Biochemical Systematics and Ecology 49 (2013) 107–114

Contents lists available at SciVerse ScienceDirect

Biochemical Systematics and Ecology

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

Genetic diversity and population structure of the endangered conifer *Taxus wallichiana* var. *mairei* (Taxaceae) revealed by Simple Sequence Repeat (SSR) markers

De-Quan Zhang [a,*], Nong Zhou [b]

[a] College of pharmacy and chemistry, Dali University, Dali, Yunnan 671000, PR China
[b] College of Life Science and Engineering, Chongqing Three Gorges University, Chongqing 404000, PR China

ARTICLE INFO

Article history:
Received 15 October 2012
Accepted 20 March 2013
Available online 13 April 2013

Keywords:
Taxus wallichiana var. *mairei*
SSR markers
Genetic diversity
Genetic structure
Endangered plant

ABSTRACT

Taxus wallichiana var. *mairei* is an endangered conifer with important medicinal value in southern China. Nuclear SSR markers were employed to assess genetic diversity and structure of 13 geographically disjunct populations. The present study revealed a moderate genetic diversity (H_E = 0.538) and low genetic differentiation (F_{ST} = 0.159). And most populations encountered in severe inbreeding and bottleneck effect. No significant genetic structure was detected by IBD and Structure analysis, which was supported by AMOVA analysis. The present results could be ascribed to an earlier period of more pronounced gene flow when the species had a more continuous distribution. However, the 13 studied populations were divided into four clusters based on the UPGMA dendrogram; these clusters were almost congruent with their geographical distributions. Vital areas such as southern mountains of Sichuan basin, Nanling Mts. and the margin of this yew's distribution range had a high priority for conservation.

1. Introduction

Genetic diversity of a species is the results of long-term evolution and plays an important role in its survival and evolution process. Rich genetic variation generally indicates stronger adaptability and potentiality in evolution, especially in case of sharply changing climates or environments (Hamrick and Godt, 1996). Estimation of genetic variation and structure using molecular markers has become a common approach in protection of endangered species (Groom et al., 2006). Simple Sequence Repeat (SSR, namely microsatellite) markers develop quickly and are widely applied in genome mapping, population genetics and related areas (Ellegren, 2004; Kalia et al., 2011; Zalapa et al., 2012). Compared with the commonly used Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeat (ISSR) and Amplified Fragment Length Polymorphism (AFLP) etc., SSR has obvious advantages, such as hypervariability, co-dominance and high reproducibility (Ellegren, 2004; Bhargava and Fuentes, 2010; Kalia et al., 2011).

Taxus wallichiana var. *mairei* (Lemée & Léveillé) L. K. Fu & Nan Li is an evergreen shrub or tree, scattered in coniferous and mixed forest in south China (Fu, 1999). It is one of the three varieties of *T. wallichiana* Zucc. according to the latest treatment (Li and Fu, 1997; Fu, 1999). However, it might be more appropriate to consider this variety as an independent cryptic species on the basis of recent molecular evidence (Liu et al., 2011). The yew has important economic values: bark is used for extracting Taxol (paclitaxel), an antitumor agent, and wood is used for high-price furniture. As a result, wild yew has encountered severe

* Corresponding author. Tel.: +86 (0) 872 2257411; fax: + 86 (0) 872 2257401.
E-mail address: zhangdeq2008@126.com (D.-Q. Zhang).

0305-1978/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bse.2013.03.030

destruction and cutting all the time. Meanwhile, yew has some congenital deficiency comparing with other species as follows: insufficiency of seedlings, weak competitiveness, rigid habitat requirements (Ru et al., 2006). Therefore, man-caused destruction and weak viability are the main factors resulting in the severe endangerment of the species. In order to protect wild resources, all species of *Taxus* L. in China are listed as first-grade State protection in the National Key Protected Wild Plants List (1999) (http://www.gov.cn/gongbao/content/2000/content_60072.htm). Moreover, *T. wallichiana* was also recognized globally as endangered A2acd in the IUCN Red List of Threatened Species (Thomas and Farjon, 2011). In all species of *Taxus* L., *T. baccata*, the only species present in Europe, was studied systematically in conservation biology, population ecology and phylogeography (Piovesan et al., 2009; Scarnati et al., 2009; Dubreuil et al., 2010; González-Martíneza et al., 2010; Chybicki et al., 2011). As for *T. wallichiana* var. *mairei*, there were only limited studies based on cpDNA gene and ISSR markers (Gao et al., 2007; Zhang et al., 2009; Liu et al., 2011). Further research using nuclear genes might reveal better the genetic diversity and population structure of this variety.

