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High quality genomic DNA from *Lens culinaris* without liquid nitrogen

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ABSTRACT

Lens culinaris Medikus also known as *Lens esculenta* Moench., lentil or masur is polyphenols, saponins and carbohydrates rich food grain. The polysaccharides and polyphenols interfere in (deoxyribonucleic acid) DNA extraction and isolation which is an important and basic step for molecular, biological and genetic study of a plant. The present study was an attempt to isolate high quality genomic DNA from *Lens culinaris* Medikus leaves using modified Cetyl trimethyl ammonium bromide (CTAB) method without using liquid nitrogen. The genomic DNA was isolated from leaves using absolute ethanol and chloroform ethanol solution as fixatives and was quantified using spectroscopic and agarose gel electrophoresis method. The concentration of DNA extracted from the leaves of the *Lens culinaris* Medikus using absolute ethanol and chloroform ethanol solution as fixatives was found to be in the range of 2.08- 2.30 μg/ ml. The DNA isolated using absolute ethanol was better in quality as it showed better optical density. DNA was observed as blue band on agarose gel. This method is rapid, simple and efficient for isolating DNA from plants rich in phenolic compounds without the use of liquid nitrogen.

KEYWORDS

Agarose gel electrophoresis;
Genomic (deoxyribonucleic acid) DNA;
Lens culinaris Medikus leaves;
Spectroscopic analysis.

INTRODUCTION

A high level of secondary metabolites such as alkaloids, flavanoids, phenols, gummy polysaccharides, terpenes and quinones are obtained from plants that possess nutritive as well as biological or pharmacological value[1-4]. The DNA acts as the regulatory code for the production of these secondary metablolites. Thus to get huge benefits from the plants, the basic need is to study their genetic based phytochemistry where, extraction and isolation of DNA is the most important step[5,6]. But, many of these constituents make the DNA unusable for downstream work in molecular biological studies[7-9]. Polysaccharides are visually evident in DNA extracted by their viscous, glue-like texture and make the DNA unmanageable in pipetting and unamplifiable in the polymerase chain reaction (PCR) by inhibiting Taq polymerase activity[10,11]. The oxidised polyphenols covalently bind to DNA giving brown colour and reduce their maintenance time[1,12,13]. Search for an efficient means of extracting DNA from plants depending upon its biochemical composition has lead to develop-

ment of a variety of protocols, though the fundamental of extraction remains the same[14]. Therefore, an effective technique is required to gain high quality and yield of DNA from lentil leaves which may help in upgrading the present status of plant biotechnology.

Lens culinaris Medikus, commonly known as masur or lentils, is rich in polyphenols, carbohydrates and saponins[15]. The delicate leaves and presence of phytoconstituents make the extraction of DNA from this plant highly difficult. The present study was an attempt to isolate the genomic DNA of Lentils from leaves.

MATERIALS AND METHODS

Collection of plant material

Plant of *Lens culinaris* Medikus was collected from Yamuna Nagar, Haryana and authenticated by Mr. S. K Srivastava, Scientist, Botanical Survey of India, Dehradun, India with the voucher specimen No. BSI/ NRC/330.

Extraction and isolation of genomic DNA

Preparation of reagents

The reagents prepared to extract and isolate the Genomic DNA were Cetyl trimethyl ammonium bromide (CTAB) 20% solution, Tris- hydrochloride (Tris-HCl) buffer (pH 8.0) - 1M solution, Ethylene diamine tetra acetic acid (EDTA) 0.5 M solution, Sodium chloride (NaCl) 5M solution, Sodium acetate solution (pH 5.2)- 3 M solution (Wash Buffer), 2- marcapto ethanol (2% solution), DNA extraction Buffer (100 ml) consisting of CTAB (20%) 10 ml, NaCl (5M) 28 ml, Tris-HCl (1M) 10 ml, EDTA (0.5M) 4 ml, Mercaptoethanol (2%) 0.2 ml and Sterile water 48 ml, Isopropanol (ice chilled), Chloroform: Isoamyl alcohol (24:1), Ethanol (80% *v/v*), 1 X TE (Tris: EDTA Buffer, 100 ml) consisting of Tris- HCl (1M) 10 ml, EDTA (5M) 0.2 ml, 10 X TBE buffer (Tris- HCl: Boric acid: EDTA Buffer, 100 ml) consisting of Tris- HCl (1M) 10.8 ml, Boric acid 5.5g, EDTA (5mM) 4 ml. The protocol has been modified from the previous studies by Doyle and Doyle[16].

