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A Simple, Efficient and Rapid Method for Good Quality DNA Extraction from Rice Grains

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Abstract: An efficient and good DNA extraction protocol should be simple, affordable and yield enough DNA with high quality. Rice (*Oryza sativa* L.) DNA extraction methods often use seedlings or leaves rather than the grains and tend to be time-consuming, involve multiple steps, and use hazardous chemicals and expensive enzymes. Rice grains offer several benefits over seedlings and leaves as a source of DNA for genetic analysis. However, these benefits are underutilized because the bulk of a rice grain is made up of starch. It is particularly important, but difficult to get rid of the starch while extracting DNA from rice grains. This co-precipitated polysaccharide is a known inhibitor of DNA polymerase activity in polymerase chain reaction (PCR). We describe here a very simple and highly affordable Chelex®-100 based DNA extraction method from rice grains. It does not require any hazardous chemicals or enzymes. This method reproducibly extracts DNA with good purity indices (A_{260}/A_{230} and A_{260}/A_{280} values), but requires only a few steps.

Key words: DNA extraction; rice; grain; Chelex®-100

DNA molecular markers have been indispensable in the last decades for the development of better crop varieties with higher yield potential and resistance to biotic and abiotic stress through genotyping, genetic relationship analysis, marker assisted selection, as well as screening of genetic transformants (Chen et al, 2006; Aliyu et al, 2013; Duan et al, 2015). Polymerase chain reaction (PCR) for its efficacy and throughput has been a key technique in these progresses (Ibemhal et al, 2015).

Highly pure DNA is essential in molecular biology research (Aliyu et al, 2013). A good and efficient DNA extraction protocol should be simple, affordable and yield enough DNA with high quality. It should also be rapid and reliable, particularly when many samples need to be analyzed (Ahmadikhah, 2009; Aliyu et al, 2013; Fouladvand et al, 2013). Except a few, the rice DNA extraction methods typically use seedlings or leaves and require fine grinding of the tissue materials using liquid nitrogen or silica (Duan et al, 2015). Most of these protocols use hazardous chemicals (e.g., phenol, choloform, cetyltrimethylammonium bromide (CTAB), isoamyl alcohol and β-mercaptoethanol) (Ahmadikhah, 2009; Sun et al, 2010; Ferdous et al, 2012; Roychowdhury et al, 2012; Mutou et al, 2014; Duan et al, 2015; Ibemhal et al, 2015; Thomas and Dominic, 2015; Vibhuti et al, 2015) and/or require expensive enzymes (e.g., α-amylase, RNase and/or proteinase K) (Roychowdhury et al, 2012; Duan et al, 2015; Liang et al, 2015). These protocols, in general, involve multiple steps and are time-consuming, as the use of the seedlings or leaves requires germination of seeds, and therefore, needs days before the actual DNA extraction process can ensue (Chen et al, 2006; Ahmadikhah, 2009; Aliyu et al, 2013; Fouladvand et al, 2013; Ibemhal et al, 2015; Vibhuti et al, 2015). Germination of seeds also calls for additional space (Duan et al, 2015). Isolation of DNA directly from rice seeds/grains accelerates the whole process (Liang et al, 2015). Seeds can also be stored for a long time without refrigeration. Commercially available kits for DNA extraction from rice grains are reliable, but expensive, especially when handling a large number of samples (Chen et al, 2006; Thomas and Dominic, 2015). These are particularly not affordable in labs with limited resources. There are only a few DNA extraction protocols from rice grains (Mutou et al, 2014; Liang et al, 2015; Thomas and Dominic, 2015). Liang et al

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(2015) extracted DNA from seeds using α-amylase, phenol, choloform and CTAB, however, the yield of DNA is very low. The method described by Thomas and Dominic (2015) requires liquid nitrogen, chloroform, phenol and sodium dodecyl sulfate (SDS). This protocol requires multiple steps, which increases the chance of cross-contamination if the study involves a large number of samples. The protocol described by Mutou et al (2014) uses hazardous chemicals, and requires couple of hours.

Chelex®-100 is a chelating resin with high affinity for polyvalent metal ions like Mg^{2+} (Walsh et al, 1991). Chelex®-100 is widely used in DNA extraction from biological samples in forensic analyses (Butler, 2005). Chunwongse et al (1993) mentioned the use of Chelex®-100 for DNA extraction from rice grains, but did not present any data. This protocol cannot remove carbohydrates from the extracted DNA samples, and therefore, has a low A_{260}/A_{230} value, and yields low amount of DNA. Here we described a simple, affordable and reliable, but rapid Chelex®-100 based extraction method that yielded good quality DNA with high purity indices (A_{260}/A_{230} and A_{260}/A_{280} values) from rice grains. In review of literatures on DNA extraction from rice grains, our protocol seems to be one of the simplest in terms of yield, purity and ease.

