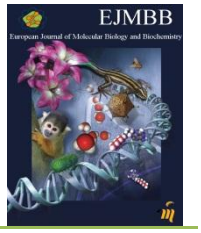
e - ISSN - 2348-2206
Print ISSN - 2348-2192

European Journal of Molecular Biology and Biochemistry

Journal homepage: www.mcmed.us/journal/ejmbb



A COMMON METHOD APPLY FOR DNA EXTRACTION FROM LEAVE OF SOME IMPORTANT PLANTS SPECIES

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Article Info
Received 23/07/2014
Revised 16/08/2014
Accepted 09/08/2014

Key words:- SDS, DNA, TBE, UV-spectroscopy.

ABSTRACT
In this investigation of a large number of samples such as Fresh leaves obtained from different tree species viz., Azadirachta indica, Bixa orellana L, Elaeocarpus ganitrus, Emblica officinalis, Mimosa pudica, Pongamia pinnata, Rosa rubigenos, Saraca asoca, require faster methods of Lin et al at SDS based applied for DNA extraction and purification method which can provide huge amount of high quality DNA determined by horizontal agarose gel electrophoresis using 1% agarose in TBE buffer at constant voltage of 100V and purity measured on 260/280nm of ratio near to 1.8 by UV-spectrometer.

INTRODUCTION

Projects that involve screening of large number of samples, such as evolutionary and breeding studies, require faster methods that reliably yield high-quality DNA [1]. Hence, there is demand for rapid, simplified and inexpensive DNA extraction/purification methods which can provide large amount of high quality DNA [2]. However, purified genomic DNA, often required for many applications in molecular genetic studies, is much more difficult to obtain from trees than other plants [3]. Studies have shown that yield and quality of DNA often varied among species within same genera as well as among tissue types from the same trees [4]. Since foliage and other tissues of trees 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.

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However, a single isolation method is unlikely to be successful for different plants [5]. 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. The relative yield and purity of genomic DNA extracted from some different plant tissues, which are potential sources of DNA, was also examined.

Brief Descriptions of Plants

Emblica officinalis is pale yellowish fleshy globose fruits, leaves are narrow and pinnate [6,7]. *Azadirachta indica* is extensively used for the treatment of several diseases, All parts of the tree i.e. leaves, flowers, seeds, fruits, roots and bark possess medicinal properties. [8,9]. *Bixa orellana* L. is known for reddish orange dye annatto that is produced in aril portion of its seeds, which is widely used in industry, as cosmeceutical and dying leather [10].

Mimosa pudica L. is quadric pinnate, often reddish, leaflets 15 to 25 pairs, Flowers of this plant are pink in colour, Fruits of mimosa are pods, mostly plants

part has medicinal properties [11]. *Pongamia pinnata L.* is found all over the world. It is commonly known as traditional system of medicines for the treatment of varied diseases of human beings [12]. *Elaeocarpus ganitrus*, is fourmal growing in tropical and sub-tropical area it's has been several types medicinal values like antibacterial. *Rosa rubigenos* has been economically values in industries and medicinal properties. *Saraca asoca* means sorrow less is an important tree in the culture of the tradition and also has been medicinal values against diabetes, fiver.

MATERIALS AND METHODS

Collection of Plants material

Fresh leaves obtained from different tree species viz., *Azadirachta indica*, *Bixa orellana* L, *Elaeocarpus ganitrus*, *Emblica officinalis*, *Mimosa pudica*, *Pongamia pinnata*, *Rosa rubigenos*, *Saraca asoca* were used as sources of DNA. All the above plants species are collected from local area of Betul district, Madhya Pradesh, India.

DNA extraction methods

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 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: chloroform: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. Genomic DNA from the leaf samples were quantified by measurement of sample absorbance at 260 nm with a spectrophotometer (UV-VIS Spectrophotometer elico, India). The purity of genomic DNA was evaluated by measuring absorbance data (A260/280 nm). The size, purity, and integrity of DNA were determined by horizontal agarose gel electrophoresis using system supplied by Genie (India), using 1% agarose in TBE buffer at constant voltage of 100V for 30min to 1 hour.