Small scale extraction and isolation of genomic DNA

The leaves were dipped in fixing solution for 30 min. Two fixing solutions i.e. chloroform and ethanol in a ratio of 3: 7 and absolute ethanol were used for the comparative study for the isolation of DNA. Fixative solutions are used to avoid the use of liquid nitrogen. The solvent was removed completely. The prechilled mortar and pestle was used to ground dried (0.5g) leaf samples. The powder was transferred in 0.75 ml of extraction buffer into micro extraction tube. The extraction buffer and frozen powder was mixed well and incubated at 65ºC for 60 min with intermittent shaking in water bath. After incubation, the mixture was cooled at room temperature and then, equal volume of chloroform: isoamyl alcohol was added and mixed by swinging for at least 15-30 min. Then mixture was centrifuged at 15000 rpm for 10 min at 25ºC. The aqueous phase was transferred to a fresh and sterile microcentrifuge tube and the DNA was precipitated by adding equal amount of ice cold isopropanol. The tube was left at room temperature for 30 min to settlement and precipitation of DNA. Precipitated DNA was centrifuged at 3000 revolution per min (rpm) for 5 min at 25ºC. Supernatant was decanted carefully and pellet washed with 80% ethanol repeatedly. The pellet was dried at 37ºC for 15 min in a laminar air flow and dissolved dry pellet in 100 µl of 1X TE.

Quantification of DNA

Reliable measurement of DNA concentration is important for many applications in molecular biology including complete investigation of DNA by restriction enzyme and amplification of target DNA by polymerase chain reaction. DNA quantification was carried out by two methods i.e. Spectrophotometric measurement and Agarose gel analysis.

Spectrophotometric measurement

1 ml TE buffer was taken in a cuvette and calibrated the spectrophotometer at 260 nm. 2-5 µl of DNA was added in a cuvette, properly mixed and optical density (OD) was recorded at both 260nm and 280 nm. Amount of DNA in µg/ml was obtained using following formula:

Concentration of DNA (µg/ml) = $(OD)_{260nm}$ / $(OD)_{280nm}$ or $OD_{260/280}$

The quality of DNA was judged from the ratio of OD value recorded at 260 and 280nm. The ratio obtained

FULL PAPER

(1.8-2.0) indicates good quality of DNA.

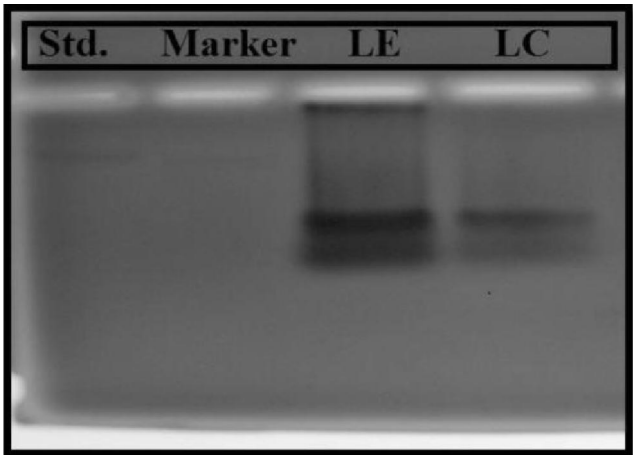
Agarose gel analysis

The purity of extracted DNA was checked by running the sample on ethydium bromide solution (0.5μg/ml) stained agarose gel or by using bromophenol dye after running the sample on unstained agarose gel. Agarose gel (0.8%) was mixed in 1X TBE (Tris- HCl - Borate-EDTA) buffer pH= 8.0 and was heated. The gel was allowed to solidify in the well plate.

