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Comparative study of genomic DNA extraction protocols in rice species.

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ABSTRACT

This study presents a rapid DNA extraction protocol for rice specie. Three published protocols (Dellaporta *et al*., 1983; Doyle and Doyle 1990; Wang *et al*., 1993) were comparatively evaluated with the 'STI' protocol for duration of extraction, purity, yield and cost of protocol. The duration and cost of extraction were significantly different (P<0.0001) for all protocols. A positive association (P<0.0001) between cost of extraction, duration of extraction and DNA purity was obtained from 5mg leaf tissue. Proposed 'STI' protocol, with good quality DNA, yield and a shorter duration of extraction would be ideal for genotyping large populations of rice genotypes, where quality, cost and time are of essence.

Keywords : DNA purity, DNA yield, Extraction protocols, Rice specie.

1 INTRODUCTION

The recent advent of techniques for DNA analysis by PCR and DNA sequencing has increased interest in plant systematic by using DNA sequencing data to study the relationships among species, thereby complementing the enormous amount of morphological data available. However, to obtain accurate DNA sequence information, it is necessary to isolate good quality DNA that is relatively free from the many contaminants found in plant cells [1]. DNA extraction is an important step in molecular assay and plays a vital role in obtaining high resolution results in gel-based systems, particularly in the case of cereals with high content of interfering components in the early steps of DNA extraction.

Biotechnology is seen as perhaps the most important new resource for achieving varietal improvement. The emergence of plant transformation and molecular marker analyses in genome studies has greatly enhanced the speed and efficacy of crop improvement and breeding programmes. A prerequisite for taking advantage of these methods is the ability to isolate genomic DNA of superior quality and quantity for analyzing through Polymerase Chain Reaction (PCR), restriction enzyme digestion and subsequent Southern blot hybridization. Rice biotechnology techniques encompass plant tissue culture (such as embryo rescue and anther culture) and molecular biology. Molecular techniques help to accelerate traditional breeding programs through germplasm management, DNA fingerprinting, gene tagging and marker-assisted selection. Research has provided 75% of improved rice varieties now grown. This has increased potential yields from four to more than ten tones per hectare. Other DNA isolation protocols are available [2], [3], [4] . These protocols involve disruption and lysis of the starting material followed by the removal of proteins and other contaminants and finally recovery of the DNA. Removal of proteins is typically achieved by digestion with proteinase k, followed by salting out, organic extraction, or binding of the DNA to a solid-phase support. DNA is usually recovered by precipitation using ethanol or isopropanol. The choice of a method depends on many factors; the required quantity and molecular weight of the DNA, the purity required for downstream applications, time and expense. Since size, content and organization of genome and contents of metabolites of different plant systems vary from each other to a great extent, a single DNA isolation protocol is not likely to be applicable for all plant systems [5]. Chemotypic heterogeneity among species may not allow optimal DNA yield with a single protocol, thus even closely related species may require different isolation protocols [6].

The problem of DNA extraction is still an important issue in the third world countries where biotechnology is yet to be fully exploited due to funding limitations and technical knowledge of extraction procedures. DNA utilized for genomic investigations are usually of low quality, impure and of low yield. This in turn affects the result obtained during genotyping and consequently wrong information about a particular genotype(s) is been passed around. Such results are not reproducible and renders the research unacceptable. Simple, high yielding, qualitative and cost effective protocols are needed to extract DNA for population studies.

2 MATERIAL AND METHODS

2.1 Plant Materials

Rice seeds of ten genotypes (two *Oryza sativa: C*isadane and Pokkali; two Interspecific hybrids: Nerica L-1 and Nerica L-41 and six *Oryza glaberrima:* TGS3, TGS25, CG14, TOG5601, CG14/TGS3 and RAM90/TGS25 were cleaned and placed in an oven at 50^{o}C for five days to break seed dormancy. The seeds were surface sterilized with 0.1% $HgCl_2$ and rinsed with distilled water. Sterilized seeds were soaked in water in a Petri-dish lined with Wattman's filter paper and incubated for 48hrs at 30^{o}C. Pre-germinated seeds were sown in loamy soil

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and watered daily. Ten days after sowing, young leaf tissues were harvested, placed on ice in eppendorf tubes and transported to the laboratory.

