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Genetic diversity of *Ulmus lamellosa* by morphological traits and sequence-related amplified polymorphism (SRAP) markers

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ABSTRACT

Ulmus lamellosa is an endangered and endemic species mainly distributed in North China. Understanding the genetic diversity of this species is useful to design the suitable conservation planning. In this study, the genetic diversity of 16 natural populations of *U. lamellosa* was evaluated by morphological traits and sequence-related amplified polymorphism (SRAP) markers. The morphological data showed that significant difference was found among 14 morphological traits. Coefficient of variation (*CV*) and phenotypic differentiation coefficient (V_{ST}) were 27.2% and 27.3% respectively. Based on SRAP markers, the percentage of polymorphic bands (*PPB*) of *U. lamellosa* populations was 82.7%. Nei's gene diversity (*He*) and Shannon's information index (*I*) were 0.22 and 0.36 respectively. High genetic differentiation was detected within populations ($P < 0.050$) and less genetic differentiation ($G_{ST} = 0.3696$) among populations by AMOVA analysis. Sixteen populations of *U. lamellosa* were gathered into three distinct clusters based on morphological and molecular data, which consistent with the result of PCoA and STRUCTURE analysis. Mantel test revealed a significant correlation between genetic and geographic distances ($r^2 = 0.1238$, $P < 0.050$). Both molecular and morphological data indicated that *U. lamellosa* was high in genetic diversity, which was probably due to the biological characteristics, distribution and modes of reproduction.

Contents

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1. Introduction

Ulmus lamellosa T. Wang et S. L. Chang (Ulmaceae) is deciduous tree with bisexual flower and key fruit (Wu et al., 1999). As an ornamentally important tree, *U. lamellosa* is widely used in the cities of North China. In addition, *U. lamellosa* is also an economically important tree, which is used in manufacture of furniture. Due to over-exploitation, the number of populations and individuals of *U. lamellosa* have dropped considerably in recent decades, which has been listed as the state second class protected species of China (Gu and Du, 1994). However, the previous studies for *U. lamellosa* were mainly focused on population structure (Ru et al., 2007), ecological characteristics of community (Bi et al., 2002), ecological niche (Bi et al., 2003), and the relationship between geographical distribution and climate conditions (Gu and Du, 1994). Very few analyses have been conducted on the genetic diversity of *U. lamellosa*. To understand the genetic diversity of *U. lamellosa* will provide useful information for its protection.

Many methods have been employed to evaluate genetic diversity of plants. Morphological traits measurement is commonly used since it provides a simple technique of quantifying genetic variation under normal growing environment (Wen and Chen, 1999). However, morphological traits are not used for reflecting the genetic identity among plants (Achtak et al., 2009). In order to overcome these limitations, molecular markers were used for evaluating the genetic diversity of plants (Achtak et al., 2009). Some researchers compared four marker systems and found the values of revealing genetic diversity power as: SRAP > SSR > ISSR > RAPD (Budak et al., 2004). Sequence-related amplified polymorphism (SRAP) is a universal molecular marker (Li and Quiros, 2001), which aims at the amplification of open reading frames (ORFs). Due to simple, reliable and moderate throughput, SRAP has been applied in molecular identification, genetic linkage map construction, gene tagging, genomic and cDNA fingerprinting, genetic diversity analysis and comparative genetics of different species (Comlekcioglu et al., 2010). The combination of molecular and morphological data may be more objective and accurate to evaluate genetic variation, which has been reported in numerous studies (Turchetto et al., 2014). It is better to analyze genetic diversity by morphological traits and molecular markers.

In this study, morphological traits and SRAP molecular markers were used to evaluate genetic diversity and differentiation among 16 *U. lamellosa* populations. Our objectives were to: (i) evaluate the morphological variability and diversity of 16 natural populations; (ii) reveal the genetic diversity and differentiation among populations; (iii) propose an appropriate conservation planning for *U. lamellosa*.

2. Materials and methods

2.1. Plant sampling

Our study was conducted in accordance with People's Republic of China laws. All researchers had introduction letters from the College of Life Science, Shanxi Normal University, Linfen to collect the samples.

From 2010 to 2014, total of 208 samples were collected from 16 natural populations of *U. lamellosa*, covering most of its entire distribution range in North China. The individuals at a location were treated as “a natural population”. Each population was positioned by GPS, and the detailed locations were listed in Table 1 and Fig. 1. Fresh leaves from 7 to 18 individuals were collected in each population depending on accessibility and population size. Each individual from same population was collected from different locations at least 20 m apart. The leaves were collected and immediately stored in the refrigerator of −80 °C and silica gel for future use.

2.2. Morphological analysis

Quantitative measurement was carried out on 30 leaves per individual. Fourteen morphological traits for leaves were evaluated to examine the phenotypic diversity. To avoid errors, each trait was measured in three replicates and the mean value was used. The morphological traits were as follow: leaf length (LL), leaf width (LW), petiole length (PL), petiole width