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Research Article

Comparison of the efficiency of the DNA extraction methods of some selected plant species

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Abstract: DNA extraction is an important aspect of plant molecular biology and the current studies require large amount of purified high molecular weight DNA with high quality. The choice of DNA extraction methods explained by Bousquet *et al*., Cheng *et al*., Doyle *et al*., and Michiels *et al*. with appropriate quantity and quality of DNA extracted from different plant species (*Oryza sativa, Nicotiana tabacum, Hibiscus esculentus, Populus alba* and *Pinus sylvestris*) grown in northern region of Sri Lanka were practiced. Based on the quantity and quality of the extracted DNA tested by measuring the absorbance of DNA at 260 nm using Nanodrop® ND-1000 spectrophotometer and measuring the ratio of A260 / A280, the efficiency of the extraction method chosen varies among plant species. Among the methods used, the method introduced by Cheng *et al*, yielded good and amplifiable quality DNA with satisfactory concentration for all the plant species tested.
Keywords: DNA Extraction, Nanodrop, PCR amplification, quality and quantity.

INTRODUCTION

Extraction of DNA is very important for the plant molecular biological investigations. Molecular marker studies in plants need large amounts of purified high molecular weight DNA. Projects that involve screening of large number of samples, require faster and high-quality DNA [5]. Hence, there is demand for rapid, simplified and inexpensive DNA extraction methods which can provide large amount of high quality DNA [6]. However, purified genomic DNA, is used in many applications in molecular genetic studies, but one method cannot be used to obtain high quality DNA from all plant species [7]. Yield and quality of DNA often varied among species within same genera as well as among tissue types from the same trees [8]. Since foliage and other tissues of trees often contain varying levels of tannins, polyphenols and polysaccharides, these impurities co-extract with DNA causing serious problems while obtaining genomic DNA and further analysis. Polysaccharides inhibit restriction enzyme digestion and Tag DNA polymerase activity [9]. Polysaccharides co-precipitate with extracted DNA and form viscous solution [9]. The DNA in viscous form cannot be used for restriction digestion reactions and Southern hybridization and it remains in the wells during electrophoresis. Consequently, many woody trees require highly complex extraction methods than the other plants [10]. There are a lot of methods being developed for isolating genomic DNA from plants in addition to the already existing methods. However, a single isolation method is unlikely to be successful for different plants [11]. Chemotypic heterogeneity among plants would not allow optimal yield with a single protocol [12]. Therefore specific protocols need to be followed for different plants. *Oryza sativa, Nicotiana tabacum, Hibiscus esculentus, Populus alba* and *Pinus sylvestris* are economically important plant species all around the globe and most of these are widely used in plant biotechnology research. These species were particularly chosen because of the great interest in their large scale plantation and utilization and molecular biological use in research. Studies at molecular level would be undertaken for determining their diversity as well as propagating for improved and economically important traits. Such studies will definitely need high yielding quality DNA. Comparative studies on evaluation of different DNA extraction methods for use in molecular biological research in these selected plant species have also not been reported. In the present study, four different DNA extraction and purification methods were evaluated for these five economically important plant species. The relative yield and purity of genomic DNA extracted from five different plant tissues, was also examined.

MATERIALS AND METHODS

Plant materials

Fresh young leaves (2^{nd} and 3^{rd} fully expanded leaves from top) from five different plant species of *Oryza sativa, Nicotiana tabacum, Hibiscus esculentus, Populus alba* and *Pinus sylvestris* were used

as DNA sources. For each method used as a first step the leaf tissue was fine ground in liquid nitrogen using mortar and pestle and resulting powder was kept in a sterile tube at -20^{o}C until use.

Extraction methods

Genomic DNA extraction methods explained by [1], [2], [3] and [4] were compared. There were three replicates for each plant species for each method and experiments were repeated.

DNA quantity and purity confirmation

Genomic DNA from the leaf samples were quantified by measuring the absorbance at 260 nm using Nanodrop® ND-1000 spectrophotometer. The ratio (A260/280 nm) was calculated to determine the purity of the DNA sample to find out whether it was contaminated with protein or not.

Gel running

The size, purity and integrity of DNA were determined by running 1 µL of total DNA from each sample on a 1% agarose gel (0.5X TBE) for 45 minutes with 60 V current and with 0.5X TBE buffer, and visualized by SYBR safe.

