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Efficient DNA Extraction Technique from Leave Tissues of Some Important Tropical Plant Species

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Abstract

Extraction of DNA is very important aspect in the plant molecular biological investigations. In this study, a large number of samples such as fresh leaves obtained from different plant species Oryza sativa, Elusine korakkana, Zea mays, Azadirachta indica, Musa sapianatum, Sesbania grandiflora were taken and faster method of Lin Rong et al (2001) applied for DNA extraction that can provide huge amount of high quality DNA determined by horizontal agarose gel electrophoresis using 1% agarose in TBE buffer at constant voltage of 60V. Purity was measured by the ratio of 260/280nm near to 1.8 by using Nanodrop® ND-1000 spectrophotometer.

Keywords: DNA Extraction; Quality; Purity.

Introduction

Studies that involve screening of large number of samples require faster methods that reliably yield high-quality DNA (Csaikl et al 1998). Hence, there is demand for rapid, simplified and inexpensive DNA extraction/purification methods which can provide large amount of high quality DNA (Weising et al 1995). However, purified genomic DNA, often required for many applications in molecular genetic studies, is much more difficult to obtain from trees than other plants (Shepherd et al 2002). Yield and quality of DNA often varied among species within same genera as well as among tissue types from the same plants (Henry, 2001). Since leaf and other tissues of plants often contain varying levels of tannins, polyphenols and polysaccharides, these impurities co- extract with DNA posing serious problems while obtaining genomic DNA. Such impurities also interfere in further DNA analysis. Several methods are available and are being developed for isolating genomic DNA from plants. However, a single isolation method is unlikely to be successful for different plants (Loomis, 1974). Chemotypic heterogeneity among plants samples also would not allow optimal yield with a single protocol, and hence, specific protocols need to be followed for different plants. Among the DNA extraction methods available in practice, the method developed in 2001 by Lin Rong et al was very efficient in terms of quality and quantity (Anil Kumar et al, 2013). In this study, relative yield and purity of genomic DNA extracted from leaf tissues of different plants using the modified method, developed by Lin Rong et al 2001, were compared.

Materials and Methods

Plants Material

Fresh leaves obtained from different plant species Oryza Sativa, Elusine Korakkana, Zea Mays, Azadirachta Indica, Musa Sapianatum, Sesbania Grandiflora were used as sources of DNA. All the above plants species are collected from local area of Northern Province, Jaffna District Sri Lanka.

DNA Extraction

The method based on SDS for extraction of genomic DNA was compared. The grind the fresh leaves 100mg with 1ml of 60ºC preheated extraction buffers; incubate the sample at 60ºC for 60 min to avoid aggregation of the homogenate. Add 500µl chloroform: Isoamyl alcohol ratio 24:1 to the extract and mix well. Now centrifuge at 12000rpm for 10min., transfer upper phase to clean tubes and mix 2/3 volume of isopropanol and incubate at in freeze for overnight

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to precipitate the nucleic acid. Again centrifuge at 12000rpm for 10min. gently pours off the supernatant and add 500µl wash buffer. Rinse the pellet with wash solution and incubate at room temperature for 15min .centrifuge at 12000rpm for 5min. Pour off supernatant and the pellet to dry at RT, Resuspend pellet in 100µl TE buffer and incubate at 37ºC with RNase A to concentration of 10µl/ml for 30 min. Add one volume of phenol: chloro form: isoamyl alcohol ratio 25:24:1 and mix vigorously to form an emulsion and centrifuge for 5min at 12000rpm. Transfer the upper phase in new tube. Add 2.5 M Ammonium acetate pH 7.7 and two volume of cold ethanol to mix and incubate on ice for 10min. Precipitate by centrifuge at 12000rpm for 10min. Rinse the pellet twice with 70% ethanol, Air dry 1-2 hours. Resusped pellet in 100µl TE buffer.

