Biochemical Systematics and Ecology 66 (2016) 265–271

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Genetic structure and diversity of *Glehnia littoralis*, an endangered medicinal plant in China

Ailan Wang, Ping Zhang, Xiao Liu, Jianguang Liang, Weiwei Li*

School of Life and Sciences, Ludong University, Yantai, 264025, Shandong, PR China

ARTICLE INFO

Article history:
Received 18 February 2016
Received in revised form 24 April 2016
Accepted 30 April 2016
Available online 19 May 2016

Keywords:
Glehnia littoralis
Genetic diversity
Gene flow
SRAP markers

ABSTRACT

The medicinal plant *Glehnia littoralis* has become increasingly endangered in China. To assist in the formulation of conservation strategies for this species, we assessed the genetic diversity of *G. littoralis* with a total of 172 samples from 10 populations using SRAP markers. The results showed that Nei's gene diversity (H) per locus ranged from 0.04 to 0.2504, with an average of 0.245, and that Shannon's information index (I) ranged from 0.233 to 0.6253, averaging 0.5516 at the population level. The total genetic diversity (Ht) was 0.2471, and 66.5% (Hs = 0.1643) of the total genetic variation was attributed to within-population diversity ($P < 0.05$), with the rest (33.5%) due to differences between populations (Gst = 0.3353), indicating that gene flow was restricted among populations (Nm = 0.9911). AMOVA also suggested that the main source of variation was within populations (77.8%), whereas 22.2% of differentiation was found among populations. Estimates of genetic distance were not correlated with geographic distance according to a Mantel test ($r = 0.0792$, $P = 0.329$). These results show that high levels of genetic variation are present within and among populations. The endangered status of this species is likely due to the destruction of the habitats of the wild populations, rather than a loss of genetic diversity.

1. Introduction

The species *Glehnia littoralis* Fr. Schmidt ex Miq is a perennial diploid (2n = 22) herb that sexually reproduces via flowers by both insects and wind pollination (self-incompatible), *G. littoralis* Fr. Schmidt ex Miq belongs to the genus *Glehnia* in the family Apiaceae (Mathias, 1928). This genus includes a single species, *G. littoralis*, which is comprised of two groups: an East Asian *G. littoralis* group and a North American group consisting of *G. littoralis* subsp. *Leiocarpa* E. Mathias (Hiroe, 1962; Shan and She, 1992; Hiraoka et al., 2002; Sun et al., 2004). The former group is distributed in the sandy seacoast of five countries in East Asia, including China, South Korea, North Korea, Japan and Russia (Hiroe, 1962; Hiraoka et al., 2002; Sun et al., 2004).

Sandy beach habitats are convenient for easily rooting deep into the soil and for expanding rhizomes, which makes this species an economically important tool for preventing sand erosion. In addition, the roots of *G. littoralis*, known as "Beishashen," are used as an important traditional medicine by Chinese people for its relatively strong inhibition of immune-related diseases, which has a history of nearly 600 years in China (Yoon et al., 2010). However, over the past several decades, wild resources of *G. littoralis* in China have declined sharply, and many natural populations have disappeared due to human

* Corresponding author.
E-mail address: lwwal@163.com (W. Li).

http://dx.doi.org/10.1016/j.bse.2016.04.019
0305-1978/

harvesting activities and the destruction of beaches by the development of houses, roads and tourist attractions. Due to its recent decline, *G. littoralis* was listed in the *Endangered Plants Chinese Chronicles* (Fu, 1992), and is known as the plant "Panda" in China.

