Plant biology and pathology/Biologie et pathologie végétales

Species identification of polygonati rhizoma in China by both morphological and molecular marker methods

Jie Jiao [a], Xiangrong Jia [a], Pei Liu [a], Qiaomei Zhang [a], Feng Liu [b], Cunde Ma [b], Pengzhou Xi [b], Zongsuo Liang [a,*]

[a] College of Life Science, Northwest of A&F University, Yangling, 712100 Shaanxi, China
[b] Research Department, Buchang Pharma, Xi'an, 712000 Shaanxi, China

A R T I C L E   I N F O

A B S T R A C T

Morphological markers as well as two types of molecular markers, inter-sample sequence repeat (ISSR) and start codon targeted (SCoT) are suitable for species identification of the polygonati rhizoma germplasms. In this paper, we adopted these methods for the identification of rhizomes collected from 47 areas in China. Based on their morphological characters, the collected germplasms were classified into two populations, one with alternate leaf arrangement and the other with verticillate leaf arrangement, and they were comprised of five species and fourteen subgroups. Of the five species identified: *Polygonatum kingianum*, *P. cirrhifolium*, *P. alternicirrhosum*, and *P. sibiricum* belonged to one cluster, and *P. cyrtonema* belonged to a different cluster. According to the analysis of both ISSR and SCoT markers, all germplasms with greater genetic similarity were classified into one group. Especially, *P. sibiricum* and *P. cirrhifolium*, which shared ∼80% similarity, were clustered together, whereas the germplasms identified as *P. kingianum* with ∼86% similarity formed a separate clade. *P. kingianum* showed a much greater genetic similarity with *P. cyrtonema* than with *P. sibiricum*. The multidimensional scaling analysis further verified the accuracy and reliability of the molecular marker-based results. Thus, both morphological and molecular methods should be combined for the differentiation of germplasms such as those of polygonati rhizoma.

© 2018 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

1. Introduction

Polygonati rhizoma (PR), the rhizomes of *Polygonatum sibiricum* Redouté, *P. cyrtonema* Hua, and *P. kingianum* Collett & Hemsley, have been used as edible products during famine [1] and by Taoists and Buddhists to survive during breatharianism in China [2]. The three species are perennial herbs belonging to the family Asparagaceae [3]. In the Tang Dynasty (618 A.D.), Zhang Ji, a famous poet, once wrote "*if you have more fields, go and plant more polygonati rhizoma*" [2,4], which indicates that PR has been cultivated since the time of the Tang Dynasty. PR can be added to many kinds of food and has become popular in China. Polygonatum polysaccharides, its dominant and effective constituents, have many pharmacological functions [5–7]. Modern pharmacology shows that PR enhances immunity [8] and memory [9], reduces blood glucose [10] and fat levels [11], and has antibacterial [12], antiviral [13], antitumor [14], and antiaging effects [15].

Germplasms collection and identification are necessary for resources conservation, improved selection of varieties, and genetic improvement of PR. Until now, only three

* Corresponding author.
   E-mail address: liangzs@ms.iswc.ac.cn (Z. Liang).

prescriptive species of *Polygonatum* rhizomes have been recorded in *Chinese Pharmacopoeia* by China Food and Drug Administration, and other species, such as the rhizomes of *P. macropodum* Turcz., *P. verticillatum* (L.) All., and *P. odoratum* (Mill.) Druce [16], are yet to be confirmed. As the phenotypic characteristics of the rhizome easily change with its growth, they may be misidentified for PR with similar rhizome traits. Especially, the rhizomes of *P. odoratum* (polygonati odorati rhizome), another traditional Chinese medicine, are similar morphologically to those of *P. cyrtonema*, but with different pharmacological values. Besides, the increasing demand for this plant decreases gradually the natural populations of PR, thereby restricting the sustainable utilization of the PR [17]. Increasing presence of fake products of PR in the market due to the globalization of traditional Chinese medicine necessitates a rapid and efficient identification method of PR rhizomes. The three species used as PR differ from *P. odoratum* in several phenotypic traits of aerial parts: verticillate vs. alternate leaves, cirrhous vs. cuspidate leaf apex, red vs. green leaf sheath. Hence, these morphological characteristics are suitable for precise identification of the content of PR. However, when the rhizomes are in a dormant state [18–20], their identification becomes complicated, time consuming, and even ineffective. Thus, the evaluation of phenotypic traits leads to limited germplasm identification due to the small number of descriptors and to insufficient quantitative traits such as plant height, stem diameter, leaf length, and leaf width.