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

Results

DNA Quantity and Quality

Fresh leaves of Sesbania grandiflora yielded maximum amount of DNA with overall mean of 2850.4 µg g^{-1} fresh leaf (**Fig. 1**) and the minimum yield was obtained from the leaves of Musa sapianatum with overall mean of 1100.3 µg g^{-1} fresh leaf (**Fig.1**). Among the plants tested, Sesbania grandiflora and Elusine korakkana yielded DNA of the highest quality with the absorbance ratio (A_{260}:A_{280}) of 1.92 and 1.84 respectively, while yielded lowest quality DNA with the absorbance ratio of 1.15 (**Fig. 2**).

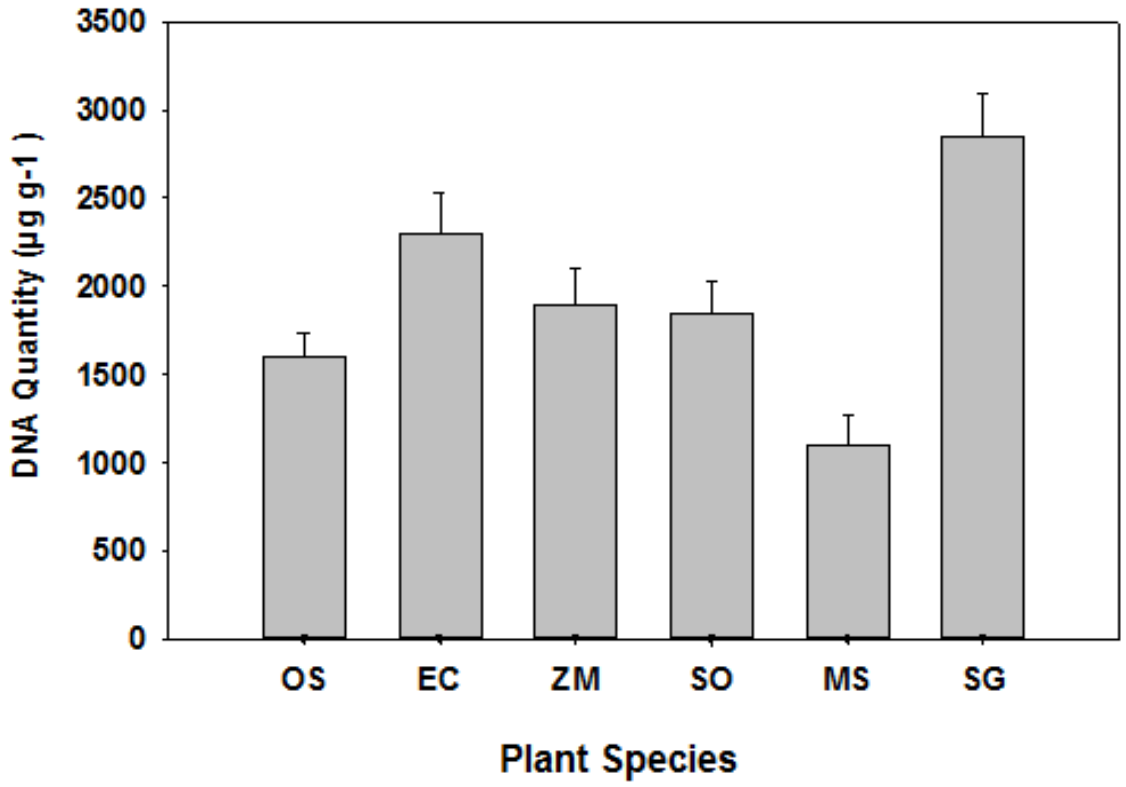

Figure 1: DNA quantity means of different plant species

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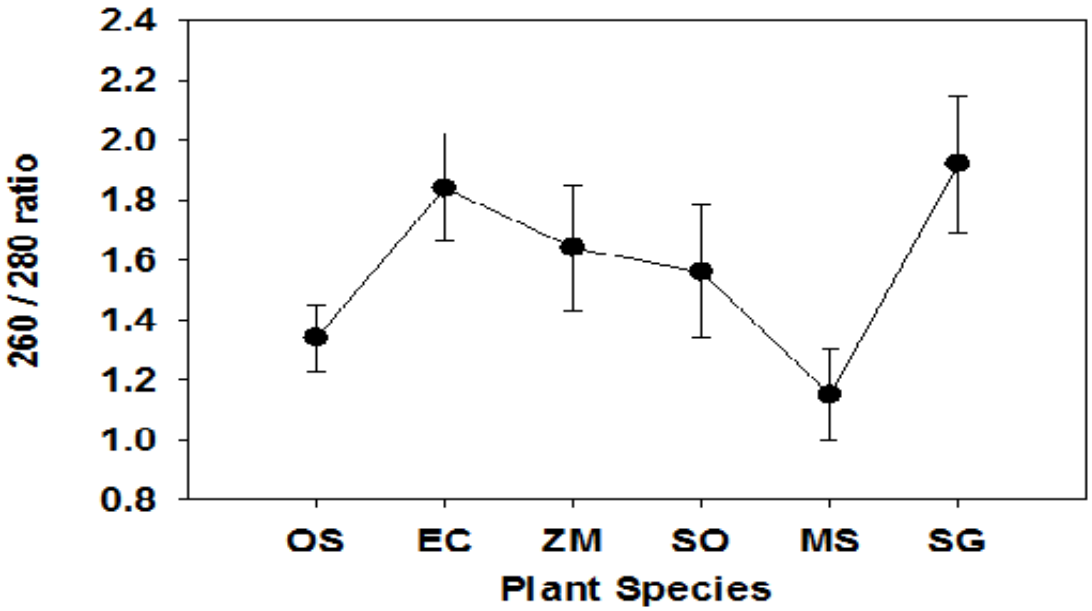

Figure 2: Means of the 260/280 ratio obtained for different plant species

Gel Running in Electrophoresis

Among the six plants investigated, all the plant species produced amplifiable DNA (Fig. 3). This method yielded DNA with high purity ratio 1.76 (**Fig 3**).

Figure 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

Discussion

Variation among extraction methods could be possibly due to varied composition of different plant tissue, extraction buffers, varied components and parameters for precipitation and purification of DNA. For example, the method of Lin Rong et al. (2001) uses SDS buffer for DNA extraction and when compared with Cheng et al (1997) few steps for completion of the entire extraction process. Quality (or purity) of DNA was examined by recording the absorbance of DNA preparations at 260 and 280 nm and computing A260:A280 ratio using Nanodrop® ND-1000 spectrophotometer. A260:A280 ratio of more than 1.8 indicates high quality whereas values less than 1.8 indicate protein contamination. DNA extraction methods and tree species were significant sources of variation for quality of extracted DNA (Arote and Yeole, 2010, Shepherd et al 2002 & Anilkumar 2013a,b). Variation in quality of DNA can be due to the genitical, 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.