MATERIALS AND METHODS

DNA extraction

A single rice grain of 10–12 mg was mechanically crushed to coarse powder. Then, 200 μL of 0.9% NaCl solution and 100 μL of 20% Chelex®-100 (C7901, Sigma) were added and vortexed vigorously for 10 s. Volume of solutions may be adjusted proportionately according to the weight of the rice grain used. This mixture was heated in a water bath at 95 ºC for 10–20 min, vortexed for another 10 s, and then centrifuged (Microfuge® 20R, Beckman Coulter) at 13 000 r/min for 10 min. An equal volume of isopropanol was added to the supernatant upon transfer to a fresh eppendorf tube and centrifuged again at 13 000 r/min for 10 min. The pellet was washed with 70% ethanol and centrifuged for another 10 min at 13 000 r/min. Afterwards, 70 μL of nuclease free water was added to the pellet and kept at 4 ºC in a refrigerator for 20–30 min. After a final centrifugation at 13 000 r/min for 5 min, the aqueous phase was collected leaving the soft spongy pellet.

Concentration and quality measurement

Concentration and purity of extracted DNA from rice grains were measured in a spectrophotometer (NanoDrop™ 2000, Thermo Fisher Scientific Inc, Wilmington, DE, USA). Chelex®-100 yields single stranded DNA upon heating at 95 ºC (Butler, 2005; Casquet et al, 2012). Therefore, the concentration of DNA in the samples was measured in the NanoDrop™ spectro-photometer based on the formula: Concentration (ng/μL) = (OD_{260} × 33). The ratios of the absorbance at 260 nm to 280 and 230 nm were used as indicators of contamination of DNA with proteins (A_{260}/A_{280}) and carbohydrates (A_{260}/A_{230}), respectively.

Amplification of extracted DNA in PCR with random amplified polymorphic DNA (RAPD) primers

DNA samples extracted using Chelex®-100 were used as templates in PCR. Sequences were amplified using RAPD primers: OPA04 (5′-AATCGGGCTG-3′) and OPA10 (5′-GTGATCGCAG-3′) (Kanawapee et al, 2011) in a thermal cycler (Gene Atlas G, Astec Co. Ltd.). Approximately 50 ng genomic DNA was used for amplification in a final reaction volume of 25 μL made of PCR buffer (EP0702, Thermo Scientific), 0.4 mmol/L dNTP mix (110-002, GeneON), 0.4 μmol/L of RAPD primer and 1.5 U *Taq* DNA polymerase (EP0702, Thermo Scientific). The cycle condition was as follows: an initial denaturation step at 94 ºC for 3 min, then 34 cycles each with denaturation at 94 ºC for 20 s, annealing at 38 ºC for 40 s, and elongation at 72 ºC for 1 min, followed by a final extension at 72 ºC for 7 min. Amplified products were resolved in 1.5% agarose gel (0710, Amresco®) using 0.5× Tris Borate EDTA (TBE) buffer in a horizontal gel electrophoresis system along with DNA marker (300003, GeneON). DNA bands were visualized in a gel documentation system (WGD-30, Witeg) following incubation with ethidium bromide (0492, Amresco®) in TBE buffer and photographed with WiseCapture II™ software.

RESULTS AND DISCUSSION

The bulk of a rice grain is made of carbohydrates (Vibhuti et al, 2015). Starch, a complex carbohydrate, makes up nearly 80%–90% of the total weight of a rice grain (Duan et al, 2015; Thomas and Dominic, 2015). It is particularly important, but difficult to get rid of the starch while extracting DNA from rice grains (Liang et al, 2015; Thomas and Dominic, 2015). This co-precipitated polysaccharide is known to inhibit DNA polymerase activity in PCR (Liang et al, 2015; Vibhuti et al, 2015). Therefore, the poor quality DNA extracted with rapid extraction methods from rice grains are often incompatible in PCR and fails to amplify products larger than 500 bp (Liang et al, 2015). This is why good-quality DNA extraction methods often use tissues other than grains and tend to be time-consuming, involve multiple steps, and use hazardous chemicals and enzymes (Ahmadikhah, 2009; Sun et al, 2010; Ferdous et al, 2012; Roychowdhury et al, 2012; Mutou et al, 2014; Duan et al, 2015; Ibemhal et al, 2015; Liang et al, 2015; Thomas and Dominic, 2015; Vibhuti et al, 2015).

The protocol described here requires fewer steps, but reproducibly yields DNA from rice grains with A_{260}/A_{280} and A_{260}/A_{230} absorbance ratios within the range of 1.8–2.2. A_{260}/A_{280} ratio that is higher than 2.0 is indicative of very pure DNA (Beyenal and Babauta, 2015). The A_{260}/A_{230} values for a 'pure' nucleic acid commonly reside in the range of 1.8–2.2. We obtained 3–6 μg DNA from one rice grain with a weight of