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Short Communication

Simple DNA extraction method for SSR-PCR analysis from different tissues of *Tapiscia sinensis*, an endangered plant species

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Abstract

A simple and rapid method to process different tissues of *Tapiscia sinensis* for effective plant DNA extraction were established in the study. DNA quality and yield obtained from bark, leaves and seeds were evaluated by Eppendorf BioPhotometer and SSR-PCR analysis. The results indicated that extracted genomic DNA based on the established method was sufficient for SSR-PCR profiling of different tree tissues. Hence, the sample treatment and extraction method can be used for SSR-PCR applications in tree genetic and molecular analysis research.

Key words: *Tapiscia sinensis*, sample treatment, DNA extraction, SSR-PCR

Tapiscia sinensis Oliv (Tapisciaceae) is a precious hardwood tree of China that is sporadically distributed in the Yangtze River valley (Bremer et al. 2009; Wu et al. 1996). As a quaternary glacial relict, *T. sinensis* is potentially a good model for inferring colonization and evolutionary histories of post-glacial plant species in subtropical China (Tang and Li 1996). Unfortunately, due to habitat fragmentation, naturally poor regeneration and the influences of human activity, *T. sinensis* is currently on the IUCN red list of threatened species in the vulnerable A1c category (http://www.iucnredlist.org, 1998).

In recent years, SSR (simple sequence repeats)-PCR has been utilized for a variety of applications, including but not limited to paternity analysis, conservation genetics and phylogenetic studies (Kalia et al. 2011; Selkoe and Toonen 2006). The extraction of genomic DNA is the first crucial step in SSR-PCR analysis. A common material used in plant genomic DNA extraction is young leaves. However, adult *T. sinensis* are usually more than 20 m in height, thus access to young leaves for sampling can be challenging. Sampling of young leaves from *T. sinensis* offspring for DNA extraction requires a wait period of at least two months because of seed dormancy and germination. In order to solve the aforementioned problems, we have developed a simple and rapid sample treatment and effective DNA extraction method for tree SSR-PCR analysis.

Five adult *T. sinensis* trees were selected from Qinling Mountains of China (N33°31', E108°35', H 1,348 m) in October 2013, in which the samples were collected for DNA extraction. Bark and leaves from the five trees were individually stored in plastic bags with silica gel at room temperature for two days to dry. In addition, five fresh seeds from the fifth tree were collected and stored at –20°C until use. For grinding purposes, 2.0 ml EP tubes, 3 mm diameter stainless steel beads and a TissueLyser II (Qiagen, Germany) were used. To fresh EP tubes, 50 mg of dried bark cut into small pieces and two beads were added or 30 mg of dried leaves and one bead were added. EP tubes with dried bark were subjected to 30 strokes per sec for 4-5 min and EP tubes with dried leaves were subjected to 25 strokes per sec for 2 min

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in the TissueLyser. For seeds, the average green-weight without testae was 53.8 mg. Seeds were cut into 4 pieces and placed in a fresh EP tube with two beads and subjected to 30 strokes per sec for 3 min in the TissueLyser.

DNA was extracted from samples using the Plant Genomic DNA Kit (Tiangen, China) according to directions. Since DNA content in bark is less compared to other tissues, the incubation step with the GP1 buffer at 65°C was prolonged to 25 minutes to increase DNA yield. To prevent sample oxidation, PVP-40 was added to the buffer up to 2.0% (w/v). DNA concentrations were determined using an BioPhotometer plus (Eppendorf, Germany), in which the average DNA concentration of bark, leaves and seeds were 32.4, 48.2 and 30.3 ng μL^{-1}, respectively.

Two SSR primer sets, TS53 and TS62 were used for PCR analysis (Table 1). A PCR reaction volume of 15 µL contained 7.5 µL of 2×PCR Mixture (Tiangen), 0.25 µM of forward and reverse primers and 20 ng of genomic DNA. The PCR reaction conditions were as follows: 95°C at 5 min followed by 30 cycles of 50 sec at 94°C, 45 sec at 61°C and 1 min at 72°C, extension at 72°C for 10 min and then 10 min at 10°C. Amplification products were run on a 10% polyacrylamide gel and visualized by silver staining. SSR-PCR amplification profiles of *T. sinensis* DNA from different tissues displayed both desired and distinct bands with good resolution and reduced background (Fig. 1). The polymorphism of primer set TS53 was greater compared to primer set TS62 and was consistent with our previous experiments. Hence, the extracted genomic DNA was sufficient for SSR-PCR analysis.

