Biochemical Systematics and Ecology 60 (2015) 193–198

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Genetic diversity and conservation evaluation of a critically endangered endemic maple, *Acer yangbiense*, analyzed using microsatellite markers

Jing Yang [a, 1], Lin-lin Zhao [a, b, 1], Jun-bo Yang [c], Wei-bang Sun [a, *]

[a] Kunming Botanical Garden, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
[b] University of Chinese Academy of Sciences, 100049 Beijing, China
[c] Germplasm Bank of Wild Species in Southwest China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China

ARTICLE INFO

Article history:
Received 2 February 2015
Accepted 18 April 2015
Available online

Keywords:
Acer yangbiense
SSR
Genetic diversity
Endangered
Conservation evaluation
Parentage analysis

ABSTRACT

The newly discovered endemic maple *Acer yangbiense* of China has only five individuals left in the wild, and thus has been classified as a plant species with extremely small populations (PSESP). PSESP species call for emergency protection procedures, such as ex-situ conservation and reintroduction. Our objectives were to examine the genetic diversity of *A. yangbiense* and to evaluate former conservation strategies from a genetic point of view. Our results suggested that *A. yangbiense* was not genetically depauperate, but its genetic loss at a species level was obvious. A parentage analysis indicated a high selfing-rate in *A. yangbiense* and suggested the existence of a previously unknown wild individual. Former conservation strategies did not include all genetic variations of the wild population, and gene diversity of the ex-situ conserved seedlings is lower than that of the wild population. From our findings, we make suggestions to guide the subsequent protection of this species.

1. Introduction

The critically endangered *Acer yangbiense* Y. S. Chen & Q. E. Yang (Aceraceae) has been recently described (Chen et al., 2003). After three separate in-depth investigations, only five individuals have been located, in a western valley of Cangshan Mountain, Yunnan Province, China. Three individuals are scattered across a sloping area mixed with farmlands and fallow lands (under daily grazing pressure) and two individuals are located in woody areas on the opposite hillsides. Three individuals have reached fertile maturity. The *A. yangbiense* distribution overlaps with a small village called Malutan, at 2200–2500 m altitude.

Based on all the available information and data from the surveys of its natural habitats, an IUCN assessment evaluated *A. yangbiense* as Critically Endangered [CR B1ab(v) + 2ab(v)] (Gibbs and Chen, 2009). Facing a very high risk of extinction, *A. yangbiense* is now listed in an emergency rescue plan for plant species with extremely small populations (PSESP) in China (Ma et al., 2013; Ren et al., 2012). This newly-initiated conservation action strategy focuses on PSESP and was approved by the

* Corresponding author. Tel./fax: +86 871 5223622.
E-mail address: wbsun@mail.kib.ac.cn (W.-b. Sun).
[1] Jing Yang and Lin–lin Zhao contributed equally to the work and are joint first authors.

http://dx.doi.org/10.1016/j.bse.2015.04.027
0305-1978/

Chinese government for immediate rescue of the most endangered plants before 2015. *A. yangbiense* was listed in the top 20 most endangered species in this PSESP plan (Ma et al., 2013). To address these aims in relation to *A. yangbiense*, in-situ conservation, seedling propagation, ex-situ conservation at the Kunming Botanical Garden (KBG) and reintroductions were made priorities.

In 2008 Dr. Chen (Institute of Botany, the Chinese Academy of Sciences) arranged for seeds of *A. yangbiense* to be collected and sent by a forest ranger to the Kunming Botanical Garden for *ex-situ* conservation. In total, 1606 seedlings were propagated and grown ex-situ in KBG. However, in order to avoid negative effects from starting with very small population sizes, such as loss of genetic diversity, and to evaluate the success of conservation actions, it is important to understand the genetic diversity of the natural population as well as these seedlings.

Although anthropogenic factors are considered to be the main cause of population extinction, genetic factors are also important (Lande, 1998). A decline in population size can cause random genetic drift, leading to allele loss (Eldridge et al., 2004; Hartl and Clark, 1997). Furthermore, a reduced population size increases the chance of inbreeding. In such cases, positive feedback between demographic and genetic decline can further reduce population size (Gilpin and Soule, 1986). Thus, inbreeding due to reduced population size lowers genetic diversity, the levels of which are positively correlated with population size in some cases (Ellstrand and Elam, 1993; Fischer and Matthies, 1998; Frankham, 1996; Linhart and Mitton, 1985; Vergeer et al., 2003). Population genetic analysis provides information on the levels of genetic diversity, which is essential for species conservation (Frankham et al., 2002). Therefore, assessing the level and distribution of genetic diversity are crucial for management and the development of effective conservation strategies for endangered species such as *A. yangbiense*.

Because microsatellites are unique in their abundance and codominant inheritance, they are powerful markers and are widely used to investigate genetic diversity, genetic structure and gene flow within populations (Nybom, 2004). For studies of contemporary gene flow as mediated by pollen and seeds, it can be employed for refined estimates of kinship and parentage (Nagamitsu et al., 2014; Schueler et al., 2003; Zane et al., 1999). Therefore 34 specific SSR primers for this research of *A. yangbiense* have been developed (Zhao et al., 2011).

In this study, the main objectives were: (1) to assess the genetic diversity of seedlings and natural population of *A. yangbiense*, (2) to assess the integrity of former conservations strategies from a genetic point of view and (3) to propose an effective conservation strategy for this species.

2. Materials and methods

2.1. Sampling methods and DNA extraction

A total of 83 samples of *A. yangbiense* were used for DNA analysis. Sampling included all five individuals from the natural population in Yangbi County (YB) and 78 samples chosen randomly from the 1606 ex-situ conserved seedlings (propagated from wild-collected seeds) in KBG. The locality information of the five wild samples (coded as Y01, Y02, Y03, Y04 and Y05) is shown in Fig. 1 and Table 1.

Total genomic DNA of *Acer yangbiense* was extracted from dry leaf tissue ground in liquid nitrogen, using a modified cetyltrimethylammonium bromide (CTAB) extraction method (Doyle, 1987). The extracted DNA was dissolved in 50 μl of Elution Buffer (Sangon Biotech, Shanghai, China) for SSR analysis.

2.2. SSR marker analysis

Nine nuclear microsatellite markers (coded as AY10, AY14, AY29, AY33, AY34, AY54, AY64, AY69 and AY74) were developed for this study (Zhao et al., 2011). Polymerase chain sequence reactions (PCRs) were performed in a 20 μl reaction volume

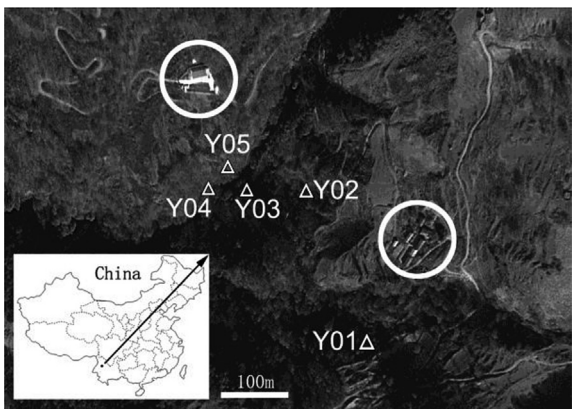

Fig. 1. The distribution of the *A. yangbiense* individuals. The white circles indicate the presence of buildings.