A high-throughput DNA extraction method from rice seeds

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ABSTRACT
Rapid and inexpensive preparation of genomic DNA from rice seeds for marker-assisted selection and seed purity estimation is a major bottleneck for plant breeders. Here, we describe a high-throughput method that provides DNA at sufficient quantity and quality for these applications. Optimization of buffer composition and individual protocol stages allow processing of 384 samples within 2 h, yielding templates that reliably support downstream polymerase chain reaction of single copy amplicons up to 1.2 kb.

KEYWORDS
DNA extraction; rice; seeds

Introduction
Rice is the most important staple food crop for more than half of the world’s population. To adapt to the world’s increasing demand for rice, several approaches are carried out to increase rice yields, including marker-assisted selection (MAS) and hybrid rice production. DNA isolation is a basic and key step for such procedures [1,2] and isolating DNA from seeds has at least two main advantages. First, part of the endosperm is sufficient for DNA isolation, leaving the corresponding viable embryo available for plant growth and phenotyping, which is important in MAS programmes. Second, isolating DNA from seeds accelerates genotyping by eliminating plant germination and growth, which can be very valuable in cases of seed purity identification before the sowing season.

Compared to leaf-based DNA isolation methods,[3–5] seed-based DNA isolation methods in rice are rather limited.[6,7] The presence of stored seed reserves, including carbohydrates, lipids, proteins and polyphenols, complicates greatly the process of isolating DNA from seeds. High-quality plant DNA extraction methods tend to be time-consuming, laborious and expensive because of the multiple manipulation steps, enzymes and chemicals involved.[6,8] Conversely, rapid rice seed extraction methods yield DNA of poor quality that is typically insufficient for generating polymerase chain reaction (PCR) products larger than 500 bp.[7,9–12] In practice, PCR markers for MAS or seed purity identification are often 1 kb or more.

[13–15] Commercial kits (e.g. Qiagen DNEasy 96 Plant Kit) are available and effective but expensive, while automated DNA extraction system are also effective but carry high initial equipment cost and high expenses per assay which typically exceed the budgets of plant molecular breeding laboratories in developing countries.

To address this problem, here we describe a simple, rapid, high-throughput and low-cost method to prepare genomic DNA from rice seeds. The method is suitable for MAS programmes and quick identification of seed purity in practice.

Materials and methods

Materials
Rice seeds of Nipponbare (japonica) and Junyou522 (indica) were obtained from Hubei Provincial Key Laboratory for Protection and Application of Special Plants in Wuling Area of China, Key Laboratory of State Ethnic Affairs Commission for Biological Technology, College of Life Science, South-Central University, China. rTaq DNA polymerase and deoxynucleoside triphosphate (dNTP) mixture (2.5 mM) were purchased from TAKARA Biotechnology Co., LTD (Dalian). Primer synthesis was carried out by Beijing Liuhe Huada Genomics Technology Co., Ltd. a-Amylase was purchased from Worthington Biochemical Corporation. All other reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd.

Reagents and consumables
The following reagents and consumables were used: CTAB (hexadecyl trimethyl ammonium bromide); chloroform;
isopropanol; ethanol; 3 mm steel beads; 96 round well blocks with silicone cover (supplied by Geno); and 96-well PCR plates.

**Solutions**

The following solutions were prepared:

- Extraction buffer: 200 mmol/L Tris-Cl (pH = 8.0), 50 mol/L ethylenediaminetetraacetic acid (EDTA; pH = 8.0), 2.8 mol/L NaCl, 4% CTAB, 2% sodium dodecyl sulphate;
- 5 mol/L potassium acetate isopropanol;
- α-amylase (15 mg/mL);
- Tris-EDTA buffer.

**Equipment**

The equipment included: Grinder (SPEX SamplePrep 2010, Geno, USA); multichannel pipettes (Eppendorf, Germany); plate mixer; plate centrifuge (Herseus Multi-fuge X1R, Thermo Fisher, Germany); water bath or oven; any type that can maintain temperature at 65°C; NanoDrop ND-1000 Spectrophotometer (NanoDrop Technology, USA).

