ARTICLE TYPE

Comparison of Methods for Genomic Deoxyribonucleic Acid (DNA) Extraction Suitable for Whole-Genome Genotyping in Traditional Varieties of Rice

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Abstract

The utilization and conservation of traditional rice genotypes have attracted global attention. Optimization of DNA isolation protocol for genetic characterization of plants is a necessary and primary step. Over the recent years, next-generation sequencing and microarray technologies have revolutionized scientific research, with their applications to high-throughput analysis of biological systems. Isolation of high quantities of pure, intact, double-stranded, highly concentrated DNA is prerequisite for successful and reliable large-scale genotyping analysis. Therefore, standardization of DNA isolation is a basic requirement. Here we employed three methods of DNA isolation, namely, Dellaporta, Hi-purA, and modified CTAB techniques for isolation of genomic DNA from 25 indigenous rice genotypes. From the results, it was found that genomic DNA isolated by a modified CTAB method to be the most appropriate for extracting high quality and maximum quantity of DNA suitable for genotyping. Spectrophotometric measurements and gel electrophoresis subsequently assessed the quality and quantity of the differentially extracted DNA.

Keywords: Isolation, CTAB, UV nano-spectrophotometer, gel electrophoresis, UV gel documentation.

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Introduction

The Asian cultivated rice (*Oryza sativa* L.) is one of the most important crops and a major food source for more than half of the global human population. Indigenous crop varieties traditionally cultivated and maintained by farmers contain a high level of genetic diversity and can serve as potential genetic resources for improving yield, resistance to pests and pathogens, and agronomic performance (Brush,1995; Hoisington, 1999: Mandel *et al*., 2011). In the present IPR regime, isolation of high quantities of pure, intact, double-stranded, highly concentrated genomic DNA is prerequisite for successful and reliable large scale genotyping analysis of very valuable biological organism like rice. Hence optimization of DNA isolation protocol for genetic characterization is the primary step in the field of molecular biology (Tan and Yiap, 2009). In the past, the process of extraction and purification of nucleic acids used to be complicated, time-consuming, labor-intensive, and limited in terms of overall throughput. Currently, many specialized methods based on the use of acid, alkali, detergents, phenol and suitable buffer are being developed (Guin, 1966). However, the fundamentals of DNA extraction remain the same. Firstly, DNA must be purified from cellular material in a manner that prevents degradation, for this even crude extraction procedures can still be adopted to prepare a sufficient amount of DNA to allow it for multiple end uses. After that, protein digestion and action of detergents during the extraction process destroy the plasma membrane and the nuclear membrane surrounding the DNA. A range of methods is available to assess the quality of the isolated DNA, which include gel electrophoresis, spectrometric analysis, restriction digestion, PCR amplification and chromatographic techniques (Varma *et al*., 2007). Agarose gel electrophoresis is a procedure used to separate DNA fragments based on their molecular weight. Quantification of nucleic acids is commonly done in molecular biology to determine the concentrations of DNA or RNA present in a mixture (Channarayappa, 2007). Spectrophotometers are commonly used to determine the concentration of DNA in a solution. It is possible to use UV-spectrophotometer to estimate the purity of a solution of nucleic acids. This method involves measuring the absorbance of the solution at two wavelengths; usually, 260 and 280 nm, calculating the ratio of the two absorbances and characteristic of pure DNA with 1.8

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of A260/A280 ratio is considered pure (Nieman and Poulsen, 1963). In this study, various genomic DNA isolation methods of Stephen *et al.* (1983), Doyle and Doyle (1990) and Suman and Khanuja(1999) were used. The objective of this work was to evaluate and compare these three different methods for extraction of genomic DNA from traditional rice genotypes in terms of the DNA quantity, concentration, and purity.

Materials and methods

All three genomic DNA isolation methods (Dellaporta, CTAB, HiPurA DNA isolation kits and Khanuja) were used to isolate genomic DNA from 25 traditional rice genotypes. However, the results for selected rice plants obtained from the modified CTAB method are discussed here as follows.

Plant Samples

A total of 25 genotypes of indigenous rice (*Oryza sativa*) were collected from the Thanjavur district in Tamilnadu, India. Wild rice (*O. rufipogon*) accessions were collected from farmers. Seeds were germinated in a nursery and transferred to the field or greenhouse.

Reagents and Chemicals

The chemicals and reagents used in the isolation of DNA were: CTAB extraction buffer [2% (w/v) CTAB; 20 mM EDTA, pH 8.0; 100 mM Tris–HCl, pH 8.0; 1.4 M NaCl]; CTAB/NaCl solution [10% (w/v) CTAB; 0.7 M NaCl mixed at 65 °C with stirring]; TE buffer [10 mM Tris–HCl, pH 8.0; 1.0 mM EDTA, pH 8.0], chloroform: isoamyl alcohol (24:1, v/v), Iso-propanol, 70% ethanol, 2-mercaptoethanol (2ME), liquid nitrogen, sodium acetate and Rnase.

