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Research Article

DNA EXTRACTION TECHNIQUE FOR DIFFERENT RICE VARIETIES GROWN IN SRI LANKA

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Abstract

Extraction of DNA is very important nowadays in bio-molecular researches. Extracted DNA should be purified and the quality of DNA should also be very high. The objective of the study was to develop a simple efficient method to isolate DNA from the rice varieties in an open laboratory environment, and to eliminate the usage of expensive chemicals and tools. The DNA extraction methods developed by the DNeasy plant kit method supplied by QIAGEN, Cheng *et al.*, Doyle *et al.* and Michiels *et al.* were applied to five different rice varieties grown in different parts of Sri Lanka. 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 and gel running on agarose, the efficiency of the extraction method chosen varied among rice varieties. Among the methods used, the methods introduced by DNeasy plant kit method supplied by QIAGEN and Cheng *et al.*, yielded good and amplifiable quality DNA with satisfactory concentration for all the rice varieties tested. Therefore the modified method of Cheng *et al.*, 1987 could be used to extract DNA from rice varieties instead of the commercially available expensive and hazardous DNeasy plant kit method supplied by QIAGEN.

Key words: DNA extraction; Rice; DNeasy plant kit; Nanodrop; ratio of A₂₆₀ / A₂₈₀

Introduction

It is very important to extract pure, intact, and high-quality DNA for molecular biology studies (Tan and Yiap, 2009). Generally extraction of DNA from plants is usually very critical because of the excessive contamination by secondary metabolites and the steps involved (Sangwan *et al.*, 1998). The DNA extraction methods are modified to improve the quality and quantity of DNA. Therefore the protocols differ among plant species and plant tissues and this may be due to the presence of secondary metabolites, structure of the plant tissues (Peterson *et al.*, 1997). The efficient DNA extraction method for diverse plant varieties is needed to generate pure DNA samples with good quality and satisfactory quantity (Peterson *et al.*, 1997; Porebski *et al.*, 1997). Some plants develop stress resistance by synthesizing higher amount of polysaccharides, polyphenols, alkaloids and flavanoids like secondary metabolites that could affect the DNA extraction (Cheng *et al.*, 2003). DNA degradation or shearing of DNA are major issues uncounted during the purification of most of the plant DNA and endonucleases directly or indirectly interfere with the DNA causing the damage (Weishing *et al.*, 1995). Polysaccharides inhibit the activities of the Taq polymerase and restriction endonucleases (Michiels *et al.*, 2003). The presence of polysaccharides in the DNA sample is identified by the formation of the viscous solution (Fang *et*

al., 1992). The oxidized form of polyphenols covalently binds to DNA giving a brown colour complex and reduces the life of DNA (Xu *et al.*, 2004). Apart from the traditional extraction approaches, diverse commercial kits are used to extract genomic DNA from plants with efficient quality (Pandey *et al.*, 1996). The chemicals used in the kits are mostly toxic, hazardous, expensive, and require special containment facilities to maximize personnel safety and minimize environmental concerns. However, the convenience provided by these methods may be cost prohibitive when considering experiments with limited financial resources. Attempts are made to eliminate the use of hazardous chemicals, expensive kits, equipment, and labor-intensive steps for high throughput DNA extraction. Demerits of these methods are limited shelf life, low purity, low recovery, and poor amplification (Kapilan, 2015a; Doyle and Doyle, 1987). The study was aimed to develop a simple efficient method to isolate DNA from the rice varieties in an open laboratory environment, and to eliminate the usage of expensive chemicals and tools.

Materials and Methods

Plant materials

Fresh young leaves (2nd and 3rd fully expanded healthy leaves from top) from five different rice plant varieties belong to *Oryza sativa* (L) such as Mooddakaruppan, H4,

Periyavellai, 500-1 and IR8 were used as DNA sources. They were harvested in the Jaffna district (300 M to 1200 M above mean sea level), and brought to the laboratory in ice bags. For each method used as a first step the leaf tissue was fine ground in liquid nitrogen using sterile mortar and pestle and resulting powder was kept in a sterile tube at -20°C until use.

