with Doyle method (Figure 3). The present study showed that there was variation in time required for different DNA extraction methods. Next to the DNA extraction kit method, modified Cheng et al method consisted of comparatively few steps for the completion of the entire extraction process. On the contrary, modified Doyle and Doyle et al. method involved several time consuming extraction

steps and took more than 10 hours to finish the entire processes. Among the three methods investigated, all the methods extracted amplifiable DNA from all the eight plant species with some exceptions of *Anacardium* and *Punica* with modified Doyle and Doyle method (Figure 3). Failure of observing clear band on the gel from samples of *Anacardium excelsum* and *Punica granatum* using modified Doyle and Doyle method may be explained by the low purity ratio of these DNA samples indicating protein co-precipitation of extracted genomic DNA.

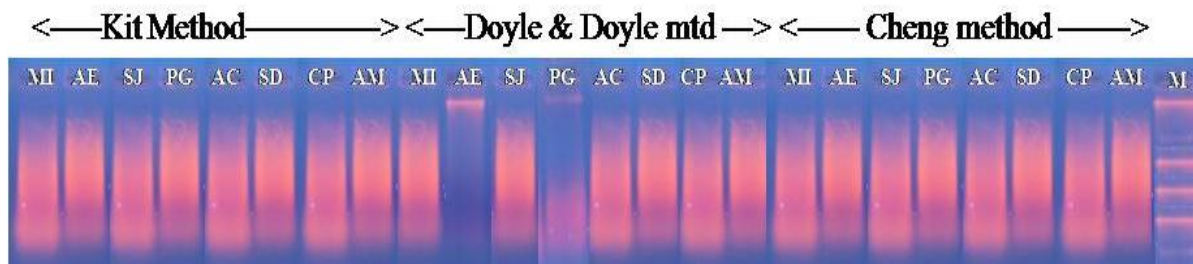


Figure 3: Bands of genomic DNA on the 1% agarose gel with 0.5X TBE buffer after visualization with UV light. Lanes are marked with the first letters of the generic and specific name of the selected fruit species. MI - *Mangifera indica* (Mango), AE - *Anacardium excelsum* (Cashew nut), SJ - *Syzygium jambos* (Rose apple), PG - *Punica granatum* (Pomaganate), AC - *Averrhoa carambola* (Star fruit), SD - *Spondias dulcis* (Ambarella), CP - *Carica papaya* (Papaya), AM - *Annona muricata* (Annona). Methods are denoted by these names K – Kit method C -Cheng et al.,2003 D - Doyle and Doyle, 1987, M - Marker

DNA quality was examined by the absorbance of DNA at 260 and 280 nm and computing A260:A280 ratio. A260:A280 ratio of more than 1.8 confirms the extraction of very good quality genomic DNA whereas values less than 1.8 indicate contamination of the genomic DNA by protein and the values more than 2.0 indicate the presence of alcohol or acetone in the DNA preparation (Ranganathan Kapilan, 2015, Webb and Knapp, 1990). DNA extraction methods and the plant species were significant sources of variation for quality of the DNA extracted (Smith et al., 2011 and Webb & Knapp, 1990). This method extracted DNA with very low purity from *Anacardium excelsum* (1.54) *Punica granatum* (1.65) which could possibly be the reason for lack of DNA amplification in these samples (Fig. 2). However reason for failure of DNA amplification from samples which had satisfactory purity ratio is not clearly understood. It is possible that such samples, even with high purity ratio, may still have trace levels of co-precipitation of phenols or

other secondary metabolites, which could not be removed by the modified Doyle and Doyle extraction method. Time and cost associated with DNA extraction and purification methods highly influence marker related studies, fingerprinting and mapping (Weising et al. 1995). Quality and quantity of DNA are critical factors in molecular marker studies. Variation among extraction methods may be due to different composition of extraction buffers, different components for precipitation and purification of DNA and the time duration to complete the procedure (Maliyakal, 1992, Weising et al., 1995). Variation in quality of DNA can be due to the genetical, structural and biochemical variation among leaf samples of different fruit species, size of the fruit that plant produce, variation in composition of the buffers used for extraction and the differences in the chemicals, their exposure time to plant tissue and the concentration of chemicals (Arumuganathan et al 1991, Maliyakal, 1992).

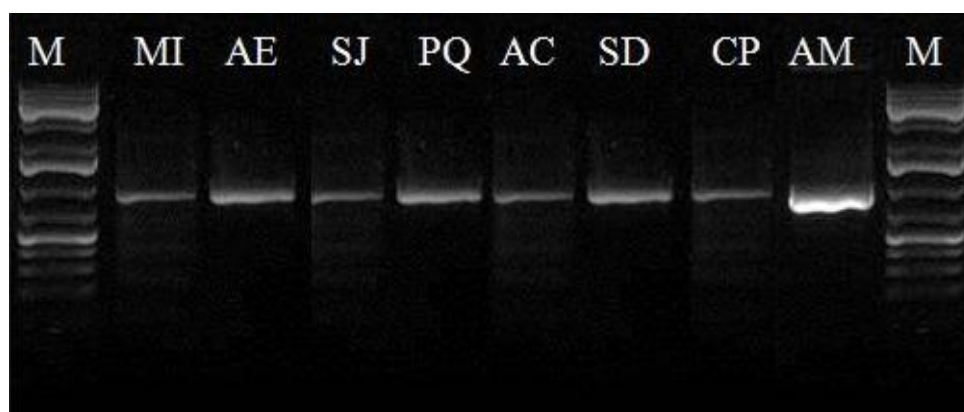


Figure 4: Gel showing the amplified PCR fragments of the genomic DNA extracted by modified Cheng et al (2003) method, using universal primers. Bands of genomic DNA on the 1% agarose gel with 0.5X TBE buffer after visualization with UV light. Lanes are marked with the first letters of the generic and specific name of the selected fruit species. MI - *Mangifera indica* (Mango), AE - *Anacardium excelsum* (Cashew nut), SJ - *Syzygium jambos* (Rose apple), PG - *Punica granatum* (Pomagranate), AC - *Averrhoa carambola* (Star fruit), SD - *Spondias dulcis* (Ambarella), CP - *Carica papaya* (Papaya), AM - *Annona muricata* (Annona). M - DNA Marker.

All the genomic DNA templates produced clear, sharp and reproducible PCR banding patterns. Figure 4 shows typical PCR results with template DNA prepared by the mini-prep method from the genotypes of the fruit species. This method has been practiced by several advanced research groups in the Europe and western America, and it has been successfully used by RAPD analyses (Tamari et al., 2013). This study recommends the need for selection of appropriate DNA extraction technique for different fruit species. A single extraction method may not be suitable to extract DNA with suitable quantity and quality from a diverse

group of economically important fruit species. Quantity, quality and amplification of extracted DNA could vary among plant species according to the extraction method chosen (Bousquet et al., 1990, Korga et al., 2007).

Conclusion

The important properties of genomic DNA such as quantity, quality, suitability for amplification and the total time required for extraction, among the three extraction methods investigated, the modified method

of Cheng et al. was the best method for all the fruit species selected for this study. Considerably high quantity of DNA was extracted using this method and it took less than six hours to complete the entire procedure. This method does not require environmentally hazardous reagents and expensive equipments and it could be performed even in low technology laboratories.

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