2.2 DNA Extraction

DNA from healthy fresh young leaf tissue (5mg) of each rice genotype was extracted following three published protocols and a derived protocol viz:

- Protocol A- Plant minipreparation [2].
- Protocol B- Cereal DNA extraction micropreparation protocol [3].
- Protocol C- Sodium hydroxide protocol [4].
- Protocol D- Sodium hydroxide, Tris HCL and Isopropanol (STI) protocol.

2.3 STI Protocol

Chemicals Utilized:

- 100 mM Tris-HCl pH 7.5
- Isopropanol
- Sodium Hydroxide (NaOH)
- Ethanol

Protocol

1. Grind samples in geno grinder.
2. Add 100 µl sodium hydroxide and 400 µl 100 mM Tris.
3. Shake by inversion for 5 mins.
4. Centrifuge at maximum speed (13000) for 10 mins.
5. Transfer supernatant into new eppendorf.
6. Add 20ul TE-RNase solution, incubate for 20 mins at 37^{o}C
7. Add 300 µl sodium hydroxide and 300 µl isopropanol.
8. Spin down at 13000 rpm for 10 mins.
9. Decant watching out for pellet.
10. Rinse in 200 µl 70 % ethanol.
11. Decant and air dry for 20 mins.
12. Resuspend in 100 µl low salt TE.

2.4 DNA Quantification

The concentration of DNA obtained (ng/µl) was determined on an ND-1000 spectrophotometer (NanoDrop inc. 2007). The purity level for all ten genotypes were accessed by obtaining the absorbance ratio $A_{260/280}$ using the Nanodrop spectrophotometer and the duration of extraction for 96 samples estimated.

2.5 PCR Amplification Using SSR Primers

DNA samples from each genotype were subjected to PCR amplification with an SSR primer. PCR was conducted in 10ul reaction mixture containing 1X PCR buffer, 1ul of 25mM $Mgcl_2$, 0.2ul of 10mM DNTP's, 0.25ul of 20pm primer pair, 0.5ul of Taq polymerase, 2.15ul milu-Q water and 5ul of l0ng/ul DNA, using Perkin- Elmer thermo cycler according to the cycle profile: (i) 2min pre heating at 94^{0}C, (ii) Denaturing for 30seconds at 94^{0}C, 34 cycles of 30sec at 94^{0}C, 30sec annealing at 55^{0}C and 67^{0}C depending on the marker used and 30sec initial extension time at 72^{0}C (iii) Final extension for 2min at 72^{0}C. The amplified products were subjected to electrophoresis in a 2% agarose gel in 1X TBE buffer at 78-80 volts for 2-3 hours for good separation. The gel was stained with 0.5ul ethidium bromide for 5mins and visualized under ultra- violet light using gel documentation system (Fig 1)

5 RESULTS AND DISCUSSIONS

DNA extracted from fresh leave samples using protocol A [2] and 'STI' (protocol D) protocol yielded good quality DNA (Table 1) . The purity of DNA is considered to be best if it ranges between 1.8-2.0, at this range DNA is absorbed most [7]. DNA purity obtained from these protocols could be as a result of the RNase treatment. It was reported that the absence of RNA are evidence of a good quality genomic DNA [8]. DNA extracted by means of protocols B [3] and protocol C **[4]** were colored (yellowish or dark green). This colored extract could be due to the presence of phenolics as the protocols did not involve SDS and RNA treatment. Report showed that in the presence of PVP , phenolics adhere to DNA in solutions forming a colored extract around the DNA [1].

In comparism of the four protocols analyzed, protocol A [2] was labour intensive as it demanded a great deal of time for extraction of samples. Protocol C, involving two basic steps, often regarded as the 'fast and dirty' extraction method was time efficient. A positive correlation was obtained for purity and time (table 2) .

