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Research Article

GENOMIC DNA EXTRACTION FROM THE BLACK SCENTED RICE (*CHAKHAO*)

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ABSTRACT
An efficient DNA isolation protocol specifically modified to get pure quality DNA required for the purpose of molecular studies has been reported in this paper. The black scented rice cultivar (*chakhao poireiton*) of Manipur, India was undertaken for the study. Black scented rice is reported to have great economical value and health benefits. The protocol thus developed will be useful in getting pure DNA. Instead of using the commercially available DNA isolation kits, this protocol can be used to get pure quality DNA, free from proteins and polysaccharide compounds. The absorbance ratio A260/A280 was 1.95 and A260/A230 was 1.73 which showed the sample genomic DNA is pure, free from the contaminants protein and polyphenolics/polysaccharides compound. The concentration of DNA was 115.7 ng/µl when measured at 260 nm. When run on agarose gel also, the extracted DNA gave a clear, bright band. Thus, the sample DNA does not need any additional purification before proceeding for molecular analysis of the isolated DNA samples as well as this protocol is very simple and economical which will find wide application in genomic study.

Keywords: *DNA, Black Scented Rice, Purity, Quantity*

INTRODUCTION
Rice is one of the most important crops in the world as half of the world's population depends on rice as a staple food (Lafitte *et al*., 2004). Black rice is popular in asian countries where it mixed with white rice prior to cooking enhanced the flavor, color and nutritional value. The black scented rice of Manipur, India is scented with dark purple color pericarp. Anthocyanins have been recognized as health promoting food ingredients due to their antioxidant activity (Nam *et al*., 2006; Philpott *et al.,* 2006) and anticancer (Hyun and Chung, 2004), hypoglycemic, and anti-inflammatory effects (Tsuda *et al.*, 2003). DNA discrimination techniques have been applied to various aspects of plant breeding such as marker-assisted selection using DNA markers, cultivar identifications of the cultivars for the protection of the breeding rights and for the prevention from the contamination of undesirable cultivars and the examination of the relationships between closely-related cultivar lines (Fukami *et al.*, 2008). Molecular marker analysis using PCR based markers in genomic studies greatly enhances speed and efficacy of crop improvement through selection of desirable traits in a genotype (Pamidimarri *et al*., 2009; Chuan *et al*., 2010). Several DNA extraction procedures for isolating genomic DNA from various plant sources have been described, including salt extraction method, CTAB method and their modifications as reported by Zhu *et al*., (1993), Liu *et al*., (1995), and Huang *et al*., (2000). There are several cultivars of the black scented rice (*Chakhao*) available in Manipur, India. *Chakhao poireiton*, a black scented rice cultivar has been reported in this paper. The protocols available in literature sometimes need modification to give quality DNA. Thus, the current study was taken up to gain quality DNA from the black scented rice for molecular biology studies with some modifications in the CTAB method.

MATERIALS AND METHODS
Plant Material
The black scented rice cultivar *chakhao poireiton* was collected from the Department of Plant Breeding and Genetics, Central Agricultural University, Imphal, India. The samples were germinated on the

petriplates and were grown in the pots; the young tender leaves were collected for genomic DNA extraction.

Required Solutions

CTAB Extraction buffer: 0.1 M Tris-HCl (pH-8.0), 1.4 M NaCl, 20mM EDTA (pH-8.0), 2% CTAB, 1% β- Mercaptoethanol (add just before use), 1% Polyvinyl pyrrolydine, PVP (add in powder form while grinding), Chloroform: Phenol (24:1), 70% ethanol , 0.1 X TE buffer (pH-8)

Protocol for DNA isolation from Rice leaves.

1. 100 mg of young leaves were ground to fine powder in liquid nitrogen using prechilled mortar and pestle and the PVP was added in powder form while grinding.
2. 1 ml of the CTAB extraction buffer was added in the sample and mixed up thoroughly with the mortar and pestle and transferred in 1.5-mL microtubes.
3. The microtubes were then vortexed for 15 s and incubated at 60ºC for 45 min.
4. 200µL of chloroform-isoamylalcohol (24:1) was added to the solution, which was vortexed for 15 s and centrifuged at 10,000 rpm for 10 min.
5. The supernatant was transferred to a fresh tube and this stage was repeated once.
6. The total volume of supernatant was collected and ½ the volume of cold isopropanol (-20ºC) was added to the supernatant samples and were gently mixed by inversion and centrifuged at 10,000 rpm for 10 min; the DNA pellet adhered to the tube was then visualized.
7. The liquid phase was then released and DNA washed twice with 500 µL 70% ethanol; the pellet was set to dry for approximately at room temperature. The pellet was resuspended in 200µL TE buffer solution with 5µL RNase (10 mg/mL); the solution was then incubated at 37ºC for 1 h, and after stored at –20ºC.

RESULT AND DISCUSSION

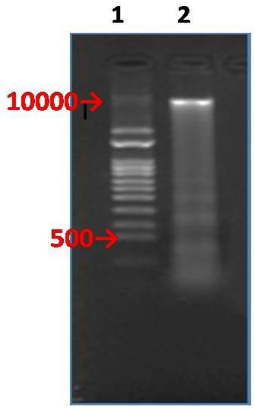

Figure 1: Extracted DNA on agarose gel. Lane1 - 1 KB ladder: Lane 2 - Rice DNA Sample

Purity of DNA was checked by the absorbance ratio A260/A280 and A260/A230 for protein and polyphenolics/polysaccharides compound, respectively using Thermo NanoDrop Spectrophotometer. The the absorbance ratio A260/A280 was 1.95 and A260/A230 was 1.73 which showed the sample genomic DNA is pure, free from the contaminants protein and polyphenolics/polysaccharides compound. The DNA quantification absorbance was measured at 260nm giving a concentration of 115.7 ng/µl. The DNA extracted was analyzed on 1% agarose gel and was visualized by staining with ethidium bromide and transillumination under short-wave UV light of BioRad gel doc system. Electrophoresis was performed at constant power of 100 Watt for 3.5 h including a 1 h pre-run to warm the gel to 50-60 °C. DNA ladder used in the electrophoresis was 1 KB ladder (Figure-1). And when run on 1% agarose, a clear bright band (Figure 1) which showed again that the DNA quantity and quality is good enough for further usage. There are only few modifications from the CTAB method by Doyle & Doyle (1987; 1990) methodology. Here,

we have added up polyvinyl pyrrolidine (PVP) in the powder form is one main modification. Almost all the steps remained same with some few modifications in the incubation period, centrifugation and the volume of the reagents taken. This method does not need proteinase in isolation step. If we are going for samples with high polyphenols we can improve by increasing the concentration of β- mercaptoethanol in extraction buffer. We can remove the polyphenols by using high levels of β-mercaptoethanol (Suman *et al*., 1990). The absorption ratio (A260/A280) of extracted DNA samples that range in between 1.8-1.9 shows that the DNA was free from protein and polyphenols. DNA yield is important in molecular studies. The results show that the quality and quantity of the DNA extracted from the black scented rice c*ultivar chakhao poireiton* in the study was pure and concentration was good enough which can be stored for further used in molecular studies like polymerase chain reaction amplification, restriction digestion, etc.

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