Biochemical Systematics and Ecology 69 (2016) 41–50

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Sequencing and phylogenetic analysis of the chloroplast genome of *Pseudosasa japonica* f. Akebonosuji

Mingbing Zhou*, Miaomiao An, Xiangwan Xia, Haiyun Yang, Minmin Cheng, Kaili Wang, Wei Fang

The Nurturing Station for the State Key Laboratory of Subtropical Silviculture, Zhejiang A & F University, LinAn, 311300, Zhejiang Province, PR China

ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form 12 August 2016
Accepted 13 August 2016

Keywords:
Pseudosasa japonica f. Akebonosuji H. okamura
Chloroplast genome
Granule-bound starch synthase I (*GBSSI*)
Evolution
Nucleotide polymorphisms

ABSTRACT

Pseudosasa japonica f. Akebonosuji H. Okamura is a bamboo species with variable leaf colors, including albino, green, and green-white stripes. To determine whether variation in leaf color is due to mutations in the chloroplast genome, we sequenced the chloroplast genomes of green and albino leaves of *P. japonica* f. Akebonosuji. The results indicated that the chloroplast genome included 86 protein-coding genes, seven ribosomal RNA genes, and 31 tRNA genes. The similarity of chloroplast genomes for the two leaf types was 99.98%, with variation between genes encoding for *trnfM* and *trnT*. We observed that the relative expression patterns of *trnfM* and *trnT* were reversed in green and albino leaves. Whether the differential expression of *trnfM* and *trnT* is involved in leaf color variation among *P. japonica* f. Akebonosuji remains unclear.

With many bamboo chloroplast genomes available, we aligned the chloroplast genomes of 28 bamboo species, including *P. japonica* f. Akebonosuji, to analyze polymorphisms. This comparison revealed that noncoding regions possessed more nucleotide polymorphisms than coding regions. Chloroplast genomes and the nuclear gene "granule-bound starch synthase I" (*GBSSI*) of 28 bamboo species were used to construct evolutionary trees. Both evolutionary trees indicated that *P. japonica* f. Akebonosuji was clustered into Subtrib. Arundinariinae.

1. Introduction

Bamboo is in the subfamily Bambusoideae of the family Poaceae. Bamboos are remarkably robust forest grasses of which more than 1400 species in 115 genera have been described (Bamboo Phylogeny Group [BPG], 2012), and global bamboo forest coverage is currently 14 million hm^2. Bamboos are widely distributed in the tropical, subtropical, and warm temperate regions between the latitudes of 46° and −47°. In China, nearly 500 bamboo species in 39 genera are primarily distributed among the provinces of Zhejiang, Fujian, Jiangxi, Sichuan, Hunan, Hubei, and Guangxi, in addition to others. The bamboo forest coverage in China is approximately 5 million hm^2. *Pseudosasa japonica* f. Akebonosuji H. Okamura is a rare, ornamental bamboo species that originated in Japan, with plants that are short and approximately 2 m in height (Yang et al., 2010). The leaf color of *P. japonica* f. Akebonosuji can change from green to a striped green-white to albino during natural cultivation, and

* Corresponding author.
E-mail address: zhoumingbing@zafu.edu.cn (M. Zhou).

http://dx.doi.org/10.1016/j.bse.2016.08.002
0305-1978/

this type of variation is unstable. Previous studies showed that the expression of chloroplast genes of albino seedlings of *Arabidopsis thaliana* (Cho et al., 2009), wheat (Xia et al., 2012), and tobacco (Bae et al., 2001) was clearly different from that of wild-type seedlings. Furthermore, a study demonstrated that a deficiency of chloroplast genes led to albinism in tobacco (Fleischmann et al., 2011). In *Pleioblastus fortunei*, another bamboo species, the expression levels of chloroplast genes in the albino mutant varied significantly from those of the wild type (Yuan et al., 2010).

Plant photosynthesis occurs in the chloroplast organelle. Chloroplast genomes are circular sequences of DNA composed of four parts: two inverted repeat (IR) regions, a large single-copy (LSC) region separating the two IR regions, and a small single-copy (SSC) region. The chloroplast genome is approximately 100–200 kb in length and contains 100–130 different genes. Among these genes, approximately 80 encode for proteins involved in photosynthesis and gene expression, whereas the others are transcribed into tRNA or rRNA (Tang et al., 2011). The chloroplast genome shows features of matrilineal inheritance and compared with the nuclear genome, has a smaller molecular weight, simpler structure, more conservative sequence, lower mutation rate, and more stable heredity (Pyke, 1999). Therefore, for investigations on phylogeny, chloroplast DNA is currently regarded as the ideal material (Badenes and Parfitt, 1995). To date, at least 27 complete bamboo chloroplast genomes are published, and entire or partial chloroplast genomes have been used to construct phylogenetic trees to determine bamboo phylogenetic relationships (Ma et al., 2014; Wysocki et al., 2015).

