Biochemical Systematics and Ecology 69 (2016) 1–5

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Genetic diversity of SSR markers in wild populations of *Tapiscia sinensis*, an endangered tree species

Xiao-Jun Zhou [a, b], Xiao-Long Ren [a], Wen-Zhe Liu [a, *]

[a] School of Life Sciences, Northwest University, 229 Taibai Bei Road, Xi'an 710069, China
[b] School of Life Sciences, Luoyang Normal University, 6 Jiqing Road, Luoyang 471934, China

ARTICLE INFO

Article history:
Received 19 May 2016
Received in revised form 4 August 2016
Accepted 13 August 2016

Keywords:
Tapiscia sinensis
Genetic diversity
Simple sequence repeat (SSR)
Conservation strategy

ABSTRACT

Tapiscia sinensis is a Tertiary relict and endangered tree species with unique scientific research value and great economic value. In this study, we assessed the genetic diversity of five wild *T. sinensis* populations from different geographical regions using 10 polymorphic simple sequence repeat (SSR) markers. Our results reveal that the natural populations of *T. sinensis* have rich genetic diversity (*PPL* = 100%, *He* = 0.6904, *I* = 1.4368), with Shannon's index indicating that the *T. sinensis* populations are at a relatively stable stage. Of the genetic relationships among populations, the distance between the Hunan Yanling (YL) and Guizhou Xifeng (XF) populations is the smallest (0.4829); the genetic distance between the Shaanxi Ningshan (NS) and the Guizhou Xifeng (XF) populations is the largest (0.9821). A Mantel test shows that there is no correlation among the populations between geographic distance and genetic distance. AMOVA suggest that 33.3% of the genetic variation arose among the populations, while 66.7% of the variation arose within them. The moderate gene flow among populations (*Nm* = 0.7274) is not sufficient to counteract genetic drift within the populations and result in significant differentiation (*Fst* = 0.2987). Our results will benefit the conservation and exploitation of *T. sinensis* and provide a theoretical basis for further study of the evolution and phylogeography of the species.

1. Introduction

In recent years, molecular markers have become popular for determining genetic diversity because of their highly distinguishable nature and relatively low cost. The advantages of their application in plants are well known. DNA-based molecular markers, such as sequence-related amplified polymorphisms (SRAPs), simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs), are relatively abundant, non-tissue-specific, suitable for early rapid assessment, and less susceptible to environmental impacts. Among these molecular markers, the SSR (also known as the short tandem repeat, or STR) widely exists in eukaryotic genomes and is commonly used because it is codominant, highly polymorphic and suitable for automated analysis (Sharma et al., 2007; Zong et al., 2015).

As a woody perennial androdioecious species, with both male and hermaphroditic individuals in populations, *Tapiscia sinensis* has unique value in research on the evolution and maintenance of plant breeding systems (Zhou et al., 2016). In addition, the species has great utility in medicine and landscaping (Xie, 2006; Zhou et al., 2015a). Therefore, *T. sinensis* is an

* Corresponding author.
E-mail address: lwenzhe@nwu.edu.cn (W.-Z. Liu).

http://dx.doi.org/10.1016/j.bse.2016.08.004
0305-1978/

excellent germplasm resource with scientific-research, economic and aesthetic value. However, because of habitat fragmentation, naturally poor regeneration and the effects of human activity, *T. sinensis* is currently on the International Union for Conservation of Nature (IUCN) Red List of threatened species (http://www.iucnredlist.org).

Previous studies of *T. sinensis* focused mainly on its introduction and domestication, developmental biology, and reproductive biology, etc. (Ma, 2013). Study of the genetic conservation of the species began over the last few years. In 2013, Wang conducted genetic diversity analysis of two natural populations, from Jiangxi Wuyi Mountain and Hunan Shunhuang Mountain in China, using 11 pairs of polymorphic SSR primers (Wang, 2013). In 2015, the genetic structure and phylogenetic relationships of 24 *T. sinensis* populations were investigated using seven SSRs and seven noncoding plastid DNA regions (Zhang et al., 2015). However, some of the important populations, such as those from Shaanxi Ningshan (the northernmost population), Hunan Yanling (the smallest population) and Hunan Yongshun (the largest population), were not included. Our study analyzed the genetic diversity and genetic structure of these populations, providing a theoretical basis for the conservation and management of this endangered species.