RESULTS AND DISCUSSION

The quantity of DNA, extracted by four different methods, significantly varied among the five plant species tested (Fig 1 – A, B, C & D). Fresh leaves of *Nicotiana tabacum*, yielded maximum amount of DNA with overall mean of 2720.2 µg g^{-1} fresh leaf (Figure 1) followed by *Hibiscus esculentus* with overall mean of 1750.2 µg g^{-1} fresh leaf (Figure 1). There was not much variation in quantities of DNA extracted from leaves of *Nicotiana tabacum, Nicotiana tabacum, Pinus sylvestris* (overall mean approx. 1500µg g^{-1} fresh leaf). The method explained by Cheng et al. [2] yielded maximum amount of DNA with overall mean of 2748.3 µg g^{-1} fresh leaf followed by the method explained by Doyle and Doyle [3] (overall mean = 1913.5 µg g^{-1} fresh leaf). DNA quantity was minimum in the method explained by Bousquet *et al* [1] with overall mean of 1258.9 µg g^{-1} fresh leaf.

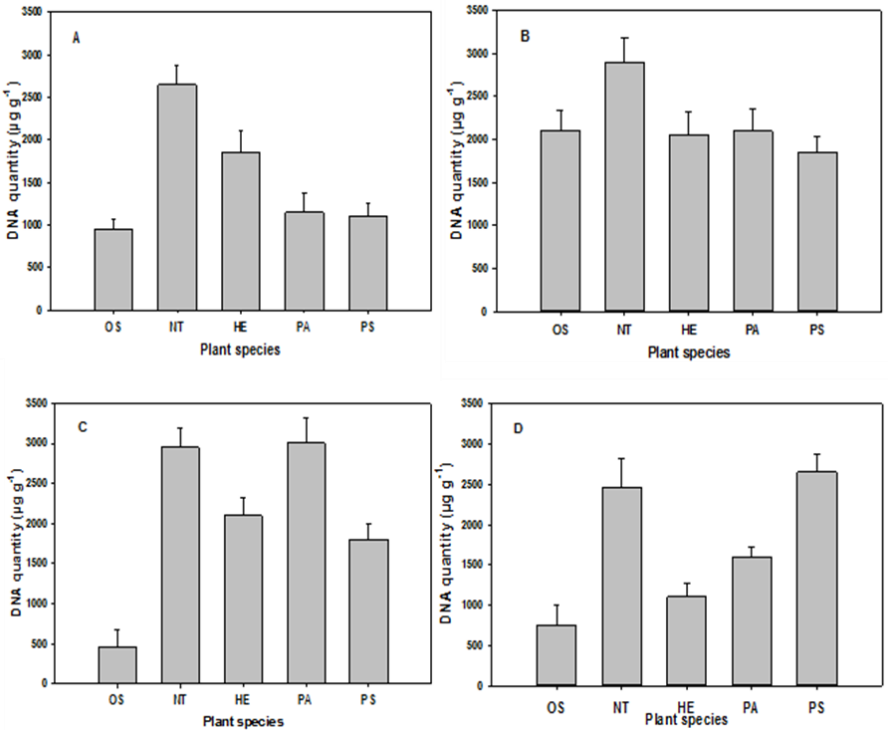

Fig-1: Quantity mean of DNA extracted from plant species using different methods. Bars are marked with the first letters of the generic and specific name of the plant species. Graphs marked with the letters corresponding to A , B, C, D. are DNA extraction methods. A - Bousquet *et al.*,1990 [1] B -.Cheng *et al.*,1997 [2] C - Doyle and Doyle, 1987 [3] D - Michiels *et al*.,2003 [4].