Extraction methods

Genomic DNA extraction methods demonstrated by (1). DNA extraction Kit (QIAGEN) (2). Cheng *et al.* 1987 (3). Doyle and Doyle, 1987 and (4). Michiels *et al.*, 2003 were employed for the comparison. There were three replicates for each rice variety 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 ($A_{260/280}$ 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, integrity and amplifiable quality of DNA were determined by running 1 μ L 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

Quantity of DNA

The quantity of DNA extracted by four different methods, significantly varied among the five rice varieties tested. Fresh leaves of H4, yielded maximum amount of DNA with overall mean of 2750 μ g/g fresh leaf followed by IR8 with the overall mean of 2460 μ g/g fresh leaf. There was not much variation in quantities of DNA extracted from leaves of other three varieties. The method of Cheng *et al.*, 1987 yielded maximum amount of DNA with overall mean of 2450 μ g/g fresh leaf followed by the DNA extraction kit (2550 μ g/g fresh leaf). DNA quantity was comparatively minimum in the method of Doyle and Doyle (Doyle and Doyle, 1987) with overall mean of 2150 μ g/g fresh leaf (Fig. 1).

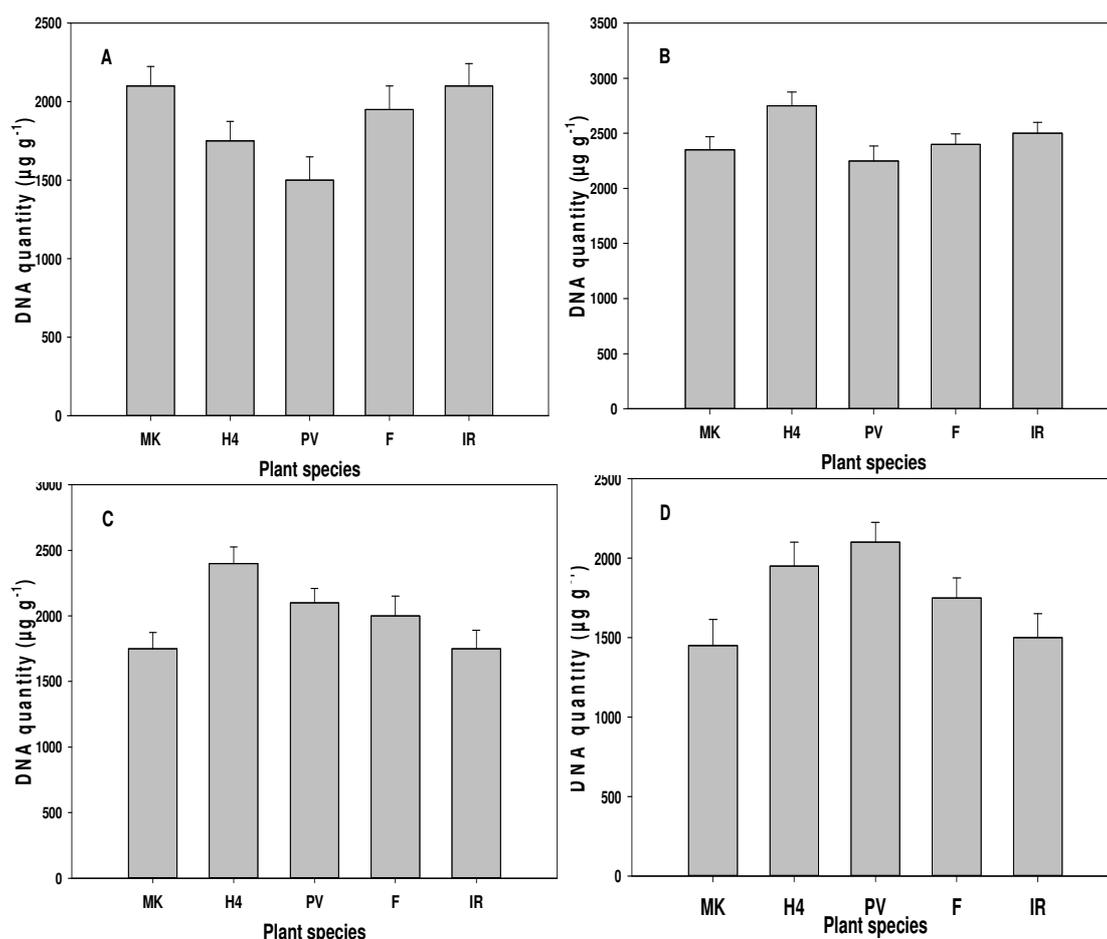


Fig. 1: Extracted DNA quantity means of different rice varieties of Sri Lanka (Letters MK, H4, PV, F and IR denotes Moondaikaruppan, H4, Periyavellai, 500-1 and IR8. A – Doyle method, B – Kit method, C- Cheng method & D Michiel method).

