Biochemical Systematics and Ecology 64 (2016) 149–155

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Amplified fragment length polymorphism analysis of the population structure and genetic diversity of *Phoebe zhennan* (Lauraceae), a native species to China

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ARTICLE INFO

Article history:
Received 19 August 2013
Received in revised form 17 October 2015
Accepted 14 November 2015
Available online 23 January 2016

Keywords:
AFLP
Genetic diversity
Phoebe zhennan
Population structure

ABSTRACT

Phoebe zhennan S. Lee et F. N. Wei (Lauraceae), is the main source of Gold Phoebe, a rare and extremely valuable wood in China. However it has undergone a dramatic decline. In this study, we used 12 amplified fragment length polymorphism primer combinations to assay 92 accessions, which were highly representative of the entire *P. zhennan* germplasm. It revealed that *P. zhennan* consisted of three genetic populations, named as SCZ (central Sichuan), CQH (eastern Sichuan, Chongqin, Hubei and Hunan) and YG (Yunnan and Guizhou), probably owing to natural selection caused by topography differences. The CQH population further diverged into two geographical sub-populations: CD-CQ (SCD and west region of Chongqin) and HB-HN (eastern side of Chongqin, Hubei and Hunan). The loci were moderately polymorphic (40.4%). The genetic distance between SCZ and YG was the highest, between CD-CQ and HB-HN the lowest. Pairwise fixation indices (Φ_{PT}) between any inferred populations were significant. This rare species exhibited low genetic diversity; therefore, the results provided significant data related to the conservation and management of *P. zhennan*. That is, with this genetic information, land managers are equipped with better tools allowing them to more effectively protect this species and its limited genetic diversity.

1. Introduction

Phoebe zhennan S. Lee et F. N. Wei, which formerly belonged in the genus *Machilus* Nees (Lauraceae), is sporadically distributed in southwest of China and is the main tree species of evergreen broad-leaved forest. This valuable plant is famous as an ornamental courtyard tree species and is widely used in urban landscaping. In particular, one major resource from this species, a type of wood known as Gold Phoebe (Tan et al., 2008), has an outstanding reputation worldwide for its high-quality of wood with dense texture, strong resistance to decomposition and shiny natural metal lines. However, over-harvesting and successive exploitation have caused the quantity of wild *P. zhennan* to decline dramatically; today, the species only grows in cultivated settings in semi-natural areas or in forests set aside for scenic protection. Thus, it has been listed as a second grade

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http://dx.doi.org/10.1016/j.bse.2015.11.001
0305-1978/

