C. R. Biologies xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Comptes Rendus Biologies

www.sciencedirect.com

Molecular biology and genetics/Biologie et génétique moléculaires

# Genetic diversity and population structure of a protected species: *Polygala tenuifolia* Willd

Yan Qun Peng [a,b], Ling Ling Fan [a,b], Fu Ying Mao [a,b], Yun Sheng Zhao [a,b,c,*], Rui Xu [a,b], Yu Jie Yin [a,b], Xin Chen [d], De Guang Wan [d], Xin Hui Zhang [a,b]

[a] *Ningxia Medical University Pharmacy College, 750004 Yinchuan, Ningxia, China*
[b] *Ningxia Research Center of Modern Hui Medicine Engineering and Technology, 750004 Yinchuan, Ningxia, China*
[c] *Ministry of Education Key Laboratory of Modern Hui Chinese Medicine, 750004 Yinchuan, Ningxia, China*
[d] *School of Pharmacy, Chengdu University of TCM, 611137 Chengdu, Sichuan, China*

ARTICLE INFO

*Article history:*
Received 29 September 2017
Accepted after revision 25 January 2018
Available online xxx

*Keywords:*
AFLP markers
Genetic diversity
Genetic structure
*Polygala tenuifolia* Willd

ABSTRACT

*Polygala tenuifolia* Willd. is an important protected species used in traditional Chinese medicine. In the present study, amplified fragment length polymorphism (AFLP) markers were employed to characterize the genetic diversity in wild and cultivated *P. tenuifolia* populations. Twelve primer combinations of AFLP produced 310 unambiguous and repetitious bands. Among these bands, 261 (84.2%) were polymorphic. The genetic diversity was high at the species level: percentage of polymorphic loci (PPL) = 84.2%, Nei's gene diversity ($h$) = 0.3296 and Shannon's information index ($I$) = 0.4822. Between the two populations, the genetic differentiation of 0.1250 was low and the gene flow was relatively high, at 3.4989. The wild population (PPL = 81.9%, $h$ = 0.3154, $I$ = 0.4635) showed a higher genetic diversity level than the cultivated population (PPL = 63.9%, $h$ = 0.2507, $I$ = 0.3688). The results suggest that the major factors threatening the persistence of *P. tenuifolia* resources are ecological and human factors rather than genetic. These results will assist with the design of conservation and management programs, such as in natural habitat conservation, setting the excavation time interval for resource regeneration and the substitution of cultivated for wild plants.


## 1. Introduction

Genetic diversity is a fundamental component of biological diversity, and how to preserve the genetic diversity of plant species can significantly affect their long-term survival and evolution in changing environments [1]. Knowledge of the genetic diversity and population structure of protected plant species is essential for their protection and management [2–4].

*Polygala tenuifolia* Willd., commonly known as "Yuanzhi" in China, is a perennial herbaceous plant within the family Polygalaceae. It is an important traditional Chinese medicine (TCM) with a history of medical use for more than 2000 years. Wild *P. tenuifolia* is widely distributed in Shanxi, Shaanxi, Hebei, Henan, and other provinces of China, and is also distributed in Mongolia, Russia, and the Korean Peninsula at elevations of 200–2300 m [5]. Cultivation of *P. tenuifolia* mainly occurs in several counties of Shanxi Province. Its root bark, called

* Corresponding author. Ningxia Research Center of Modern Hui Medicine Engineering and Technology, Ningxia Medical University, Pharmacy College, No. 692 Shengli South Street, Yinchuan 750004, Ningxia, China.
*E-mail addresses:* 372529865@qq.com (Y.Q. Peng), 303511080@qq.com (L.L. Fan), mfyzys@126.com (F.Y. Mao), zhaoyunsheng1986@163.com, zwhjzs@126.com (Y.S. Zhao), 1807308241@qq.com (R. Xu), g0dloveyyj@foxmail.com (Y.J. Yin), 40071413@qq.com (X. Chen), wandeguang@163.com (D.G. Wan), 271684389@qq.com (X.H. Zhang).

https://doi.org/10.1016/j.crvi.2018.01.007
1631-0691/

Polygalae radix, is an important crude drug [6]. There is increasing commercial demand for this plant due to its excellent prevention and treatment of mental diseases (e.g., amnesia, anxiety, and insomnia) and respiratory tract ailments (e.g., asthma, bronchitis, and allergic respiratory disorders) [5]. Its medical importance generates great interest among researchers and companies to assess the existing active constituents and pharmacological effects [7,8].