Quality of DNA

Among the extraction methods tested, DNA extraction Kit (QIAGEN) method, Chang method and Doyle and Doyle method yielded DNA of highest quality with the absorbance ratio ($A_{260}:A_{280}$) of 1.72, 1.74 and 1.69 respectively. Doyle and Doyle method resulted in the lowest quality of DNA with absorbance ratio of 1.52 (Fig. 2). However, with this method, except for IR8 and Periya Vellai that yielded DNA with satisfactory quality, other varieties yielded high quality DNA with absorbance ratio of about 1.7. Among the rice varieties, H4 consistently yielded DNA with high purity ratio ($A_{260}:A_{280} \geq 1.8$) with all the four methods investigated. Gel running of samples from all the plant species using all the four methods showed considerable amount of amplifiable quality DNA except Periya vellai with Doyle method. The present study showed that there was variation in time required for different DNA extraction methods. The method of Cheng *et al.*, 1987 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 of Bousquet *et al.*, Doyle and Doyle, 1987 and Michiels *et al.*, 2003 involved several time consuming extraction steps and took more than 16 hours to finish the entire processes. The protocols differ among rice varieties and plant species and the tissues and this may be due to the production of secondary metabolites and complicated plant structures (Peterson *et al.*, 1997). DNA isolated by Chang method and Kit method yielded, reproducible and consistent amplification products proving its compatibility for PCR applications using SSR primer. Long-tail surfactants such as CTAB in the Doyle method change the conformation in the DNA from “random coil” to “compact globule”, facilitating the DNA precipitation efficiently. Phenols and related compounds are very good oxidizing agents and bind covalently to the extracted DNA. This will make the DNA useless for most of molecular manipulations (Doyle and Doyle, 1987; Padmalatha and Prasad, 2006).