DNA Extraction Procedure

Leaf samples from seedlings were harvested, air dried, and used for the study. For the preparation of CTAB buffer (100 mL working solution), 12 mL of Tris-HCl was mixed with 30 mL of 5 M NaCl, and 4.5 mL of 0.5M EDTA and mixed thoroughly. Then 2.4 g of CTAB powder and 48.9 mL of double distilled water were added to the solution, and it was kept in a water bath at 60°C for 10-15 min. Only the young leaves (1g) of the plants were collected, added liquid nitrogen before grinding. Then 2 ml of CTAB extraction buffer was added. After grind well with the help of mortar and pestle, each sample solution was taken in eppendorf tube and kept in a water bath at 65°C for 30 min. The samples were taken out of water bath and kept in room temperature for 10 min and added an equal amount of chloroform: Isoamyl alcohol (24:1), mixed by inversion and centrifuged at 12000 rpm for 10 mins. The aqueous layer was collected in a fresh tube. DNA was precipitated by adding 1/5th volume of ice-cold isopropanol and 100uL of sodium acetate (pH 5.2). It was mixed well and kept at -20 °C for 20 minutes, centrifuged at 12000 rpm for 10minutes and the supernatant was discarded. The pellet was washed with 70% ethanol twice, air-dried and dissolved in 200µL TE buffer. 3µl Rnase was added and incubated at 37°C for 30 minutes. Then the aqueous layer was transferred into a fresh tube and double volume of ice-cold ethanol was added. The content was mixed slowly, centrifuged at 10000 rpm for 5 minutes and washed the pellet with 70% ethanol. Then it was dried, and dissolved in 100µl TE.

Checking of quality and quantity of isolated genomic DNA

The quality and quantity of isolated genomic DNA was achieved using a spectrophotometer (UV–visible spectrophotometer) and agarose gel electrophoresis. The yield was determined by measuring the absorbance at A260, A280 and A320 nm. The level of DNA purity was determined

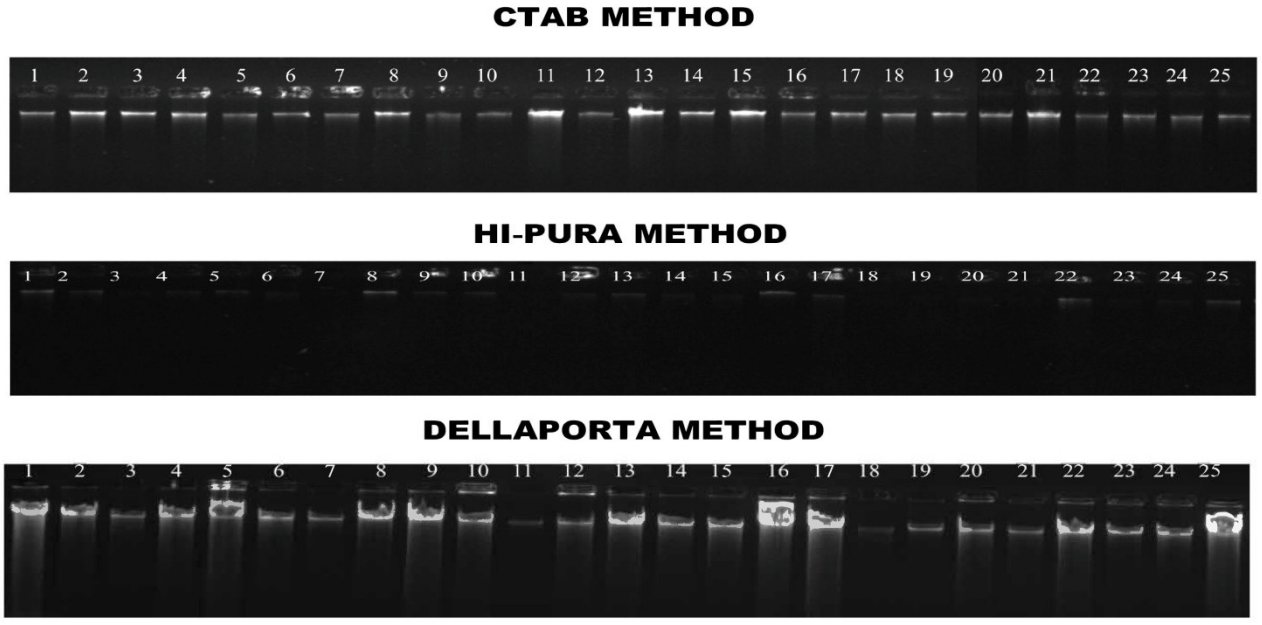


Figure 1: Comparison of genomic DNAs on agarose gel using three DNA extraction methods.

by the A260/A280 and A260/A230 absorbance ratios (Nieman and Poulsen, 1963).