2. Materials and methods

2.1. Collection of plant samples

This study selected five wild *T. sinensis* populations in China, from Shaanxi Ningshan (NS), Hunan Yongshun (YS), Hunan Yanling (YL), Hubei Shennongjia (SN) and Guizhou Xifeng (XF). At each site, we randomly sampled adult individuals at three-meter intervals. There were only 12 adult in the YL population, and all were sampled. Because of the small number of individuals in these populations, a total of 91 individuals were sampled for analysis. Sampling data are shown in Table 1.

2.2. DNA extraction, SSR-PCR amplification and genotyping

Whole genomic DNA was extracted from leaf or bark tissue using the Plant Genomic DNA Kit (Tiangen, China), according to directions. Eleven polymorphic SSR markers (*TS013, TS024, TS040, TS053, TS060, TS062, TS103, TS116, TS119, TS126* and *TS149*) that we previously developed were used for genotyping the 91 sampled individuals (Zhou et al., 2015a). Details of SSR-PCR amplification and genotyping are described by Zhou et al. (2015b).

2.3. Data processing and statistical analysis

Most EST-SSR markers are located in the coding regions of functional genes and may be evolutionarily selected along with non-neutral traits. To identify suitable primers for subsequent genetic analysis, we first conducted neutrality tests for the 11 SSR primers. All of the alleles that were amplified in the 91 individuals by the 11 primers were analyzed with GenAlEx v6.5 using specific base pairs, and their format was then transformed for further analysis by Popgene v1.32 (Yeh et al., 1999). The amplification data obtained using the neutral primers were used in subsequent analyses.

The genetic diversity indices of the five populations were calculated using Popgene v1.32 and included the percentage of polymorphic loci (*PPL*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), Nei's expected heterozygosity (*H*), and Shannon's information index (*I*), etc. Genetic variation among and within populations was calculated using the analyses of molecular variance (AMOVA) function of Arlequin v3.5 (Excoffier and Lischer, 2010). The average differentiation coefficients (*Fst*) and gene flow (*Nm*) were calculated using GenAlEx v6.5 (Peakall and Smouse, 2012).

To determine the genetic relationships among populations more directly, Nei's genetic distances were imported into MEGA6 software and used to construct a dendrogram using UPGMA (Tamura et al., 2013). Correlation analysis between the genetic and geographical distances of the populations was performed with the Mantel test matrix feature of *PASSaGE* 2 (Rosenberg and Anderson, 2011). The genetic structure of the populations was calculated using Structure v2.3 (Pritchard et al., 2010). The length of the burnin period was set to 100,000, and the number of MCMC Reps after burnin was set to 500,000. The *K* value was set to 1–5, and each *K* value was repeated five times. The remaining parameters were left at their default values. The most suitable *K* value was determined using STRUCTURE HARVESTER (Earl and vonHoldt, 2012). In addition, based on Nei's genetic distance of the individuals in different populations, the genetic relationship among populations was further analyzed using the principal coordinate analysis (PCoA) function of GenAlEx v6.5.

Table 1
Information from the five *T. sinensis* sampling sites.

Population ID	Sampling localities	Sample size	Altitude m	Coordinate
NS	Ningshan, Shaanxi	20	1385	N33° 31′ E108° 34′
YS	Yongshun, Hunan	19	496	N28° 47′ E110° 15′
YL	Yanling, Hunan	12	1152	N26° 27′ E114° 01′
SN	Shennongjia, Hubei	20	1035	N31° 26′ E110° 26′
XF	Xifeng, Guizhou	20	1410	N27° 08′ E106° 38′