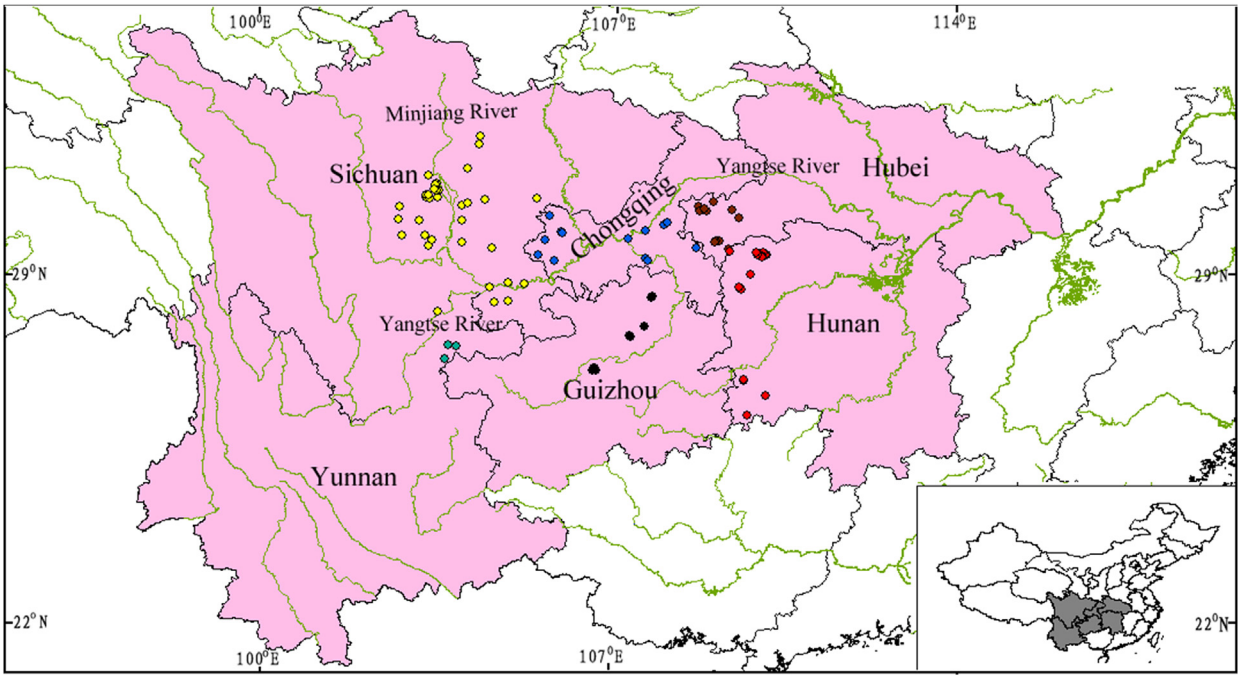

Fig. 1. Geographical distributions of the 92 *P. zhennan* accessions in China. The coloured dots represented of *P. zhennan* in China. The green coloured lines represented water systems of six provinces (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

national key protected plant in China. Any wild species must have an available pool of genetic diversity to survive and it is crucial for an endangered species to retain as much genetic variation as possible to enhance the chance for its recovery. The conservation of genetic diversity is important for the long-term survival of any species (Cruz et al., 2012). However, almost no published reports were found that discuss the genetic diversity of *P. zhennan*. Therefore, it is necessary and urgent to investigate the genetic diversity using appropriate molecular markers. Amplified fragment length polymorphism (AFLP) (Money et al., 1996; Vos et al., 1995) depends on the reliability of restriction fragment length polymorphism (RFLP) and the high efficiency of PCR to amplify digested genomic DNA segment selectively, and is highly reliable for the assessment of genetic variation among and within populations (Keiper and McConchie, 2000). Compared with the RAPD technique, AFLP has more stability and amplifies more loci so can be applied widely. Some researchers have simplified, optimized and improved the AFLP protocol so that it can be easily performed and had higher resolution to polymorphic loci than other methods (Suazo and Hall, 1999; Vekemans et al., 2002).

In this study, we collected 92 *P. zhennan* accessions from six provinces of China, and then used 12 AFLP markers to determine their population structure and the level of genetic diversity. This study will provide reliable information that will aid land managers and researchers in the protection and restoration of *P. zhennan* by providing details related to the genetic diversity of this species.

Table 1
The 12 AFLP primer combinations used for selective amplification.

Selective primer combination	Number of total markers	Number of polymorphic markers	Percentage of polymorphism
P1 (EACA/MCGA)	42	21	50
P2 (EACA/MCGG)	30	11	43.3
P3 (EACC/MCCT)	33	7	21.2
P4 (EACG/MCCT)	41	9	26.8
P5 (EACG/MCCC)	22	6	27.3
P6 (EACG/MCCG)	21	5	33.3
P7 (EAAC/MCCT)	43	19	44.2
P8 (EAAC/MCCC)	41	21	51.2
P9 (EAGG/MCTT)	39	17	43.6
P10 (EAGG/MCCC)	57	31	54.4
P11 (EATC/MCGT)	21	9	42.9
P12 (EATC/MCGC)	32	15	46.9
Total	422	177	485.1
Mean	35.2	14.8	40.4

E = GACTGCGTACCAATTC; M = GATGAGTCCTGAGTAA