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DNA extraction methods and plant species influenced PCR amplification of extracted DNA and gel running (Anil kumar 2013a,b). Production of good amplification from all the samples using this method may be demonstrated by the high purity ratio of these DNA samples indicating very low or no protein co-precipitation of extracted DNA. The equipment needed for electrophoresis basically consists of two items, a power pack and an electrophoresis unit. The equipment described here is for low voltage work. The power pack provides a stabilized direct current and has controls for both voltage and current output. For the low voltage use, power packs are available with an output of 0-500V and 0-150 mA and can give either constant voltage or constant current. The electrophoresis unit contains the electrodes, buffer reservoirs, a support for the electrophoresis medium and a transparent insulting cover. Stainless steel electrodes can be used, but some buffers cause corrosion and platinum electrodes are more satisfactory. The two buffer reservoirs are normally portioned into two sections, the electrode and wick compartments. Electrical contact between the buffers in the two compartments was maintained by small holes or slots in the partition between the compartments or by mean of porous contact between the supporting medium always saturated in buffer prior to electrophoresis and the buffer in the reservoirs was normally maintained.

Conclusion

In this study, a single method of DNA extraction was applied from leaves of different selected plant species. That way some steps were modified in present SDS base DNA extraction method of Lin Rong et al 2001 and it turned out to be a suitable method for extraction of DNA from leaves. While various plants leave have different chemicals contained molecules and hardness such as mucilage and phenolic compounds create difficulty in DNA extraction. Usage of this modified method of DNA extraction improved the quality and quantity of extracted DNA in Sesbania grandiflora than any other plants tested.

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