Extracting DNA from seeds and bark for PCR analysis is straightforward based on a number of methods (Lin and Walker 1997; Kang et al. 1998; Halilu et al. 2013). However, grinding of a large number of samples is time-consuming and the rate-determining step in DNA extraction. Dry seeds of *T. sinensis* are too hard to be broken and using liquid nitrogen grinding causes material loss considering the small size of the seed. In our study, we divided single fresh seed into small pieces and placed them in an EP tube with metal beads and subjected the tube to the highest frequency in a TissueLyser. Situations in which divided seed could not be ground, seed material were soaked in 50 µL of water overnight at 4°C. Compared to other methods, we extracted DNA from 0.05 g of single fresh seed; however, less material could have satisfied experimental needs. The tenacity and length of *T. sinensis* bark fibers make the material hard to grind;

Table 1. Characterization of two polymorphic microsatellite loci in *Tapiscia sinensis*

Locus	Repeat motif	Primer sequences (5' to 3')	Ta (°C)	Product size (bp)
TS53	(TCA)6	F: GACTTCCTCCTC TGCCACTG	62	227
		R: AGGATCGCGAG AGATTTCCT		
TS62	(TTC)7	F: GGCGAGTGTTC TTTCGTAGC	60	181
		R: TTTCACGCAAC AAGAACGAG		

Ta = annealing temperature

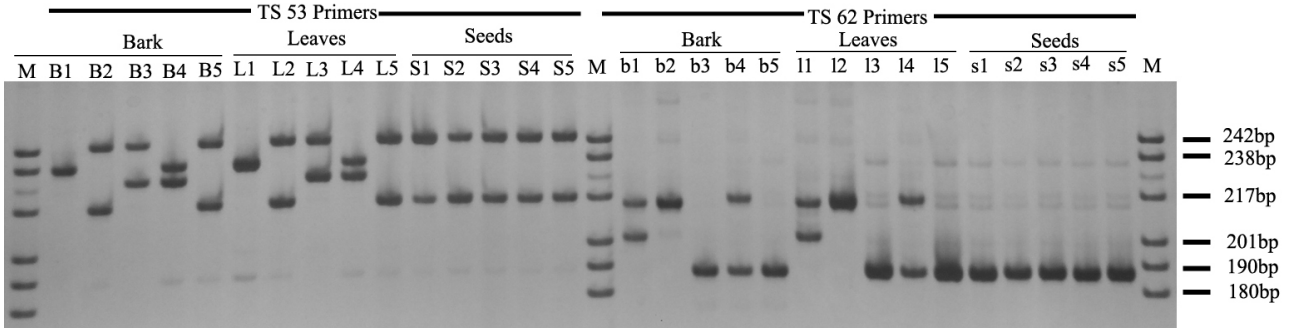

Fig. 1. SSR-PCR amplification profile of *T. sinensis* DNA from different tissues using TS53 and TS62 primers. Tissues sampled for SSR-PCR are designated with B/b = bark, L/l = leaves and S/s = seed. Numbers 1-5 for B/b and L/l are in reference to the five trees sampled for bark and leaves, and 1-5 for S/s are in reference to five seeds from the fifth tree. As observed in the SSR-PCR profile, DNA extracted from bark and leaves of the same tree, displayed similar band patterns. M is the DNA marker pBR322 DNA/MspI

therefore, cutting the bark into small fragments was necessary. Based on traditional methods involving liquid nitrogen grinding, an approach followed by DNA extraction of 96 samples would require a daylong effort. However, using a high throughput TissueLyser approach, the grinding process and DNA extraction of equivalent samples can be completed in half the time. Thus, our simple and rapid treatment process and effective DNA extraction method provides a reliable means for SSR-PCR applications in trees paternity analysis and genetic diversity research.

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