After the gel solidification, the well plate was transferred to the electrophoresis tank having tank buffer i.e. 1X TBE. 2-5 µl extracted DNA was mixed with 2 µl bromophenol dye and loaded in to the wells. Electrophoresis was run at 110- 120V for 30 min. The quality of DNA was judged by visualizing the gel under UV fluorescence apparatus with presence of single compact band after staining the gel with dye.

RESULTS

The DNA extracted from the sample of leaves with absolute ethanol as fixative (LE) showed $(OD)_{260nm}$= 0.931 and $(OD)_{280nm}$= 0.448. Therefore, $OD_{260/280}$= 2.08.



Std: Standard; LE: Lentil leaf sample using Ethanol as fixative; LC: Lentil leaf sample using Chloroform Ethanol solution as fixative

Figure 1 : Bands of genomic DNA on agarose gel

The DNA extracted from the sample of leaves with chloroform ethanol solution as fixative (LC) showed $(OD)_{260nm}$= 0.127 and $(OD)_{280nm}$= 0.055. Therefore, $OD_{260/280}$= 2.30.

This revealed that the concentration of DNA (μg/ml) in the leaves of the *Lens culinaris* Medikus was found to be in the range of 2.08- 2.30.

Figure 1 represents the isolation of DNA of *Lens esculenta* Moench. leaves as a band after staining the agarose gel with Bromophenol dye. The DNA appeared as dark blue coloured bands.

DISCUSSION

Medicinal plants are important part of biotechnological studies but are often limited by poor extraction of plant DNA due to the presence of major contaminants such as ribonucleic acid (RNA), Protein, and polysaccharides; it is an important step for genetic studies[17,18]. The extraction of DNA is desirable for screening accessions, choosing parents and selection of progeny. It can be done by various methods such as DNeasy Plant Mini Kit, Wizard extraction, Liquid nitrogen method etc. All the methods differ in their efficiencies of removing non- DNA substances. But the contamination of extracted DNA with kit extraction reagents can induce error in PCR analysis hence reduce the efficiency of procedure. The presence of certain plant chemicals can hamper DNA isolation procedures[17,19]. Polyphenolics and flavonoids also co-precipitate with DNA after alcohol addition during DNA isolation and lead to viscous solutions, making DNA unsuitable for restriction and Southern hybridisation[20]. CTAB is frequently used as a surfactant in DNA extraction. Inclusion of CTAB in DNA extraction buffer helps in elimination of polysaccharides from DNA preparation to a large extent[16].

The key step in this protocol was complete disruption of plant cells in fixatives as the liquid nitrogen is not safe to use[21]. High concentration of sodium chloride was used to precipitate high levels polyphenols and flavonoids[22]. The concentration of DNA in the leaves of *Lens culinaris* Medikus was found to be 2.08-2.30 μg/ ml. The $OD_{260/280}$ ratio indicated the absence of contaminants in DNA and a good quality DNA[23]. It was also observed that the use of absolute ethanol as fixative is better for Genomic DNA extraction as it showed better optical density. Poor leaf DNA quality was observed when chloroform and ethanol solution was used as a fixative which could be due to slow tissue death as a result of slow fixing solution penetra-

tion[14]. It is a modified CTAB procedure used to isolate DNA from leaves of polyphenols, flavonoids and saponin rich lentil plant without using liquid nitrogen to overcome problems such as low yield, degradation and poor PCR amplification. It does not require complicated and long ultracentrifugation and can be performed at room temperature. This method is rapid, simple and efficient for isolating DNA from plants rich in phenolic compounds.

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