DNA purity was further tested by running the extracted genomic DNA samples on 0.8% agarose gel (Fig.1) stained with 6ul/100ml ethidium bromide in 1×TBE (Tris base, Boric acid, 0.5M EDTA) gel buffer. The gels were visualized and photographed under UV light (vilber lourmat).

Results and discussion

Indigenous rice varieties are treasure houses of traits which can be used to develop genotypes in the future to combat climate change and nutritional challenges. In the present Intellectual Property Rights Regime, basic techniques and tools of biotechnology have a great role in distinguishing the precious variations in crops for registration and establishing rights to the stakeholders. One of such basic technique needed for any genetic level of studies is the isolation of DNA.

In the present study, 25 indigenous rice varieties were used for DNA extraction. The CTAB protocol (Doyle and Doyle, 1990) was modified so that a good DNA yield from rice plants could be obtained in such a way that all the rice plants gave good DNA yield. We increased the centrifugation speed, time, and concentration of certain chemicals for obtaining a good yield. By comparing all the three methods, we observed that the purity and concentration of DNA were higher found in the modified CTAB method of isolation. The OD260/280 value obtained in the CTAB method ranged from 1.8 to 2.0. This protocol showed a 260/280 nm ratio above 1.8, which is considered standard for pure DNA. In the other two methods, OD values were frequently deviating from the optimum of 1.8. The DNA quality was tested and confirmed by agarose gel electrophoresis.

Table 1: Comparison of DNA concentration absorbance ratios A260/A280 of three methods.

S.No	*Genotypes*	*CTAB*	*Dellaporta*	*Hi-PurA*
1	M1	1.8	1.6	2.2
2	M2	2.1	1.7	2.3
3	M3	1.9	1.6	1.8
4	M4	2.0	1.7	1.9
5	M5	2.0	2.2	2.1
6	M6	2.0	2.1	2.0
7	M7	1.8	1.7	2.0
8	M8	1.8	1.8	2.2
9	M9	2.0	1.6	1.7
10	M10	2.0	1.5	1.9
11	M11	1.9	1.2	1.6
12	M12	2.1	1.7	2.3
13	M13	1.9	2.2	2.1
14	M14	1.9	1.6	2.2
15	M15	1.9	1.8	1.6
16	M16	2.0	1.9	2.2
17	M17	2.0	2.1	1.7
18	M18	2.0	2.0	1.3
19	M19	2.0	2.0	1.6
20	M20	1.8	2.2	1.7
21	M21	1.9	1.7	1.6
22	M22	1.8	1.9	2.2
23	M23	1.8	1.6	1.7
24	M24	1.7	1.4	2.1
25	M25	1.8	2.1	1.7

Residual chemical contamination from nucleic acid extraction procedures may result in an overestimation of the nucleic acid concentration. High 260/280 purity ratios are not necessarily indicative of a problem. Although purity ratios are important indicators of sample quality, the best indicator of DNA or RNA quality is functionality in the downstream application of interest.

Out of the three techniques we compared for DNA isolation in 25 genotypes of traditional rice varieties, DNA isolated by a modified CTAB method gave good yield and high purity of DNA than the other two methods. In the Dellaporta method, 13 genotypes had OD values less than 1.8 and the lowest is 1.6. In HipurA method, 20 genotypes had exceeded the optimum range of 1.8 to 2.0. An appreciably lower ratio may indicate the presence of protein, phenol, or other contaminants that absorb strongly at or near 280 nm. Residual chemical contamination from nucleic acid extraction procedures may result in an overestimation of the nucleic acid concentration. Unusual high rate 260/280 may be due to co-extraction of RNA along with DNA extraction system. But we have eliminated that by analyzing the extracts on the agarose gel. Modification of the centrifugation speed and time, the addition of chloroform isoamyl alcohol after the centrifugation only, increasing the time of after adding the ice-cold propanol, addition of sodium acetate after adding the Rnase, continue to the incubating after only and given the ethanol wash at one time only. Although purity ratios are important indicators of sample quality, the best indicator of DNA or RNA quality is functionality in the downstream applications. A pure and efficient DNA isolation protocol should be simple and yield enough DNA with high quality. It should also be rapid and reliable. The rice DNA extraction methods typically use seedlings or leaves and require fine grinding of the tissue materials using liquid nitrogen. Different methods of DNA isolation have been reported in many studies (Ahmadikhah, 2009, Aliyu *et al.*, 2013; Fouladvand *et al.*, 2013 and Duan *et al.*, 2015).

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