RESULTS AND DISCUSSIONS

DNA quantity

Variation among extraction methods could be possibly due to varied composition of extraction buffers, varied components and parameters for precipitation and purification of DNA. For example, the method of Lin et al. (2001) uses SDS buffer for DNA extraction and comparatively few steps for completion of the entire extraction process.

DNA quality

DNA quality (or purity) was examined by recording the absorbance of DNA preparations at 260 and 280 nm and computing A260:A280 ratio. 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. [13,14].

Figure 1. Quantities of DNA

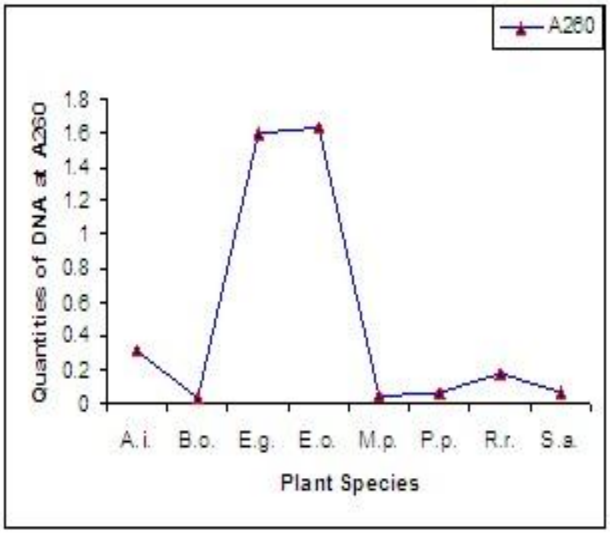


Figure 2. Quality of DNA

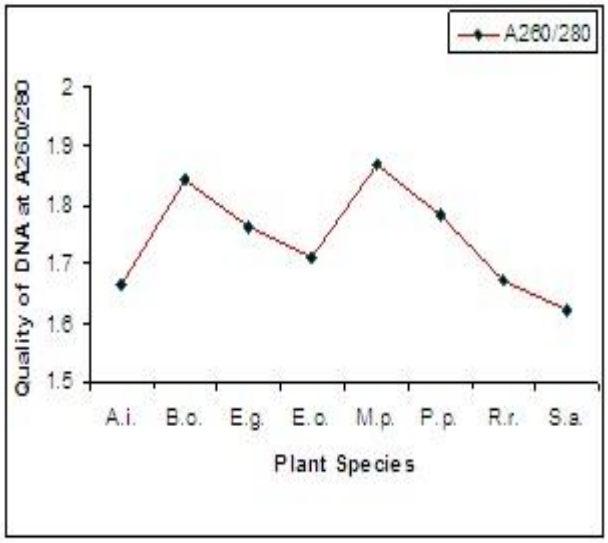
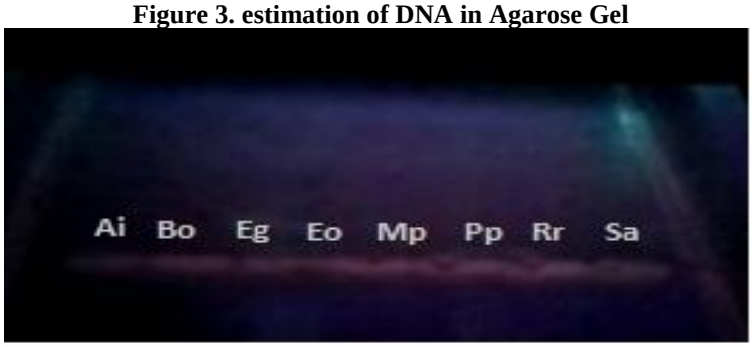


Figure 3. estimation of DNA in Agarose Gel



Estimation of DNA by 1D Gel Electrophoresis

The equipment required for electrophoresis consists basically 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 low voltage use ,power packs re 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 is 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 is normally maintained.

CONCLUSION

In this research investigation applied a single method of DNA Extraction from different selected plant species leaves. That way some steps modified in present SDS base DNA Extraction method of Lin et al [15]. it is suitable 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. The modified method are applied to DNA extraction and their good quality and quantity improved by using spectrophotometer and also estimation of DNA band in 1D Agarose gel electrophoresis visualized under UV Trans-illuminator.

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