In this paper, SSR markers were used to detect genetic diversity and population structure of the 13 studied populations of *T. wallichiana* var. *mairei*. The main aims were to (i) assess genetic diversity and spatial genetic structure of the 13 studied populations; (ii) detect possible inbreeding and bottleneck effect in each population and (iii) propose feasible strategy for this endangered species.

2. Materials and methods

2.1. Plant sampling

A total of 130 individuals from 13 geographically separate populations of *T. wallichiana* var. *mairei*, almost covering the entire distribution range, were sampled in this study based on the former genetic and morphological researches (Gao et al., 2007; Möller et al., 2007) (Table 1 and Fig. 1). Fresh and clean leaves were collected from adult trees and dried by silica gel in fieldwork. To avoid sampling posterity from the same maternal, distance between these individuals was at least 50 m. All voucher specimens were deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Science (KUN).

2.2. DNA extraction and PCR amplification

Total genomic DNA was extracted from dried leaf tissue of all individuals using the CTAB method with minor modifications (Liu et al., 2011). Quantity and quality of DNA was detected by 1% agarose gel, and adjusted to a final concentration of about 30–50 ng/μl as working solution.

Eight SSR primer pairs with high PCR success and polymorphism, were selected from loci developed in relative *Taxus* species (Dubreuil et al., 2008; Huang et al., 2008; Yang et al., 2009; Zhou et al., 2009). Reaction systems and procedures of PCR amplification were conducted on the basis of above literatures with minor modification and optimization such as annealing temperatures. DNA amplification were performed in 20 μl reaction volumes containing 10 μl 2 × Taq PCR MasterMix (TIANGEN), 0.15 μM of each primer and about 50 ng genomic DNA. And the reactions were conducted on GeneAmp® PCR System 9700 (Applied Biosystems, Inc.) under the following profile: 94 °C for 2 min following by 35 cycles at 94 °C for 30 s followed by the annealing temperature for each specific locus (Table 2) for 30 s, 72 °C for 45 s, and a final extension step at 72 °C for 4 min. PCR products were detected by ABI PRISM® 3100 Genetic Analyzer with the Saga^{GT} Microsatellite Analysis Software (Version 3.2). And the gathering data were transformed into peak graph by software GeneScan 3.0 and GenoTyper 2.0. In this study, data collection for all loci was performed by the Dingsheng Science and Technology Co., Ltd (Beijing). Amplified fragments for all loci were scored as alleles on the basis of sizes in the peak graph, and modified for ambiguous bands according to graphs of individuals within population or lineages. Individuals with no peak graph or doubtful bands were reexamined again by the same methods.

Table 1
Collecting information and genetic parameters revealed by 8 SSR loci in 13 populations of *T. wallichiana* var. *mairei*.

Population	Location	*N*	Longitude/Latitude	Altitude (m)	N_A	N_E	H_O	H_E	F_{IS}
CH	Chishui, Guizhou	10	106°07′ E/28°37′ N	920	5.750	3.631	0.475	0.641	0.268
DG	Daguan, Yunnan	10	103°56′ E/28°04′ N	1480	4.250	2.681	0.400	0.561	0.325
FQ	Fuqing, Fujian	10	119°08′ E/25°46′ N	535	3.875	2.805	0.425	0.576	0.282
FRB	Yinjiang, Guizhou	10	108° 41′ E/27°59′ N	950	4.125	2.290	0.238	0.388	0.321
GJ	Jinxiu, Guangxi	10	110°12′ E/24°14′ N	1002	3.625	2.359	0.425	0.511	0.173
JG	Jinggangshan, Jiangxi	10	114°10′ E/26°34′ N	1377	4.375	2.443	0.400	0.499	0.179
JX	Longnan, Jiangxi	10	114°38′ E/24°50′ N	680	3.500	2.501	0.350	0.531	0.336
LA	Linan, Zhejiang	10	119°30′ E/30°19′ N	470	3.875	2.504	0.488	0.524	0.157
LS	Leishan, Guizhou	10	99°01′ E/26°16′ N	840	4.250	2.754	0.438	0.546	0.270
LZH	Lianzhou, Guangdong	10	112°23′ E/25°08′ N	470	4.500	3.280	0.400	0.603	0.380
TQ	Tianquan, Sichuan	10	102°43′ E/30°08′ N	1200	4.750	3.044	0.363	0.625	0.400
WXX	Weixin, Yunnan	10	104°58′ E/27°48′ N	1300	3.500	2.080	0.275	0.445	0.387
ZX	Zhongxian, Chongqing	10	107°54′ E/30°16′ N	860	3.625	2.440	0.400	0.541	0.302
Mean					4.154	2.678	0.390	0.538	0.290

Note: *N*, Sample size; N_A, No. of different alleles; N_E, No. of effective alleles; H_O, Observed heterozygosity; H_E, Expected heterozygosity; F_{IS}, Fixation index.