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SHORT COMMUNICATION

Standardization/optimization of High Quality DNA Isolation Protocal by using CTAB Method

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ABSTRACT

The rice is important food in world population need to improved crops and food productivity. DNA based breeding selection is much more reliable other than traditional breeding methods. Howere, mangroves and salt marsh species are known to synthesize a wide spectrum of polysaccharides and polyphenols including ûavonoids and other secondary metabolites which interfere with the extraction of genomic DNA. The extraction of DNA is often the most time consuming and laborious step in high-through put molecular genetic analysis. This study a rapid and reliable cetyl trimethylammonium bromide (CTAB) protocol suited specifically for extracting genomic DNA from rice plants. The purity of extracted DNA was excellent as evident by A260/A280 ratio ranging from 1.78 to 1.84 and A260/A230 ratio was good quality and yield assessments, electrophoresis was done of all DNA samples in 0.8% agarose gel, stained with Ethidium Bromide and bands were observed in gel documentation system.

Key words *DNA extraction, high-throughput PCR, marker assisted selection, gene mapping*

Rice has become an important food for the people all over the world. The world population is rapidly growing day by day, researchers are trying to improve rice production. Therefore, scientists have started to use breeding program based on molecular marker for development of new variety. Another major goal of breeding programs is investigate genetic diversity and relationships among breeding lines in rice. So many factors are responsible for shearing of DNA during isolation and extraction time. DNA degration due to endonucleases is one such problem for the isolation and puriûcation of high molecular weight DNA from rice plant, which directly or indirectly interfere with the enzymatic reactions.

The extraction of DNA from plant tissue can vary depends on the ege of plant material used. Any mechanical means of breaking down the cell wall and membranes to allow access to nuclear material. Several researchers have attempted to eliminate the use of hazardous chemicals, expensive kits, equipment, and labour-intensive steps for high throughput DNA extraction. In most cases this involves the use of liquid nitrogen flash freezing followed by grinding the frozen tissue with a mortar and pestle. Liquid nitrogen is difficult to handle and it is dangerous in an open laboratory environment such as a classroom. For this reason we have modified a very simple plant DNA extraction protocol to use fresh tissue. The protocols and results are presented here.

MATERIAL AND METHODS

Plant materials

Rice seeds were germinated in an incubator for 2 to 3 days. After germinating the seeds were sowed into paddy fields in the department of Plant Molecular Biology and Biotechnology IGKV, Raipur. C. G.

Reagents

1. Micro-centrifuge tubes
2. Mortar and Pestle
3. Absolute Ethanol (ice cold)
4. 70 % Ethanol (ice cold)
5. 55 °C water bath
6. Chloroform: Iso Amyl Alcohol (24:1)
7. Autoclaved Water (sterile)
8. Agarose
9. 6x Loading Buffer
10. 1x TBE solution
11. Agarose gel electrophoresis system
12. Ethidium Bromide solution.

CTAB Buffer

5 g CTAB and 20.35 g NaCL were dissolved in 200 ml double distilled water. Later 25 ml 1 M tris HCL and 10 ml 0.5 M EDTA was added and stirred vigorously on a magnetic stirrer. Volume was made up to 250 ml and stored at room temperature. 20 ul/ 20 ml 2- mercaptoethanol was added prior to used.

1 M Tris pH 8.0

Dissolve 121.1 g of Tris base in 800 ml of H2O. Adjust pH to 8.0 by adding 42 ml of concentrated HCL. Allow the solution to cool to room temperature before making the final adjustments to the pH. Adjust the volume to 1 L with H2O. Sterilize using an autoclave.

TE Buffer (100 ml)

10 mM Tris (pH 8.0) (Use 1 M stock)

2 mM EDTA (Use 0.5 M stock)

5x TBE buffer

54 g Tris base

27.5 g boric acid

20 ml of 0.5M EDTA (pH 8.0)

Make up to 1L with water. To make a 0.5x working solution, do a 1:10 dilution of the concentrated stock.

Table 1. DNA concentration by using Nano Drop Spectrophotometer

Sample Name	Nucleic acid	Unit	Ratio 260/280
Sample-1	2012.3	ng/ul	1.95
Sample-2	1216.5	ng/ul	1.85
Sample-3	500.1	ng/ul	1.83
Sample-4	637.4	ng/ul	1.86
Sample-5	450.2	ng/ul	1.82
Sample-6	400.3	ng/ul	1.76

