Journal of the Arkansas Academy of Science

Volume 44 Article 18

1990

Comparison of Methods for Purification of DNA From Rice

Theresa L. Hines
University of Arkansas at Little Rock

Neela R. Patel
University of Arkansas at Little Rock

Daoud Abu-Diab
University of Arkansas at Little Rock

Michael Sikes
University of Arkansas at Little Rock

Alvan A. Karlin
University of Arkansas at Little Rock

A COMPARISON OF METHODS FOR PURIFICATION OF DNA FROM RICE

THERESA L. HINES, NEELA R. PATEL, DAOUD ABU-DIAB,
MICHAEL SIKES, and ALVAN K. KARLIN
Department of Biology
University of Arkansas at Little Rock
Little Rock, AR 72204

ABSTRACT

Three techniques were employed to purify genomic DNA from deomstic rice (*Oryza sativa* L.). Following extraction, the DNA was electrophoresed through agarose to determine its integrity. We determined that spooling yielded better quality, through lower quantity DNA than either of the other two techniques.

INTRODUCTION

Rice (*Oryza sativa* L.), a major cereal grain crop in world food production, is a dietary staple in many third world countries. Arkansas supplies 60% of the nation's rice for exportation, thus higher yields from genetically engineered rice would not only benefit third world countries, but would also economically benefit the United States and subsequently Arkansas.

Rice has been difficult to engineer genetically due to the barriers related to *Agrobacterium*-mediated transfer in many monocot species. Rice tissue culture techniques have proven quite effective, although other gene transfer systems need to be explored (Dekeyser *et al.*, 1989). One of the first steps in genetically engineering rice is developing a method for purification of genomic DNA.

In this study, we extracted DNA from the major rice cultivars (Huey, *et al.*, 1987) in production in Arkansas (Newbonnet, Lemont, and Tebonnet). The DNA was further purified by ethanol (Et-OH) precipitation, spooling, or cetyl-trimethyl-ammonium bromide (CTAB) extraction and the methods were compared. The DNA obtained was quantified using diamino-phenyl-indole (DAPI) and electrophoreses through agarose to determine the degree of shearing. From the results, we were able to determine which extraction/purification method is most effective for obtaining rice genomic DNA.

MATERIALS AND METHODS

We obtained 3 rice cultivars, Newbonnet, Lemont, and Tebonnet, from the University of Arkansas Rice Research and Extension Center in Stuttgart, Arkansas. Each cultivar was grown in laboratory flats under artifical lighting until the leaves were approximately 13 cm long. Two grams of leaf material were harvested for each analysis. The leaf was finely chopped and pulverized in liquid nitrogen. While still frozen, the tissue was transferred to a 50 ml Falcon tube. To the tube, 15 ml of extraction buffer (10 mM Tris, 50 Mm EDTA, 500 Mm sodium chloride, and 1 Mm beta-mercaptoethanol) and 1 ml of 20% lauryl sulfate (SDS) were added. After a 10 minute incubation at 43 °C, 10 units (100 ul) of Proteinase-K were added to the tube, and the tube was allowed to incubate overnight at 43 °C.

The next day, following addition of 5.0 ml of 5 M potassium acetate, the tube was incubated at 0 °C for 20 minutes. After the incubation period, the sample was centrifuged at 10,000 g for 30 minutes. The supernatant was decanted through Miracloth into a Corex tube containing 10 ml of cold isopropyl alcohol. The tube was gently shaken, and then incubated at −20 °C for 20 minutes. Following extraction of the DNA, 3 different purification methods were used.

SPECIFIC PURIFICATION METHODS

For the Et-OH precipitation method, the sample was centrifuged at 10,000 g for 30 minutes. The pelleted DNA was allowed to dry. The pellet was redissolved in 1 ml of TE (10 mM Tris, 1 mM EDTA, pH 7.5). The DNA was microfuged for 10 minutes to remove any insoluble debris. The sample was divided between 2 microfuge tubes and 50 ul of 3 M sodium acetate and 1 ml of ethanol were added to each tube. The tubes were then shaken well, and the DNA was pelleted for 5 minutes in the microfuge. The pellet was rinsed with 70% ethanol before the tube was microfuged for another 5 minutes. The DNA pellet was allowed to dry and resuspended in 100 ul of TE and stored at room temperature.