In the study, we sequenced the chloroplast genomes of both green and albino leaves of *P. japonica* f. Akebonosuji and then systematically analyzed the structures of the chloroplast genomes of differently colored leaves. We also analyzed the evolutionary position of *P. japonica* f. Akebonosuji in Bambusoideae based on the nucleotide polymorphisms of chloroplast genomes and *GBSSI* of *P. japonica* f. Akebonosuji and 27 other bamboo species available from NCBI.

2. Materials and methods

2.1. Plant materials

Pseudosasa japonica f. Akebonosuji H. Okamura green and albino leaves were collected from the bamboo garden of Zhejiang A & F University, China. The leaves of *Bambusa emeiensis* L. C. Chia *et* H. L. Fung, *Phyllostachys propinqua* McClure, and *Ph. sulphurea* (Carr.) A. *et* C. Riv. were collected from the Anji Bamboo Garden in Zhejiang, China.

2.2. DNA extraction

Total DNA of green and albino leaves of *P. japonica* f. Akebonosuji was extracted using the CTAB method (Doyle, 1987). Genomic DNA of *B. emeiensis*, *Ph. propinqua*, and *Ph. sulphurea* was also extracted using this method. The total DNA concentration of each species was determined using ultraviolet spectrophotometry and electrophoresis with a 0.7% agarose gel.

2.3. Sequencing of chloroplast DNA of P. japonica f. Akebonosuji green and albino leaves

We obtained the chloroplast genome sequences of Bambusoideae from NCBI, in addition to those of rice, maize, and other gramineous plants for reference. The chloroplast genome was divided into 37 areas (Fig. 1) based on conserved regions, which were defined by homologous analysis using Clustal software (Chenna et al., 2003). Two adjacent areas showed an overlapping region of >100 bp. Primers were designed according to the highly conserved regions and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) (Supplementary table S1). High-fi ity polymerase chain reaction (PCR) was conducted using the extracted total DNA as the template in a total reaction volume of 50 μL, which contained 0.5 μL of LA Taq polymerase (5 U/μL), 5 μL of 10 × LA buffer II, 8 μL of dNTPs (2.5 mM each), 2 μL of each primer (10 μM), 1 μL of the extracted DNA (<1 μg), and ddH_2O to a total volume of 50 μL. PCR conditions consisted of an initial denaturation step at 94 °C for 1 min, followed by 30 cycles at 98 °C for 10 s and at 68 °C for 4 min (which extended approximately 1000 bp of DNA per minute), and a final extension step at 72 °C for 10 min. The amplified products were run on 0.7% agarose gels to verify the quality of the PCR products, and the PCR fragments were later purified and sequenced using Sanger sequencing technology.

Sequence differences in the chloroplast genome sequences of green and albino leaves of *P. japonica* f. Akebonosuji were investigated by sequence alignment using DNAMAN software.

2.4. Analysis of gene expression levels

RNA was extracted from tender green and albino leaf samples of *P. japonica* f. Akebonosuji. The cDNA was generated using a PrimeScript™ II 1st Strand cDNA Synthesis Kit (TaKaRa, Japan). The cDNA was then subjected to quantitative reverse transcription/real-time PCR (qRT-PCR) (TaKaRa, Japan). The reaction conditions were those recommended by the SYBR® *Premix Ex Taq*™ II Kit (Tli RNaseH Plus) (TaKaRa). The melt curve was obtained by heating the amplicon from 65 °C to 95 °C. The qRT-PCR was performed in 96-well plates with a CFX96 Real-time PCR Detection System (Bio-Rad). The reverse primer sequence of *trnfM* was 5′-GAGACGGGAATCGA-3′, and the forward primer sequence was 5′-AGCGGAGTAGAGCA-3'. Reaction conditions consisted of 30 s at 95 °C and 40 cycles at 95 °C for 5 s, at 55 °C for 20 s and at 72 °C for 20 s. The reverse primer sequence of *trnT* was 5′-ATTTGAACCGATGA-3′, and the forward primer sequence was 5′-GCCCTTTTAACTCA-3'. Reaction conditions consisted of 30 s at 95 °C and 40 cycles at 95 °C for 5 s, at 50 °C for 20 s and at 72 °C for 20 s. All qRT-PCR reactions