RNase A

Stock solutions

1. 10 mM Tris HCL (pH 7.5)
2. 15 mM Nacl

1 % Agarose gel

1 g Agarose dissolved in 100 ml TBE

CTAB Method

Procedure

1. Cut leaves to small pieces in a mortar and pestle; add 1 ml of CTAB buffer (Add 500 ul more CTAB if required) to CTAB add 1% PVP (1g in 100ml) and Beta-mecaptoethanol 1 ul/ml of buffer.
2. Transfer homogenate to 2 ml tubes; incubate at 65 °C on water bath for 15-20 minutes.
3. Allow to cool & then add 700 ul of Chloroform: Isoamyl Alcohol (CIA-24:1) shake vigorously to mixed and leave for another 10 minutes.
4. The contents were shaken by hands intermittently and kept at room temperature for 15minutes.
5. Then tubes were centrifuge at 14,000 rpm for 5 minutes and collect the supernatant in fresh 1.5 ml Eppendorf tube
6. Add double volume of 100% chilled Iso-Propanol and mix it by inverting the tube & Incubate for 1 hr at -20 °C or 4 °C over night.
7. The sample was centrifuge for 10 min at 14000 rpm at 5 ^{0}C. & Wash the pellet with 70% ethanol & Centrifuge it for 3 min (14000 rpm), decant the ethanol
8. Pallets were dissolved in 200 ul of TE buffer and add 1 ml of absolute ethanol.
9. Then again centrifuge at 14000 rpm at 5 ^{0}C for 3 minutes.
10. DNA pellet was air dried for 30 minutes then dissolved in 50-100 ul of TE buffer.
11. Proceed for DNA quantification.

DNA Quantification

The DNA concentration was quantified using a Nano Drop Spectrophotometer and some statistical analysis was performed to investigate the optimum age and EDTA concentration among all the treatments. The quality of genomic DNA was determined in ratio absorbance of A260/ A280 range of 1.85 (Table.1) is the good qulity of DNA using NanoDrop reading.

DNA Concentration and Quality

The concentration and quality of extracted rice DNA were confirmed by using 0.8% (w/v) agarose gel.

Agarose Gel

1. Cast a 0.8 % (w/v) regular agarose gel in 1X TBE
2. Place 2 µL of extracted DNA and 3 µL loading dye.
3. Run the gel for 30 min. at 65v.
4. Stain gel and view result.

RESULT AND DISCUSION

Plant genomic DNA extraction did not show promising results for mangroves and salt marsh species as evident by

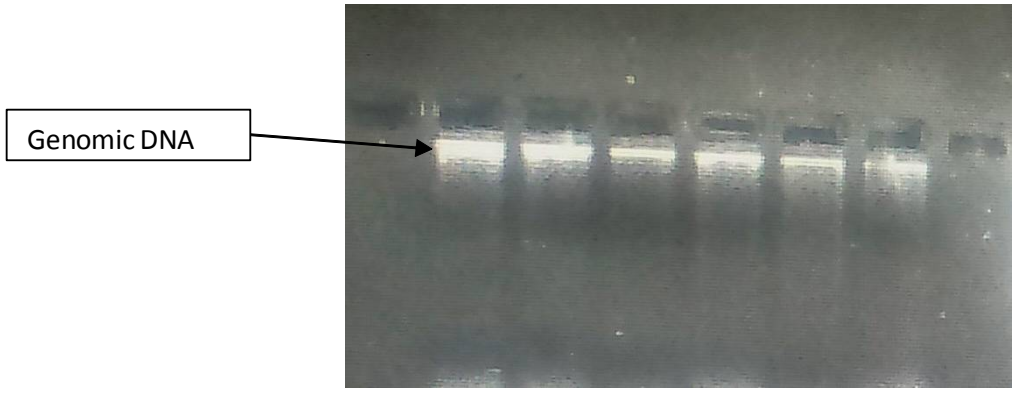

Fig. 1. Preparation of genomic DNA by CTAB buffer.

the presence of sticky polysaccharides in the pellet and sheared band in the agarose gel. We encountered so many difficulties from the first step of cell lysis to DNA separation in the supernatant and subsequent reactions when the CTAB DNA extraction method.

The highly viscous and sticky pellets were difficult to handle and brownish pellet indicated contamination by phenolic compounds in your sample. Therefore, high concentration of *â*-mercaptoethanol was use in protocol for good and high quality DNA extraction by using CTAB method.

The success of the optimized extraction method in obtaining high quality genomic DNA from rice plants. The addition of NaCl at concentrations higher than 0.5 M, along with CTAB is known to remove polysaccharides during DNA extraction procees.

CONCLUSSION

A simple, safe, reliable and cost effective CTAB method of DNA extraction that provides high quality DNA isolation from mangroves and salt marsh plants containing high concentrations of polysaccharide and polyphenolic compounds. The resulting optimized CTAB protocol for without used of liquid nitrogen with good quality and quantity of genomic DNA extraction for sequencing purpose. This method is recommded of low technology laboratories for high throughput sample preparation suitable for various molecular study.

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