



Genetic diversity of endangered *Manglietia patungensis* assessed by inter simple sequence repeat and sequence-related amplified polymorphism markers



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ARTICLE INFO

Article history:

Received 15 May 2014

Accepted 23 August 2014

Available online

Keywords:

Manglietia patungensis

Genetic diversity

Genetic differentiation

ISSR

SRAP

conservation strategy

ABSTRACT

Manglietia patungensis Hu is an endangered plant native to China. Knowledge of its genetic diversity and structure would aid its conservation. This study assessed nine natural populations of *M. patungensis* using two methods: inter simple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP) markers. Using 10 ISSR primer pairs, 334 bands were generated, and 10 SRAP primer pairs generated 276 bands. The percent of polymorphic bands (91.32% and 93.48%), Nei's genetic diversity (0.3448 and 0.3323), and Shannon's information index (0.5075 and 0.4935) revealed a high level of genetic diversity at the species level. Total heterozygosity was 0.3439 by ISSR and 0.3281 by SRAP. The mean heterozygosity was 0.2323 by ISSR and 0.2521 by SRAP. The coefficient of genetic differentiation among natural populations was 0.3245 by ISSR and 0.2316 by SRAP. These data indicated higher levels of genetic diversity of *M. patungensis* within, rather than among, populations. Estimates of gene flow among natural populations were 1.0411 and 1.0589, which implied a certain amount of gene exchange among populations. A Mantel test revealed no significant correlation between genetic and geographic distance. ISSR and SRAP markers are both effective for genetic diversity research in *M. Patungensis*. Based on these results, conservation of *M. patungensis* should be performed both in situ and ex situ.

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1. Introduction

The Magnoliaceae are important plants for phylogenetic and evolutionary research in angiosperms because of their relatively primitive status. In China, there are 11 genera and more than 130 species of Magnoliaceae (Liu et al., 1997). Thirty-nine of those species are listed in the "China Plant Red Data Book" as rare and endangered plants deserving national protection (Fu and Jin, 1992). One such species, *Manglietia patungensis* Hu., grows in evergreen broadleaves forestry at altitudes ranging from 700 to 1000 m, and is distributed in Badong and Lichuan, as well as the Shennongjia forestry of Hubei Province, Sangzhi and the Xiaoxi Natural Reserve area of Hunan Province, and the Guanshan Natural Reserve area in Jiangxi Province.

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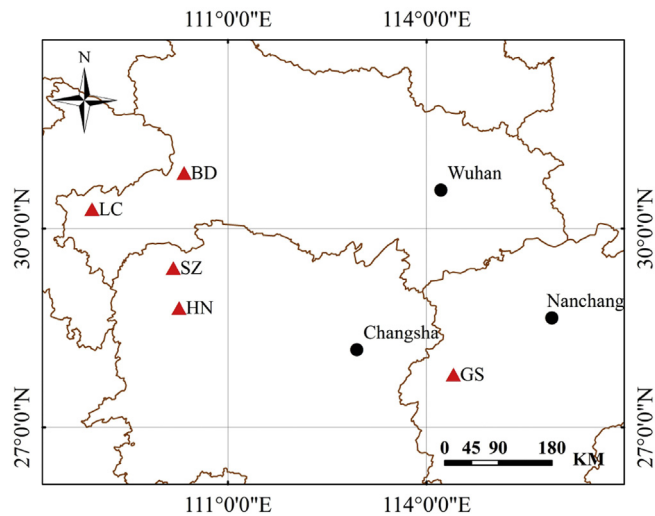


Fig. 1. Locations of *M. patungensis* populations.

It has a restricted distribution because of small populations and slow reproduction. Previous research on *M. patungensis* focused on its propagation (Huang and Li, 2002; Huang et al., 1998), biocoenosis structure (Li et al., 2006; Ge et al., 2009), distribution (Li et al., 2004) and allozymes (He et al., 2005). As the next step toward the conservation of this species, the present study examined the genetic diversity and population structure using inter simple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP) molecular markers, which are widely used to study endangered plants (Agostini et al., 2010; Yu et al., 2011; Trindade et al., 2012; Ferreira et al., 2013; Manners et al., 2013; Harish et al., 2014).

2. Materials and methods

2.1. Plant material

According to the distribution range and geographic locations of *M. patungensis*, five populations were chosen: Badong (BD), Lichuan (LC), Sangzhi (SZ), Guanshan (GS) and Hunan Xiaoxi (HN). The largest population, Hunan Xiaoxi, was divided into five subpopulations along elevation and distance, HN1, HN2, HN3, HN4 and HN5 (Fig. 1). Fresh unfolding leaves were randomly picked from mature trees (each tree was more than 50 m apart), labeled with the population name, and stored at −70 °C. Information concerning the samples is provided in Table 1.

2.2. Methods

2.2.1. DNA extraction and measurement

An improved cetyl trimethylammonium bromide protocol was used to extract the genomic DNA (Wan et al., 2008), which was detected by 1.0% agarose gel electrophoreses, purified and quantified by UV spectroscopy.

2.2.2. ISSR and SRAP analyses

ISSR primers were designed following the standards provided by the University of British Columbia (UBC801-UBC900) and synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). The optimized ISSR-PCR reaction system for *Manglietia patungensis*

Table 1
Information of samples.

Name	Location	Longitude	Latitude	Attitude(m)	Sample size
BD	Badong, Hubei	110°20'17.62"	30°50'19.96"	415	3
LC	Lichuan, Hubei	108°56'51.51"	30°17'31.78"	1230	20
SZ	Sangzhi, Hunan	110°10'34.31"	29°24'16.26"	722	12
GS	Guanshan, Jiangxi	114°24'20.52"	27°47'47.66"	406	24
HN1	Xiaoxi Xiannvchi, Hunan	110°15'44.53"	28°48'8.89"	545	27
HN2	Xiaoxi Badongmulian waterfall, Hunan	110°15'44.53"	28°48'8.89"	538	35
HN3	Xiaoxi Xiaohexi, Hunan	110°15'44.53"	28°48'8.89"	515	15
HN4	Above of Xiaoxi Badongmulian waterfall, Hunan	110°15'44.53"	28°48'8.89"	550	21
HN5	Xiaoxi Paohuxi, Hunan	110°15'44.53"	28°48'8.89"	540	11

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