Methods combining morphological and molecular markers have been widely used for the identification of a number of germplasms, such as yardlong bean [21], mung bean [22], artichoke [23], haploid maize [24], Spanish olive cultivars [25], durum wheat [26], and *Curcuma comosa* Roxb. [27]. Molecular markers, such as restriction fragment length polymorphism, amplified fragment length polymorphism, random amplification of polymorphic DNA, inter-simple sequence repeat (ISSR), single-nucleotide polymorphism, start codon targeted (SCoT), and others, are powerful tools for the identification of species at the DNA level. Molecular markers are not dependent on the environment and can delineate accurately the plants by describing the genetic relationships among taxa. Of the different methods implementing molecular markers, ISSR and SCoT have been widely used for germplasm identification of different taxa because they require low quantities of plant material, they are simple to operate, and do not require prior information about plant genomic sequences, while providing effective information that can be implemented in genetic breeding, genomic mapping, and species identification. In addition, its operation is not limited in time and space. For example, the ISSR markers have been used to identify *Polygonatum sibiricum*, *P. cirrhifolium* (Wall.) Royle, *P. filipes* Merr. ex C. Jeffrey & McEwan, and *P. cyrtonema* in China [28,29]. The SCoT markers have been used for evaluating genetic diversity in medicinal *Chrysanthemum morifolium* Ramat. [30], *Dendrobium* [31], Tunisian citrus [32], chickpea [33], Chinese *Elymus sibiricus* L. [34], and *Polygonatum* germplasms [35]. However, the SCoT markers have never been used for identification of PR species.

The aim of the present study was to test whether the ISSR and SCoT markers can be used to identify the PR species. To that end, we adopted both morphological characteristics analysis and ISSR and SCoT methods to identify PR samples collected from 47 different regions in southern China. The fresh rhizomes were identified based on their morphological characteristics and cultivated to score and analyze morphological characters. The ISSR [36] and SCoT [32,37] markers were developed and implemented for the identification of the specimens. Finally, the identification results by both morphological characteristics and molecular markers schemes were compared.

2. Material and methods

2.1. Plant material

Polygonatum rhizomes were collected from 47 different germination areas in China: Anhui (5), Chongqing (2), Fujian (1), Guangdong (2), Guangxi (4), Guizhou (4), Henan (7), Hubei (2), Hunan (1), Jiangxi (3), Shaanxi (2), Sichuan (2), Yunnan (6), and Zhejiang (6) Provinces. Table 1 shows the information on the preliminary taxonomic classification, the geographic origin, and the phenotypic traits of rhizomes. Approximately 100 rhizomes free of lesions and mildew infection were selected from each region and screened primarily for morphological features such as their thickness, number of nodes, and diameter of tubercle. Fresh tissue samples (0.5 g) were cut out from five rhizomes from each region, rinsed with distilled water, dried with filter paper, and preserved at $-80\,^\circ\text{C}$ for DNA isolation.

Almost 80 rhizomes from each region with similar characters were selected and cultivated in a PR GAP planting base (Buchang Pharma, Hanzhong, China). The experimental plots were located in Zhongchuanba Village, Wulongdong Town, Lveyang County ($106^\circ12'30''$E, $33^\circ30'9''$N; 897 m above sea level). The area of each plot was $1.2\,\text{m} \times 5.0\,\text{m}$, and the spacing between each plot was 1.2 m. The rhizomes were planted in rows; the distance between the rows and that between rhizomes was 25 cm.

2.2. DNA extraction

Genomic DNA was isolated according to the CTAB method [38]. The quality of the extracted DNA was verified by a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA), diluted to $20\,\text{ng}\cdot\mu\text{L}^{-1}$ and stored at $-20\,^\circ\text{C}$ for molecular marker analysis.

2.3. ISSR primers screening and amplification

A total of 100 ISSR primers, designed at the University of British Columbia, were synthesized by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China). All primers were screened for their amplification efficiency and 15 primers (Table 2) with high amplification efficiency and reproducibility were selected.

PCR amplification was performed in a 96-well Veriti Thermal Cycler (Applied Biosystems, Foster City, CA, USA) in 20-μL reaction volumes comprised of 10 μL of $2 \times$ Es