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Assessment of genetic diversity and population differentiation of *Achyranthes bidentata* (Amaranthaceae) in Dao Di and its surrounding region based on microsatellite markers

Shuang-Xi Yan [1], Ling Zhang [1], Run-Li Mao, Hong Zhu, Yong Li*

College of Forestry, Henan Agricultural University, Zhengzhou 450002, China

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ABSTRACT

Identifying genetic diversity patterns is fundamentally important for effective species management and conservation. In this study, we used five microsatellite loci to investigate the genetic diversity and population differentiation of *Achyranthes bidentata* in Dao Di and its surrounding region. Our analysis of microsatellite data indicated the level of genetic diversity of *A. bidentata* ($H_T = 0.333$) was lower than other plants. The results showed no significant genetic diversity differences and population genetic differentiation between the Dao Di and surrounding region. Significant temperature differences (Bio2: mean diurnal range and Bio7: temperature annual range) were found between the Dao Di and surrounding region, which may improve the accumulation of medicinal ingredients of populations in the Dao Di. The populations of *A. bidentata* were divided into two genetic groups, which was caused by five temperature variables (Bio1, Bio4, Bio7, Bio9, and Bio11). This study thus provides an important case for over-collecting within limited ranges in affecting population diversity and bioclimate variables for different producing area in driving population differentiation.

1. Introduction

An understanding of the genetic diversity and population structure of natural populations is fundamental in conservation genetic studies (Lande, 1988; Schmitt, 2007). Generally, the genetic diversity and population structure of species are influenced by population size, mating system, environmental conditions, and historical events (Hewitt, 2004; Nybom, 2004; Ohsawa and Ide, 2008). The consequences determine the species evolutionary potential. The investigation of genetic diversity and population structure will provide conservation biologists more information on population dynamics and help them devise appropriate conservation and management strategies.

Achyranthes bidentata Blume., commonly known as one of the most important herbal medicines, is a diploid ($2n = 2x = 42$) species belonging to Amaranthaceae. It is widely distributed in India, Java, China, and Japan (Yang et al., 2012). In China, this species grows in the warm-temperate and subtropical regions, with elevations from 200 m to 1750 m above sea level (Li et al.,

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* Corresponding author.
E-mail address: liyongrui1@126.com (Y. Li).
[1] These authors contributed equally to this work.

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2012). The root of *A. bidentata* is used as an anti-inflammatory drug because of its high concentrations of bioactive chemical compounds, such as rutin, saponins, achyranthine, caffeic acid, oleanolic acid, inokosterone, ecdysterone, rubrosterone, and physcion (Bishit et al., 1993; Nguyen et al., 1995; Chang et al., 1997). Although a wide growth area for *A. bidentata* exists in China, only the area in Henan province is considered to be the Dao Di, where it is known as "Huai Niuxi" (Li, 2008). The Compendium of Materia Medica in the Ming Dynasty (in the year 1578) recorded that the "Huai Niuxi" has greater medicinal functions. Modern studies have shown that some of the medicinal component content of "Huai Niuxi," such as oleanolic and ecdysterone, are higher than those, in the other areas (Zhang et al., 2001; Wang et al., 2012; Liang et al., 2014). Thus, the wild population size of *A. bidentata* in the Dao Di has been seriously affected by the over-collecting for its roots in recent years. Prior to this work, two studies on the genetic diversity of *A. bidentata* in the Dao Di have been reported (Li et al., 2012; Kong et al., 2014). However, these studies did not compare the population genetic diversity in the Dao Di with that in the surrounding areas. Thus, the population dynamics in the Dao Di could not be fully understood.

Microsatellites, also known as simple sequence repeat (SSR) or short tandem repeat (STR) markers, are noncoding tandem repeats of short nucleotide motifs (1–6 bp) dispersing widely prokaryotic and eukaryotic genomes. Currently, microsatellites have been widely employed in conservation genetic studies because of their codominance inheritance, high variability, and easy genotyping (Rakoczy-Trojanowska and Bolibok, 2004; Sun et al., 2008; Gyorgy et al., 2014).

In the present study, we used five microsatellite loci to investigate the genetic diversity and population differentiation of *A. bidentata* in Dao Di and its surrounding region. The specific objectives of this study were (1) to assess whether the long-term over-collecting for its roots has resulted in lower genetic diversity in the Dao Di than that in the surrounding region and (2) to elucidate whether populations in the Dao Di have significant genetic differences compared with those in the surrounding region. We hypothesized that genetic diversity would decline with the long-term over-collection of *A. bidentata* roots. Because of the differences of medicinal functions and components, we also hypothesized that significant genetic differences exist between the populations in the Dao Di and the surrounding region.

2. Materials and methods

2.1. Sample collection

Seventy-nine accessions of 8 populations from the Dao Di and 140 accessions of 12 populations from the surrounding region were collected in the present study (Fig. 1 and Table 1). Fresh leaf tissues were dried in silica gel immediately after collection and stored at room temperature until DNA extraction and genotyping.

2.2. Molecular protocols

Genomic DNA were extracted from approximately 30 mg of dried leaves using the plant genomic DNA kit (TIANGEN, Beijing, China) according to the manufacturer's protocol, and the DNA quality was determined in 1.5 agarose gels. Five microsatellite markers from Li et al. (2015) were selected for polymerase chain reaction (PCR). Ab059F were 5′ fluorescent

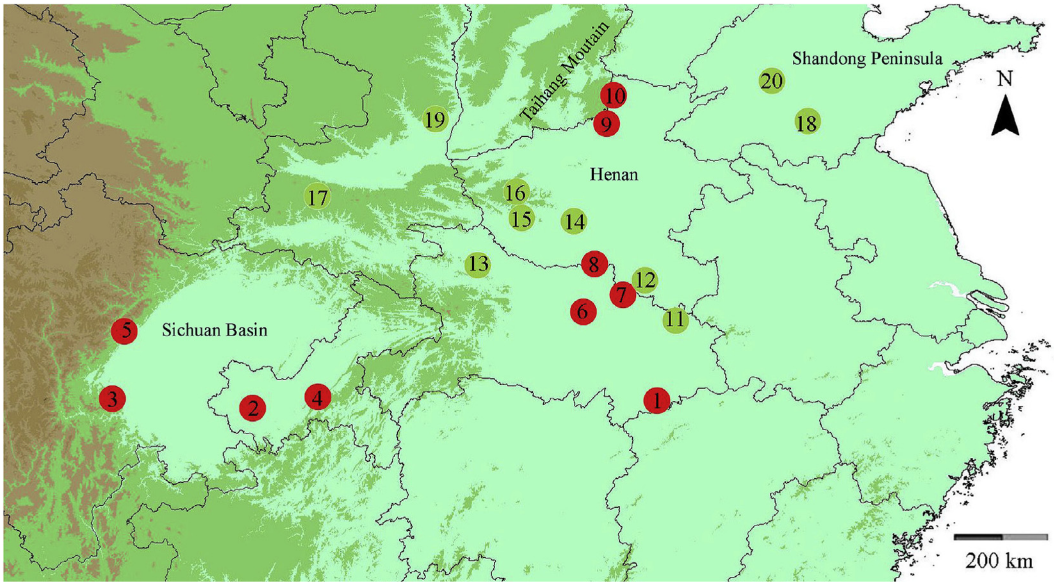

Fig. 1. Locations of the 20 sampled *A. bidentata* populations. Map produced by software DIVA-GIS. The colors (red for Group I and green for Group II) correspond to two genetic clusters identified by STRUCTURE program (for population numbers see Table 1). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)