Biochemical Systematics and Ecology 57 (2014) 332–337

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

DNA fingerprints of living fossil *Ginkgo biloba* by using ISSR and improved RAPD analysis

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ARTICLE INFO

Article history:
Received 2 July 2014
Accepted 20 September 2014
Available online

Keywords:
Ginkgo biloba
Molecular markers
Inter-simple sequence repeat
Random amplified polymorphic DNA
RAMP
Genetic variation

ABSTRACT

In this study, we have aimed to genetically characterize *Ginkgo biloba*. Nine *G. biloba* samples from different places of China were collected, and DNA was extracted from the leaves of these samples for inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) analysis. ISSR analysis showed high genetic variation among the nine varieties of *G. biloba*; the polymorphism and similarity coefficients were 87% and 0.40 −0.84, respectively. RAPD analysis also showed 93% polymorphism, and the similarity coefficients ranged from 0.44 to 0.87. Persistent genetic isolation that developed for millions of years might influence the genetic variability between the samples of *G. biloba*. This study generates a genetic map of *G. biloba*, and reports the highly variable intra-species genetic characteristics of this living fossil among different geographical locations of China. Our study also suggests that ISSR and the improved RAPD markers are useful molecular tools for the genetic characterization of plants.

1. Introduction

Ginkgo biloba (commonly called 'ginkgo' or 'maidenhair tree'; in Chinese, 'yín xìng') is a large beautiful tree, with a range of height of 20–35 m or more. *G. biloba* is the only living representative of Ginkgoales order, for which, it is often called as 'living fossil'. *G. biloba* is native to China, originated in the Paleozoic era, which is being also planted in Japan and Korea from ancient times. In addition to beautifying nature, *G. biloba* has importance as a source of food and medicine (Encyclopedia Britanica, 2014; Mustoe, 2002). Different parts of this plant have been used as traditional medicines to treat several disorders for thousands of years. Recent scientific studies have reported that the *G. biloba* extracts or the active ingredients from this plant have memory enhancement efficiency and beneficial effects against neurodegenerative disorders. *G. biloba* is also known for its anticancer, antidiabetic, antihypertensive, immunestimulative, antimicrobial hepato-protective and antioxidant activities (Man et al., 2012; Unger, 2013; Mohanta et al., 2014).

Genetic diversity within a species is a common feature, usually developed by the generation of genetic differences, a process by which a species adapt with the changing environment. Genetic diversity plays an important role in the survival

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http://dx.doi.org/10.1016/j.bse.2014.09.007
0305-1978/

and adaptability of a species, and it also saves the species from extinction with the change of environment (Frankham, 2008). Analysis of genetic diversity is important for the identification and authentication of a species. This is also important for the genetic profiling and conservation of any organism. Since *G. biloba* has a long evolutionary history, certain genetic changes are speculated within this plant species. In this study, we have aimed to genetically characterize *G. biloba* from nine geographically isolated regions of China. We have used inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) analysis for the characterization of these *G. biloba* samples. Along with some other molecular techniques, like amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) analysis, these ISSR and RAPD are important and popular markers, which have been used for more than last three decades for the genetic characterization of organisms (Agarwal et al., 2008; Shinzato et al., 2009; Fu et al., 2013; Mei et al., 2014a; Mei et al., 2014b). This study evaluates the genetic information on, genetic variation among the different samples of *G. biloba* from different locations in China, which might be beneficial for evolutionary, ecological, genetic and molecular studies of plants and other organisms.

2. Methods

2.1. Chemicals and reagents

The UBC primers (for ISSR analysis) were synthesized at Beijing DNA chem. Biotechnology Co., Ltd (Beijing, China) and RAPD primers were purchased from SBS Genetech Corporation. 2 × PCR Taq Mastermix was purchased from TianGen Biotech Co. Ltd (Beijing, China). DL2000 DNA Marker was purchased from Takara Biotechnology Co. Ltd. (Dalian, China). Other reagents used in this study, were analytical grade reagents, and used as our previous experiments (Fu et al., 2000, 2013).

2.2. Collection of plant samples and extraction of DNA

We have collected the *G. biloba* samples from nine different locations of China, which are geographically isolated, namely Beijing, Lasha in Xizang, Shenyang in Liaoning, Changsha in Hunan, Nanjing in Jiangsu, Chengdu in Sichuan, Kunming in Yunnan, Guilin in Guangxi and Shenzhen in Guangdong (Fig. 1, Table 1). The leaf samples of *G. biloba* were first fixed in fixing solutions containing chloroform (without liquid nitrogen), and grinded into small pieces by silica (SiO_2), and DNA materials were extracted by using cetyl trimethylammonium bromide (CTAB), a previously described method with slight modification

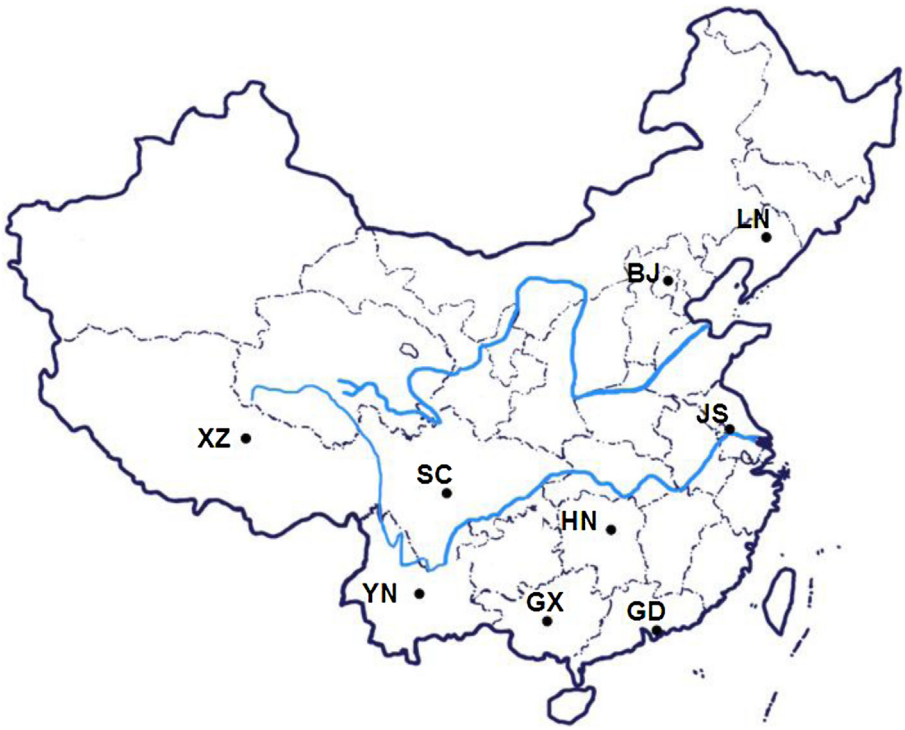

Fig. 1. Map of P. R. China showing the geographic locations of the *G. biloba* samples used in this study. The sources of the samples were from Beijing city (BJ), Lasha city of Xizang (XZ), Shenyang city of Liaoning province (LN), Changsha city of Hunan province (HN), Nanjing city of Jiangsu province (JS), Chengdu city of Sichuan province (SC), Kunming city of Yunnan province (YN), Guilin city of Guangxi (GX) and Shenzhen city of Guangdong province (GD).