The second method was the spooling technique. A glass hook was prepared using a disposable Pasteur pipette. Each DNA sample was removed from the Corex tube by twirling in onto the glass hook. The DNA was rinsed in cold 100% ethanol, dried and redissolved in 100 uL of TE.

In the CTAB procedure, the DNA was centrifuged at 10,000 g for 20 minutes, and the DNA pellet was dried. The dry pellet was resuspended in 0.5 ml of TE. The DNA was centrifuged for 10 minutes to remove any debris, and 50 ul of 3 M sodium acetate and 100 ul of 1% CTAB were added. The DNA was incubated at 50 °C for 15 minutes, then extracted in an equal volume of phenol-chloroform-isoamyl alcohol (24:24:1). After centrifuging, the upper layer was recovered, and re-extracted with chloroform-isoamyl alcohol (24:1). The DNA was precipitated by adding twice the volume of cold 100% ethanol, and microfuged for 5 minutes. The DNA pellet was washed with 70% ethanol, dried, and resuspended in 100 ul of TE.

The concentration of DNA in each sample was determined by comparing a 2X serial dilution of rice DNA to known concentrations of salmon sperm DNA in 1% DAPI. To determine the degree of damage to the rice DNA, a 5 ul sample was electrophoresed on a 1% agarose gel in Tris-Borate-EDTA at pH 8.8 (Maniatis, *et al.*, 1982) using .75 volts/cm of agarose for 3 hours.

STATISTICAL METHODS

The experimental design used 3 purification methods for each of the 3 cultivars of rice. This produced a 3 X 3 factorial Analysis of Variance (Steele and Torrie, 1960). The design was replicated twice.

RESULTS AND DISCUSSION

The results of the three purification methods are shown in Table 1. It is apparent the Et-OH method yields more DNA/ml than either spooling the DNA or the CTAB method. When purification method is not considered, the 3 cultivars yield approximately the same amount of DNA/ml with Lemont slightly below the other 2 cultivars (Table 1). The observed difference between purification methods is significant ($F = 18.99$, 2:9 df, $P < 0.001$, Table 2). No significant difference was observed between cultivars or for the interaction between cultivar and purification method ($P > 0.129$ and $P > 0.223$, respectively).

The results of the agarose electrophoresis were somewhat surprising. Although the greatest amount of DNA was obtained using the Et-OH precipitation method, it was also the most sheared (*i.e.*, least useful) when compared to the DNA obtained from the other purification methods. For one cultivar (Tebonnet), the CTAB method yielded

A Comparison of Methods for Purification of DNA from Rice

Table 1. Amount of rice genomic DNA (ug/ml) extracted from rice categorized by purification method and cultivar.

Cultivar	Et-OH Precipitation	Spooling	CTAB
Lemont			
Trial 1	5.0	1.25	0.62
Trial 2	5.0	0.31	1.25
Tebonnet			
Trial 1	10.0	1.25	0.62
Trial 2	20.0	0.0	1.25
Newbonnet			
Trial 1	20.0	1.25	2.5
Trial 2	10.0	2.5	2.5

Table 2. Summary of amount of DNA (ul/ml) extracted from rice categorized by purification method and cultivar.

	Average Concentration	Standard Deviation
Method:		
Et-OH	11.67	6.83
Spooling	1.09	0.88
CTAB	1.49	0.86
Cultivar (combined across methods):		
Lemont	2.24	2.17
Tebonnet	5.52	8.01
Newbonnet	6.41	7.35

sheared DNA, as evidenced by DNA streaked through the entire lane, although it was not as sheared as the Et-OH method. Overall, we found less smearing through the agarose electrophoresis for DNA prepared by the spooling method. Thus we judge the spooling method to yield the best quality DNA for further analysis.

LITERATURE CITED

DEKEYSER, R., B. CLAES, M. MARICHAL, M. VAN MONTAGU, and A. CAPLAN. 1989. Evaluation of selectable markers for rice transformation. Plant Physiol. 90:217-223.

HUEY, B.A., K.K. MOLDENHAUER, and R.S. HELMS. 1987. Rice varieties in Arkansas. Cooperative Extension Service. Leaflet #518. 4 pp.

MANIATIS, T., E.F. FRITSCH, and J. SAMBROOK. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory. New York. 545 pp.

STEEL, R.G.D. and J.H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Book Co. New York. 481 pp.