**Protocol**

The DNA extraction protocol includes the following stages:

1. Cut the rice seeds into two halves (if necessary), grind the embryo-free portion in the 96 round well block (two steel beads in each well) twice for 4 min in Geno grinder.
2. Add 150 μL of extract solution and 20 μL of α-amylase (15 mg/mL) to each well, keep the plates at 65°C for 30 min.
3. Centrifuge plate at 1660 g for 10 min at room temperature.
4. Add 20 μL of chloroform and mix for 10 s by plate mixer.
5. Centrifuge plate at 1660 g for 1 s at room temperature, then add 20 μL of 5 mol/L potassium acetate and mix for 10 s.
6. Centrifuge plate at 1660 g for 10 min at room temperature.
7. Carefully transfer 100 μL of supernatant to a 96-well PCR plate.
8. Add 80 μL of isopropanol and mix by inversion several times.
9. Centrifuge plate at 3000 g for 15 min at room temperature.
10. Discard the supernatant and wash the pellet with 70% ethanol (v/v).
11. Dry the pellet by centrifugation at 17 g for 10 s at room temperature.
12. Dissolve in 50 μL of Milli Q water and directly use as template for PCR amplification.

**PCR analysis**

PCR analysis was performed in 10 μL of reaction mixture. A reaction tube contained 1.0 μL of template DNA, 0.2 U of rTaq, 100 μmol/L of each dNTP, 1× TAKARA rTaq buffer, and 0.5 μmol/L primers (Table 1). Amplifications were performed in the PTC-100TM PCR system (MJ, USA) with touchdown PCR with the following conditions: 94°C for 3 min; 15 cycles at 95°C for 30 s, annealing temperature decrement of 0.7°C every cycle (from 65°C to 54.5°C) and 72°C for 1.5 min; 20 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 1.5 min; and a final extension at 72°C for 10 min.

Amplification products were separated in a 1% agarose gel, stained with ethidium bromide and visualized on TFL-40 gel documentation system (Synoptics Ltd, UK).

**Results and discussion**

Total DNA isolated from embryo-free halves of rice seeds was checked by a NanoDrop ND-1000 Spectrophotometer. Our procedure yielded 3.07 ng/μL DNA. The ratio of absorbance at 260 nm to 280 nm was 1.8—2.1, which indicated insignificant levels of contaminating proteins and polysaccharides but high RNA content without RNase A treatment. Our method significantly minimizes
the time and the use of laboratory materials. A single person can process as many as 384 samples within 2 h.

To test the suitability of the purified DNA for PCR-based analysis, several single-copy gene targets were amplified (Table 1). As shown in Figure 1, the PCR amplifications were all successful, using specific primers JU-1 to identify the seed purity of hybrid rice JY522. Only one out of 47 amplicons yielded two bands, showing that seed purity for the sample was 98%. PCR amplifications were all successful when PCR product size was less than 1.2 kb, and the success rate was 50% for PCR product sizes above 1.6 kb (Table 1).

α-Amylase is a critical component of our methodology, as it significantly improves the quality of the isolated rice DNA. Contamination with high concentrations of polysaccharide, which is an inhibitor for the enzymes in the case of downstream processes such as PCR, is a big problem with many seed DNA extraction methods. α-Amylase has proven to be efficacious and is used commercially to remove polysaccharide contamination and increase PCR amplification rates in DNA extraction of corn, wheat and potato (α-Amylase Ultrapure, Nippon Gene, Japan).[16] In our study, PCR amplification using the primer pair Pibdom (Table 1) was very unstable using DNA isolated by the same method but without the application of α-amylase (data not shown). Applying an appropriate concentration of α-amylase in the extraction buffer resulted in the purification of DNA that supported PCR amplification using primer Pibdom in all cases.[17] Furthermore, the success rate of PCR amplification could be significantly increased for amplicons larger than 1 kb (data not shown). This indicates that our method could be applied to the DNA extraction of other cereal grains.

As mentioned above, the DNA quality of rapid isolation methods from rice seeds is typically insufficient to support PCR amplifications greater than 500 bp.[20,21] Even using a commercial kit (QuickExtract™ Seed DNA Extraction Solution, Epicentre, Madison, WI), the reproducibly successful PCR product sizes are still less than 750 bp.[22] This could be due to DNA degradation and more contamination in these methods. PCR amplifications of DNA samples obtained by our method all succeeded for PCR product sizes up to 1.2 kb, which has high practical value in MAS programmes and quick identification of rice seed purity.

Conclusions

Overall, the method described here provides an efficient way for rapid isolation of DNA from rice seeds. The method is simple, reasonably high-throughput in nature, cost competitive compared to many other rice DNA extraction methods and, importantly, workable in laboratories lacking specialized or sophisticated instrumentation. Time and resources are saved by our method for at least two reasons: (1) the time needed for seed germination is eliminated; and (2) greenhouse and/or field space is saved. For molecular breeding applications such as MAS, positional cloning, allele mining or seed purity identification, we believe our method could be immensely helpful in DNA extraction from a large number of rice seeds.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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