The cost of extracting DNA from the leaf tissues positively and negatively correlated with duration of extraction and purity respectively (Table 2). Protocols B,C and D were cost effective for DNA extraction compared to Protocol A. This is probably because a lot of chemicals, reagents and equipments were utilized for DNA extraction with protocol A.

It was observed that DNA yield from protocols B (207.90ng/ul) and C (220.87ng/ul) were higher than that obtained from protocols A (60.02ng/ul) and D (92.76ng/ul). This is probably due to the absence of contaminants and RNA treatment in protocols A and D. Rreports showed that since the spectrophotometer takes reading of the nucleic acids, the high DNA yield obtained with protocols B and C could have been a combined reading of DNA and RNA [8]. Nucleic acids (DNA and RNA) absorb light at 260 nm so it is possible to calculate the concentration of DNA in a sample by measuring the A_{260}. Certain proteins also absorb light at this wavelength [8] and so high level of protein contamination in a sample can give a false result. However, proteins also absorb light at 280 nm; so by measuring both A_{260} and A_{280} it is possible to calculate the ratio of nucleic acid to protein in the solution and thus estimate the accuracy of DNA concentration. A ratio of $A_{260}/ A_{280} = 1.8$ is considered acceptable. A lower value indicates high level of protein contamination and the estimation of the DNA concentration will not be accurate.

The amplification of DNA from PCR analysis with SSR primer

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was clear as shown in Fig 1. The absence of RNA, polysaccharides and the amplification of molecular bands are evident of a good DNA quality with 'STI' protocol.

The satisfactory quality and yield of DNA obtained from 'STI' protocol is therefore essential where a simple, cost effective and time saving protocol is needed to extract DNA from large populations of rice genotypes.

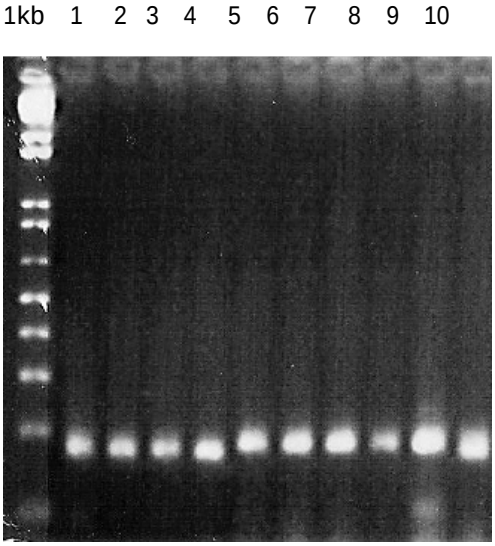

Fig 1: Amplified DNA of ten rice genotypes extracted by 'STI' protocol.

(1- Cisadane, 2- Pokkali, 3- Nerica L-1, 4- Nerica L-41, 5- TGS3, 6- TGS25, 7- CG14, 8- TOG5601, 9- CG14/TGS3, 10- RAM90/TGS25)

Table 1: Evaluation of samples extracted using protocols A-D according to DNA purity, extraction time, cost of extraction and yield(ng/ul).

PROTOCOL	PURITY ($A_{260/280}$)	TIME (hrs)	COST (N)	YIELD (ng/µl)
A	1.86c	15.05a	5.27a	60.02a
B	2.17**a**	6.00b	3.60c	207.90c
C	2.00**b**	3.09d	3.71bc	220.87cd
D	1.90**bc**	5.30bc	3.94b	92.76b
S.E	0.33	1.01	1.67	46.68
CV	20.66	4.75	2.21	17.01
R2	0.73	1.00	1.00	0.65
P VALUE	0.006	0.0001	0.0001	0.018

Values followed by the same letters are not significantly different at 1% Duncan Multiple Range Test (DMRT).

Table 2: Correlation coefficients of DNA extraction factors for protocols A-D.

	PURITY	TIME	COST	YIELD
PURITY	1			
TIME	0.58*	1		
COST	-0.58*	0.51*	1	
YIELD	0.11	-0.18	-0.48	1

* - Significant. P < 0.0001

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