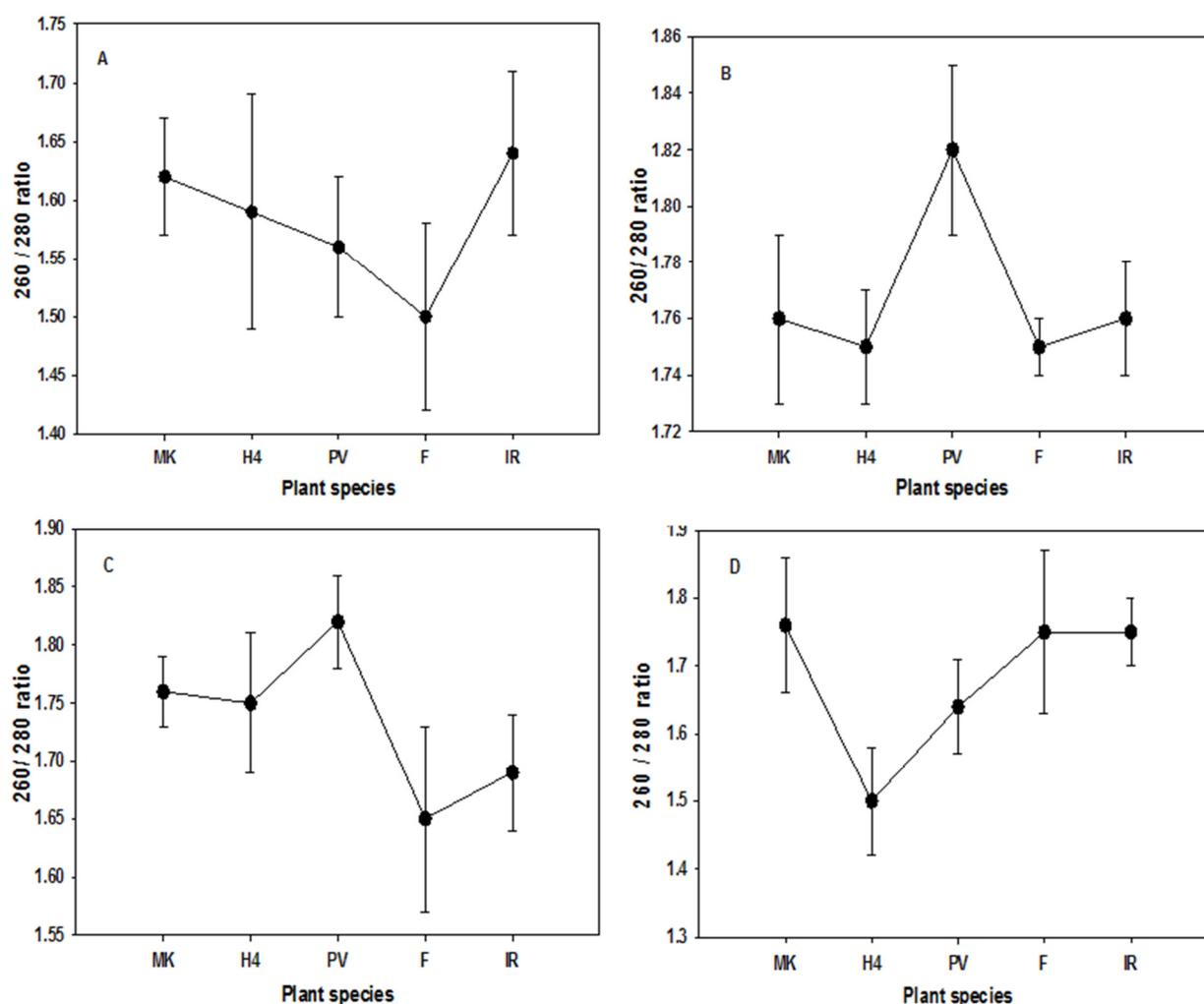


Fig. 2: Means of the 260/280 ratio for DNA obtained for different rice varieties (Letters MK, H4, PV, F and IR denotes Moondaikaruppan, H4, Periyavellai, 500-1 and IR8. A – Doyle method, B – Kit method, C- Cheng method & D – Michiels method).

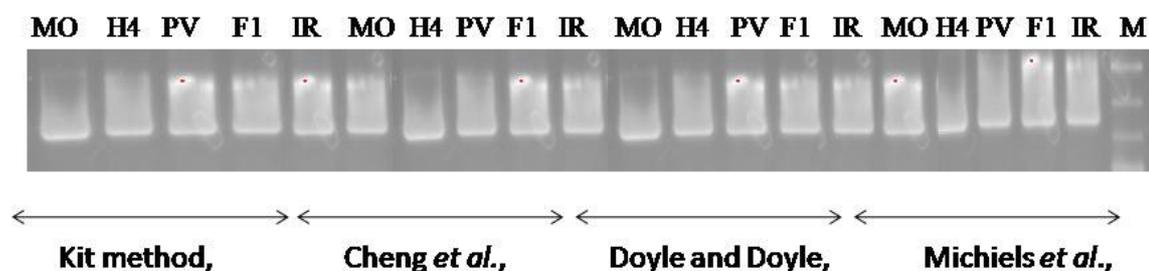


Fig 3: Bands of DNA on the 1% agarose gel with 0.5X TBE buffer after visualization with SYBR safe. M- Marker Other alphabets indicate the first letter of the generic and species names of the plants used (Letters MK, H4, PV, F and IR denotes Moодаikaaruppan, H4, Periyavellai, 500-1 and IR8. M - 1 kb DNA ladder)

Amplification quality DNA

Gel running of samples from all the rice varieties using all the four DNA extraction methods showed satisfactory amplifiable quality of DNA (Fig 3). This study showed that there was variation in time required for different DNA extraction methods. The method of Cheng *et al.*, 1987 and DNA extraction Kit (QIAGEN) method both consisted of comparatively few steps for the completion of the entire extraction process (less than seven hours). Variation in quality of DNA can be due to the genetics, 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 (Kapilan, 2015b).

Conclusion

Among the methods used, the method introduced by DNeasy plant kit method supplied by QIAGEN and Cheng *et al.*, yielded good and amplifiable quality DNA with satisfactory concentrations for all the rice varieties tested. This modified method of Cheng *et al.*, 1987 could also be used to extract DNA from other plants after testing the quality of DNA, instead of the commercially available expensive and hazardous DNeasy plant kit method supplied by QIAGEN.

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