DNA preparation of Amorphophallus paeoniifolius for SSR evaluation Version 2

edi santosa

Abstract

The corms and leaves of elephant foot yams (*Amorphophallus paeoniifolius* (Dennst.) Nicolson) are important foods in the local diet in many Asian regions. The crop has high productivity and wide agroecological adaptation and exhibits suitability for the agroforestry system. Although the plant is assumed to reproduce via panmixia, a comprehensive study on the genetic background across regions to enhance wider consumer palatability is still lacking. Here, ten informative microsatellites were analyzed in 29 populations across regions in India, Indonesia and Thailand to understand the genetic diversity, population structure and distribution to improve breeding and conservation programs. The genetic diversity was high among and within regions. Some populations exhibited excess heterozygosity and bottlenecking. Pairwise F_{ST} indicated very high genetic differentiation across regions (F_{ST} = 0.274), and the Asian population was unlikely to be panmictic. Phylogenetic tree construction grouped the populations according to country of origin with the exception of the Medan population from Indonesia. The current gene flow was apparent within the regions but was restricted among the regions. The present study revealed that Indonesia and Thailand populations could be alternative centers of the gene pool, together with India. Consequently, regional action should be incorporated in genetic conservation and breeding efforts to develop new varieties with global acceptance.

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Guidelines

Leaf collection

Leaf of Amorphophallus paeoniifolius was collected by using dry method.

DNA extraction

DNA was extracted using the modified CTAB method from the original protocol described by Lian et al. [2002]. Modification was required because the leaf of *A. paeoniifolius* contained glucomannan, a kind of high molecular of carbohydrate that difficult to dissolve in the water. Presence of glucomannan in the solution might cause DNA impurity, resulting on low PCR amplification. To separate DNA from the glucomannan, we used fishing method. Glucomannan pellet commonly transparent but still visible

to be observed at the bottom of the extraction tube after we added water. After about 40 minutes, and DNA was expected dissolve completely, we pick up the glucomannan by fishing. For SSR analysis, DNA from approximately one gram of dry leaf was then preserved in a final volume of 200 µL of water and was stored at -30 °C until ready for use.

SSR primer optimation and reagent

Polymerase chain reaction (PCR) was carried out in a 5-µL tube. For large sample, we recommend to use PCR plate with 48 wells. A 0.5-µL aliquot of extract was used in each PCR amplification to approximately contain 5-20 ng of DNA. SSR marker and DNA were amplified with a PCR reagent mix of 0.5 µM of the Forward primer, 0.1 µm of the Reverse primer tailed with U-19 (Fastac Co, Tokyo-Japan), 0.5 µM of the U-19 primer labeled with Texas Red, 0.2 mM of each dNTP, 1× PCR buffer (Mg^{2+} free, Applied Biosystems), 2.5 mM $MgCl_2$, and 0.5 U of Ampli Taq Gold DNA polymerase (Applied Biosystems).

Protocol

Leaf collection

Step 1.

Harvest a fresh leaf of Amorphophallus paeoniifolius at green stage.

Step 2.

Clean the leaf initially both abaxial and adaxial sides using moist tissue paper.

Step 3.

Clean additionally using a mild detergent.

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The cleaning step was important to ensure extracted DNA only from the A. paeoniifolius.

Step 4.

Then, remove the leaf midrib/vein using a sterile knife.

Step 5.

Before storing in the silica gel, cut the leaf into small sections facilitating quick drying.

Step 6.

One gram of fresh leaf requires approximately 20-30 gram of silica gel, and store in air tight box.

DNA extraction

Step 7.

Glucomannan pellet commonly transparent but still visible to be observed at the bottom of the extraction tube after adding water. After about 40 minutes, and DNA is expected dissolve completely, pick up the glucomannan by fishing.

Step 8.

For SSR analysis, preserve DNA from approximately one gram of dry leaf in a final volume of 200 µL of water and store at -30 °C until ready for use.

SSR primer optimation and reagent

Step 9.

Perform the PCR using hot start to increase reproducibility of the product.

Step 10.

Perform the PCR as follows: **one cycle** of **9 min at 94 °C** followed by **30 s at the locus-specific annealing temperature (T_a)** plus **1 min at 72 °C**, and then **38 cycles of 30 s at 94 °C**, **30 s at the locus-specific T_a** plus **1 min at 72 °C**, followed by **one cycle of 30 s at 94 °C**, **30 s at the locus-specific T_a** plus **5 min extension at 72 °C**, and ending at 4 °C.

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In the optimation step, we mixed some SSR primers that had similar *Ta* and the target bands that had distant allele size.

Gel eletrophoresis

Step 11.

Finally, mix 1.5 µL of the PCR product with 3.5 µL of loading dye, denature using the Thermal cycle TAKARA for **five minutes at 95 °C**, and then was place **on ice** immediately for **five minutes** prior to loading.

AMOUNT

1.5 µl Additional info: PCR product

AMOUNT

3.5 µl Additional info: loading dye

Step 12.

Load two-microliter aliquots onto a 6% denaturing polyacrylamide gel and were run using the SQ-5500 sequencer (Hitachi Co., Tokyo). The band patterns were analyzed with FRAGYLS ver. 2 (Hitachi Electronics Engineering Co., Japan).

AMOUNT
2 µl Additional info: aliquots

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Two bands were considered different alleles if the difference was larger than two base pairs.

Step 13.

Two bp different is a matter of technical procedure, not related to nucleotide repeat motif. Determine allele size using FRAGYLS software.

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The software has automatically detected homo or hetero alleles, successfully in most individuals. In some cases, hetero alleles had only one base in different of the first and the second alleles. In this case, we checked the size manually considering the bands quality and concluded the alleles size as homo or hetero to avoid bias estimate of FRAGYLS software. Theoretically, we concluded the hetero alleles if 2 or more bp in different.