Assessing genetic diversity is considered to be vital for formulating conservation strategies of endangered species. Although *G. littoralis* has become increasingly endangered, there are still few relevant studies of the population structure for this species (Hui et al., 2001; Huh et al., 2003; Song et al., 2014). To understand its overall genetic diversity and distribution to inform conservation efforts for *G. littoralis* in China, we assessed the genetic diversity among different populations of *G. littoralis* across large geographical distances and measured the genetic variation at within- and among-population levels using sequence-related amplified polymorphism (SRAP) molecular markers. SRAP markers detect genetic variations in open reading frames (ORFs) of genomes of plants and related organisms based on two-primer PCR (Li and Quiros, 2001) and has been demonstrated to be a powerful tool for genetic diversity analysis in many plant species (Chen et al., 2013; Babaei et al., 2014; Peng et al., 2015). In this study, we attempted to understand the following: 1) What is the state of genetic diversity in this endangered species? 2) Which factors affect the genetic structure of this species? 3) Are genetic patterns associated with geographic distribution? 4) What measures should be adopted in the future for the conservation of this species given the results of the genetic structure analysis?

2. Materials and methods

2.1. Plant materials

We sampled 172 individuals from 10 populations of *G. littoralis* (Table 1). All samples were collected from natural populations. The number of samples and origin of each population are listed in Table 1 and Fig. 1. These populations cover nearly the entire existing range, including Liaoning Province, Shandong Province, Hebei Province, and Zhejiang Province, in which Pop3, Pop4, Pop6, and Pop8 are collected from the island adjacent to the mainland. Silica gel was used to rapidly dry fresh leaf tissues in the field.

2.2. DNA extraction, amplification

Genomic DNA was extracted from dried leaves using the CTAB method (Doyle and Doyle, 1987). Concentrations of DNA samples were estimated by gel electrophoresis and adjusted to 20 ng/μL for subsequent PCR reactions.

A total of 30 SRAP primers were first used to choose suitable primers with a relatively high level of polymorphism and banding reproducibility. A final set of 10 primers was selected to detect polymorphisms, and these primer sequences are shown in Table 2.

PCR reactions were performed in 25-μL reaction volumes containing 18.25 μL of sterile double-distilled water, 2.5 μL of 10×Taq polymerase reaction buffer, 0–1.5 mM Mg^{2+}, 0.5 μL of 10 mM dNTPs, 1 μL of each primer at 5 μM, 1 unit of Taq DNA polymerase, and 2 μL of plant DNA. The thermal cycling profile was as follows: initial template denaturation at 94 °C for 4 min, 5 cycles of 94 °C for 1 min, 35 °C for 1 min, and 72 °C for 1 min, followed by 36 cycles of 94 °C for 1 min, 48–60 °C for 1 min, and 72 °C for 1 min, with a final extension of 72 °C for 10 min. PCR products were examined by electrophoresis on 1.5% w/v agarose gels stained with ethidium bromide in 0.5×TBE buffer and were viewed under ultraviolet light and photographed using a UV Transilluminator.

2.3. Analysis of sequence data

Only clear and unambiguous bands were counted and scored as 1 for the presence and 0 for the absence of a band. The data obtained were entered into a binary matrix for analysis. The percentage of polymorphic loci (PPL), effective number of alleles (Ne) (Kimura and Crow, 1964), Shannon's information index (I) (Lewontin, 1972) and Nei' gene diversity (H) (Nei, 1973) were

Table 1
Profiles of the sample populations.

Population ID	Location	No. of samples	Latitude	Longitude
Pop1	Haiyang, Shandong	17	121°15′	36°41′24″
Pop2	Qinhuangdao, Hebei	16	119°18′	39°37′12″
Pop3	Changhai, Liaoning	15	122°19′12″	39°10′12″
Pop4	Changhai, Liaoning	35	122°40′12″	39°15′36″
Pop5	Wafangdian, Liaoning	15	121°31′48″	39°41′24″
Pop6	Zhuanghe, Liaoning	22	122°57′35″	39°31′12″
Pop7	Bayuquan, Liaoning	4	122°4′12″	40°12′36″
Pop8	Xingcheng, Liaoning	11	120°48′36″	40°30′36″
Pop9	Zhoushan, Zhejiang	13	122°24′36″	29°52′48″
Pop10	Rizhao, Shandong	24	119°34′48″	35°26′24″
Total		172		