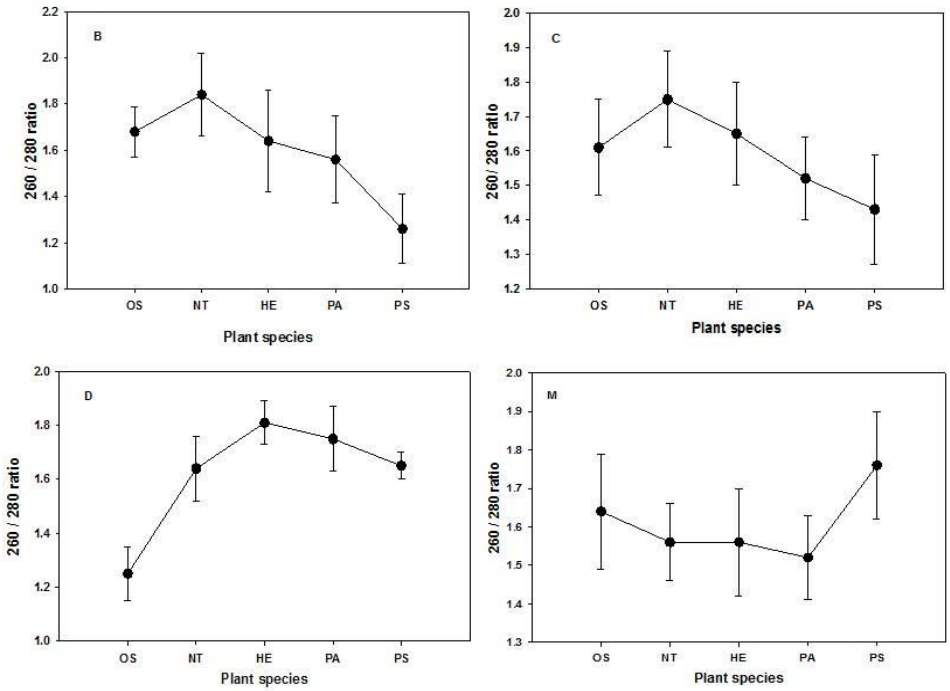

Fig-2: Quality mean of DNA extracted from plant species using different methods. Dots are marked with the first letters of the generic and specific name of the plant species. Graphs marked with the letters corresponding to the methods B - Bousquet *et al.*,1990 [1] C -Cheng *et al.*,1997 [2] D - Doyle and Doyle, 1987 [3] M - Michiels *et al.*,2003 [4].

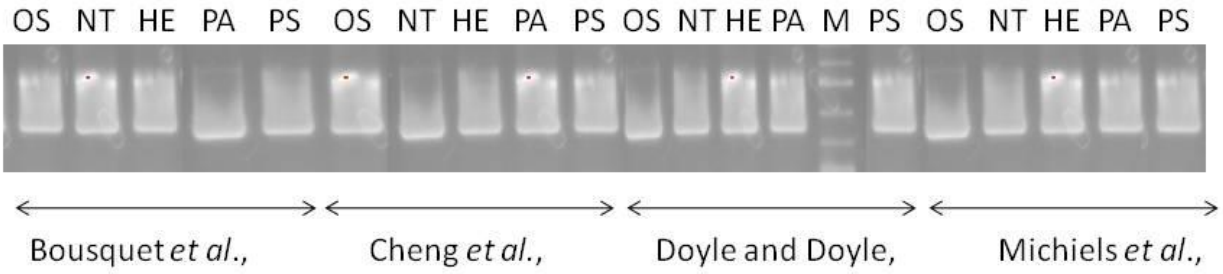

Fig-3: Genomic DNA extracted from different plant species by different methods on agarose gel. Bands are marked with the first letters of the generic and specific name of the plant species. Lanes are marked with the letters corresponding to the methods B - Bousquet *et al.*,1990 [1] C -Cheng *et al.*,1997 [2] D - Doyle and Doyle, 1987 [3] M - Michiels *et al.*,2003 [4], M – Marker.

Among the extraction methods tested, those of Bousquet et al. Michiels et al. and Cheng et al. yielded DNA of highest quality with the absorbance ratio (A_{260}:A_{280}) of 1.71, 1.64 and 1.77 respectively (Figure 2 - B, C, D M). Doyle and Doyle method resulted in the lowest quality of DNA with absorbance ratio of 1.51. However, with this method, except for *Pinus sylvestris* and *Oryza sativa*, that yielded DNA with satisfactory quality, other plant species yielded high quality DNA with absorbance ratio of about 1.7. Among the plant species, *Nicotiana tabacum* consistently yielded DNA with high purity ratio (A_{260}:A_{280} $\geq$ 1.8) with all the four methods investigated. However, *Oryza sativa* yielded DNA with the lowest purity ratio (overall mean of 1.3) (Figure 2). The quantity and quality of DNA extracted by methods other than Cheng et al, were comparatively lower in *Oryza* and this may be due to the small size of the leaves and the time and complications involved in the grinding and extraction process.

Gel running of samples from all the plant species using all the four methods showed considerable amount of amplifiable quality DNA except *Oryza* with Doyle method (Figure 3). The present study showed that there was variation in time required for different DNA extraction methods. The method explained by Cheng et al [2] consisted of comparatively few steps for the completion of the entire extraction process and was the most rapid extraction method requiring less than seven hours. On the contrary, the method explained by Bousquet [1] Doyle and Doyle[3] and Michiels et al.[4] involved several time consuming extraction steps and took more than 16 hours to finish

the entire processes. Among the four methods investigated, all the methods extracted amplifiable DNA from all the five plant species with some exceptions of *Oryza* (Fig. 3). The methods of Cheng et al., Doyle and Doyle and Michiels et al. could extract amplifiable DNA from *Nicotiana tabacum, Hibiscus esculentus, Populus alba* and *Pinus sylvestris*. Failure of observing clear band on the gel from samples of *Oryza sativa* using Doyle et al method may be explained by the low purity ratio of these DNA samples indicating protein co-precipitation of extracted DNA.