However, *P. tenuifolia* resources are increasingly difficult to find in the wild and *P. tenuifolia* faces the danger of resource depletion. Slow growth has also exacerbated the depletion of wild resources [9]. Thus, it has long been listed among the Grade-III Key State-Protected Wild Medicinal Species in China [10], and strengthened protection and restoration is imminent. Most studies of *P. tenuifolia* have focused on its pharmacological activity [11], phytochemical properties [12], taxonomy [13], and cultivation technology [14]. Limited information is available about its genetic diversity and population structure [15], although such knowledge is fundamental to its effective conservation and sustainable utilization.

In the present study, amplified fragment length polymorphism (AFLP) analysis were employed to investigate the genetic variability in the wild and cultivated populations of *P. tenuifolia*. AFLP is a PCR-based marker for the rapid screening of genetic diversity and intraspecific variation without the need for prior sequence knowledge. It is a potent fingerprinting technique for genomic DNAs of any source or origin, and it can rapidly generate many highly replicable markers that allow high-resolution genotyping. Apart from AFLP, a variety of PCR-based DNA fingerprinting techniques (random amplified polymorphic DNA [RAPD], restriction fragment length polymorphism [RFLP], and simple sequence repeat [SSR]) is currently in use for molecular characterization and screening of genetic diversity [16–23], but these techniques have limitations and shortcomings, such as a lack of polymorphism (RFLP and RAPD) and the relatively low number of loci assayed (SSR) [24]. Both these limitations are removed with the use of AFLP and can be tailored to generate large numbers of polymorphisms [25]. AFLP reproducibility, reliability, resolutions, stringency, and number of loci detected per reaction do enhance its overall application. AFLP marker has largely taken over other markers for diversity, phylogenetic, and population genetics study. AFLP is very efficient in revealing polymorphisms even between closely related individuals. It has been used for recognition of individuals (paternity analysis, selfing rates, identification of cultivars, clones, etc.) and studying genetic diversity of Chinese medicinal plants [26–32].

In this study, we investigated the genetic diversity and genetic structure of *P. tenuifolia*. Our study aimed to:

- reveal the level of genetic diversity in *P. tenuifolia*;
- explore the distribution of genetic variation within and between the wild and cultivated populations;
- discuss possible implications of genetic data for its management and protection.

## 2. Material and methods

### 2.1. Plant materials

We collected 382 samples representing wild and cultivated populations of *P. tenuifolia* from different areas of China, including Shanxi, Shaanxi, Sichuan, and Gansu Provinces. The samples were collected from 24 populations. Among these populations, six were collected from the cultivated population, 17 from the wild population and one was *P. sibirica*, collected as a reference substance. Collected samples were identified by Professor Wan Deguang of the School of Pharmacy, Chengdu University of TCM. The distance between the collected individual samples was at least 200 m. We collected fresh young leaves from shoots, which were dried in silica gel and stored at −80 °C before DNA was extracted. Detailed information regarding locations and codes of the study samples is shown in Table 1.

### 2.2. DNA extraction

We extracted genomic DNA from dried leaves using a modified cetyltrimethylammonium bromide protocol [33]. The extracted DNA was detected using 0.8% agarose gel electrophoresis and stored at −20 °C until use.

### 2.3. AFLP fingerprinting

AFLP fingerprinting was performed in accordance with the method described by Vos et al. (1995) [24] with minor modifications. The AFLP protocol followed kit instructions (Ding Guo Biotechnology Company, Beijing China) for PCR amplification. An initial screening was performed using three individuals from wild *P. tenuifolia* using 64 primer combinations for selective amplifications. A total of 12 primer combinations (Table 2), which generated clear and abundant bands, were chosen for selective PCR.

Amplified DNA products (4.8 μL) were mixed with 98% formamide loading buffer (1.8 μL), heated at 95 °C for 8 min and immediately cooled in an ice bath for 30 min. The resulting products were resolved by 6% polyacrylamide gel electrophoresis in 1 × Tris–borate–EDTA buffer using a 2000 bp DNA marker (TakaRa Bio, Dalian China) for 2 h at 2500 V and then 0.1% silver nitrate was used for staining.

### 2.4. Analysis of AFLP data

All samples were manually scored as either '0' or '1' corresponding to the absence or presence of AFLP bands (100–2000 bp), respectively, to construct a binary matrix. The binary matrix was edited using Microsoft Excel 2003® [34]. The percentage of polymorphic loci (PPL), effective number of alleles (Ne), Shannon's information index (I), Nei's genetic diversity (**h**), total gene diversity ($H_t$) genetic diversities within populations ($H_s$), coefficient of genetic differentiation ($G_{st}$), and level of gene flow ($N_m$) were calculated using POPGENE version 1.31 [35] with manual corrections.

Please cite this article in press as: Y.Q. Peng, et al., Genetic diversity and population structure of a protected species: *Polygala tenuifolia* Willd, C. R. Biologies (2018), https://doi.org/10.1016/j.crvi.2018.01.007