DNA quality was examined by the absorbance of DNA at 260 and 280 nm and computing A_{260}:A_{280} ratio. A_{260}:A_{280} ratio of more than 1.8 indicates high quality whereas values less than 1.8 indicate protein contamination. DNA extraction methods and the plant species were significant sources of variation for quality of the DNA extracted [7,8]. This method extracted DNA with very low purity from *Oryza sativa* (1.25) *Populus alba* (1.5) which could possibly the reason for absence of DNA amplification in these samples (Fig. 2). However reason for failure of DNA amplification from samples which had high 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 extraction method like that of Lin et al. Usually different (tricky) tissues required different protocols and different tissue preparation steps. The need for a universal procedure is urgent especially when hundreds of samples need to be analyzed. Time and cost associated with DNA extraction and purification methods highly influence marker related studies, fingerprinting and mapping [11]. 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 [12,13]. Variation in quality of DNA can be due to the genetical, structural and biochemical variation among leaf samples of different plant species, variation in types of buffers used for extraction and the difference in the extraction with varying parameters and chemicals [13,14].

CONCLUSION

This study confirms the need for selection of appropriate DNA extraction for different plant species. A single extraction method may not be suitable for extraction of DNA with suitable quantity and quality from a diverse group of plant species. Quantity, quality and amplification of extracted DNA could vary among plant species according to the extraction method chosen. The important factors such as quantity, quality, suitability for amplification and the total time required for extraction, among the five extraction methods investigated, the modified method of Cheng et al. was the best method for the plant species *Oryza sativa*, *Nicotiana tabacum, Hibiscus esculentus, Populus alba* and *Pinus sylvestris*. 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 expensive and environmentally hazardous reagents and equipment and it could be performed even in low technology laboratories [9]. The methods of Bousquet et al, Doyle and Doyle and Michiels et al. were also good to extract amplifiable DNA from above plant species except *Oryza* but they took long time to complete the entire extraction processes.

REFERENCES

1. Bousquet J, Simpson I, Lalonde M; DNA amplification from vegetative and sexual tissues of trees using polymerase chain reaction. Canadian journal of forest research, 1990; 20:254-257.
2. Cheng FS, Brown SK, Weeden NF; DNA extraction protocol from various tissues in woody species. Hort Science, 1997; 32(5):921-923.
3. Doyle JJ, Doyle JL; A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Photochemical Bulletin, 1987; 19:11-15.
4. Michiels A, Van den Ende W, Tucker M, Van Riet L, Van Laere A; Extraction of high-quality genomic DNA from latex-containing plants. Analytical biochemistry, 2003; 315(1):85-89.
5. Masuda N, Ohnishi T, Kawamoto S, Monden M, Okubo K; Analysis of chemical modification of RNA from formalin-fixed samples and optimization of molecular biology applications for such samples. Nucleic Acids Res, 1999; 27:4436–4443
6. Korga A, Wilkolaska K, Korobowicz E; Difficulties in using archival paraffin-embedded tissues for RNA expression analysis. Postepy Hig Med Dosw; 2007; 61:151–155.
7. Do N, Adams RP; A simple technique of removing plant polysaccharides contaminants from DNA. Biotechniques, 1991;10:162-166.
8. Smith B, Li N, Andersen AS, Slotved HC, Krogfelt KA; Optimising bacterial DNA extraction from faecal samples: comparison of three methods. Open Microbiology Journal, 2011;5:14–17.
9. Kapilan R; Efficient DNA Extraction Technique from Leave Tissues of Some Important Tropical Plant Species. Journal of progressive research in biology, 2015;1(1):29-32.
10. Maliyakal EJ; An efficient method for isolation of RNA and DNA from plants containing polyphenolics. Nucleic Acids Res, 1992; 20:2381-2381
11. Claassen S, du Toit E, Kaba M, Moodley C, Zar HJ, Nicol MP; A comparison of the efficiency of five different commercial DNA extraction kits for extraction of DNA from faecal samples. Journal of microbiological methods, 2013; 94(2):103-110.

12. Webb DM, Knapp SJ; DNA extraction from a previously recalcitrant plant genus. Plant Mol. Biol. Rep, 1990; 8: 180-185.
13. Arumuganathan K, Earle ED; Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep, 1991; (9):208-218.
14. Haugland RA, Brinkman N, Vesper SJ; Evaluation of rapid DNA extraction methods for the quantitative detection of fungi using real-time PCR analysis. J. Microbiol. Methods, 2002; 50**:**319-323.