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Research article

Study on the grading standard of *Panax notoginseng* seedlingsLijuan Chen^{1,2,3}, Ye Yang^{1,2,3}, Jin Ge^{1,2,3}, Xiuming Cui^{1,2,3,*}, Yin Xiong^{1,2,3,**}¹ Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, China² Yunnan Key Laboratory of *Panax Notoginseng*, Kunming 650500, China³ Laboratory of Sustainable Utilization of *Panax Notoginseng* Resources State Administration of Traditional Chinese Medicine, Kunming 650500, China

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ABSTRACT

Background: The quality differences in seedlings of medicinal herbs often affect the quality of medicinal parts. The establishment of the grading standard of *Panax notoginseng* seedlings is significant for the stable quality of medicinal parts of *P. notoginseng*.

Methods: To establish the grading standard of *P. notoginseng* seedlings, a total of 36,000 *P. notoginseng* seedlings were collected from 30 producing areas, of which the fresh weight, root length, root diameter, bud length, bud diameter, and rootlet number were measured. The K-means clustering method was applied to grade seedlings and establish the grading standard.

Results: The fresh weight and rootlet number of *P. notoginseng* seedlings were determined as the final indices of grading. *P. notoginseng* seedlings from different regions of Yunnan could be preliminarily classified into four grades: the special grade, the premium grade, the standard grade, and culled seedlings.

Conclusion: The grading standard was proven to be reasonable according to the agronomic characters, emergence rate, and photosynthetic efficiency of seedlings after transplantation, and the yields and contents of active constituents of the medicinal parts from different grades of seedlings.

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1. Introduction

Panax notoginseng is a well-known traditional Chinese medicinal herb belonging to the ginseng family (Araliaceae). Sanqi ginseng (*P. notoginseng*), American ginseng (*Panax quinquefolius*) and Chinese and Korean ginseng (*Panax ginseng*) are highly prized medicinal herbs, with *P. notoginseng* being the most expensive [1]. *P. notoginseng* has been cultivated and medicinally used since ancient times for its remarkable and valuable properties such as dispersing blood stasis, hemostasis, detumescence, and analgesia [2,3]. The species is scarcely found in the wild, but with the heaviest production in Yunnan and some in Guangxi, Guizhou, and Sichuan Province, China [4]. Pharmacological research studies and clinical trials indicated that *P. notoginseng* possesses effects of hemostasis, reducing blood fat, antithrombosis, and also enhances immunity, with antifibrosis and antineoplastic functions [5,6]. *P. notoginseng* is the major source of famous Chinese invented medicinal formulas

such as Yunnan Baiyao, Compound Danshen Dripping Pills, and Pian Zai Huang [7].

P. notoginseng seedlings are involved in the whole productive process of medicinal parts, which therefore play a significant role in the yield and quality of medicinal materials [8]. However, there is no comprehensive and normative quality standard for *P. notoginseng* seedlings at present. For a long time, farmers kept seeds and cultivated seedlings by themselves, which were then circulated in the market. This production system could be problematic for the following reasons. First of all, the uneven quality, unclear sources, and uncertain production areas of seedlings result in low rates of emergence and acclimatized seedlings, as well as high ratio of inferior seedlings. Second, as there is no quality standard to follow, supervision and management of *P. notoginseng* seedlings is difficult to achieve. Thus, establishment of seedlings grading standard can be beneficial for the normative development of the *P. notoginseng* planting industry. From our previous investigation on the biological

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characteristics of *P. notoginseng* seedlings [9] and the effect of grading seedlings on the growth and production of medicinal parts [10], the quality differences in *P. notoginseng* seedlings had considerable impact on the quality stability of medicinal parts, which also provided some theoretical and practical evidence for the breeding of superior *P. notoginseng* seedlings.

In this study, the grading standard of *P. notoginseng* seedlings was preliminarily established through sample collection, grading, and verification test of *P. notoginseng* seedlings, of which correlations with yields and levels of active constituents of medicinal parts were also investigated. We hope that this research can lay the foundation of the production and standardized management of *P. notoginseng* seedlings.

2. Materials and methods

2.1. Study on grading standard of *P. notoginseng* seedlings

2.1.1. Samples collection

A total of 36,000 healthy *P. notoginseng* seedling samples without root disease, pest, and mechanical damages were collected from 30 producing areas of Yunnan Province in late December 2014 to mid-January 2015. The geographical distribution of all seedlings samples were in E104.19°–E105.6°, N23.01°–N24.05°, at an altitude of 1,380–1,991 m. Further information on *P. notoginseng* seedlings is shown in Table 1.

2.1.2. Measurement indices

The appearance of *P. notoginseng* seedlings is shown in Fig. 1. The indices included the root length (the length from the base to the end of main root), root diameter (the longest diameter of main root), bud length (the length from the base to the end of the bud), bud diameter (the longest diameter of bud), rootlet number, and fresh weight.

2.2. Plots verification of seedlings grading

2.2.1. Testing materials

According to the grading standard proposed by our study, different grades of seedlings were planted in plots to verify the reasonability of the grading standard. Plots for the test were located in the greenhouse (E102.73°, N25.04°) of the Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming, China. The orthogonal design of $L_9(3^4)$ was used in the plots for the verification test. Twelve plots randomly permuted were set up. The area per plot was 30 m². All seedlings were managed under the general field management methods. Seedlings of different grades were transplanted on January 19, 2015. The planting spacing was 10 cm × 15 cm. Two thousand strain seedlings

were planted in each plot. Samples of different grades of seedlings were collected, and the agronomic characters and photosynthetic rate were measured after transplanting seedlings for 60 days, 150 days, and 240 days, respectively.

2.2.2. Testing methods

2.2.2.1. Agronomic characters of seedlings. Fifty seedlings samples were sampled by the S-shaped sampling method at different growth stages in each plot, in which the agronomic characters were measured. The measured items included the rate of emergence, chlorophyll content [evaluated by Soil and Plant Analyzer Development (SPAD)] value, plant height (the length from the base of root to the top of the longest leaf), stem height (the length from the base of stem to the petiole bottom), the mid-leaf length (the length from the base to the end of maximum mid-leaf), and mid-leaf width (the length of the widest part of the maximum mid-leaf). In addition, the fresh weight of root, stem, and leaves were measured after the seedlings, with the soil on the surface of roots washed, respectively. Moreover, the dry weight of roots, stems, and leaves were also measured after the deactivation of enzymes at 105°C for 30 min and constant-temperature drying to constant weight at 50°C, respectively.

2.2.2.2. Photosynthesis indices of seedlings. The photosynthesis indices of 10 samples were measured. The samples were randomly sampled from different grades at different growth stages and measured by CIRAS-3 Portable Photosynthesis System (PP Systems, Amesbury, MA, USA). The indices included the net photosynthetic rate, transpiration rate, stomatal conductance, intercellular CO₂ concentration, and water use efficiency in leaf.

2.3. Yield and saponins contents of *Notoginseng Radix*

Notoginseng Radix samples, the medicinal parts of *P. notoginseng*, were collected from different plots in late April 2016, and the yields, size, and roots number per 500 g from different grades of seedlings were measured. Meanwhile, the contents of five saponins in *Notoginseng Radix* from different grades of seedlings were determined according to the method described in Chinese Pharmacopoeia of 2015 edition [2]. A high performance liquid chromatography (HPLC) system equipped with LC-20AB Binary HPLC pump, SPD-20A absorbance detector, and SIL-20A autosampler was used to perform the HPLC analysis and to determine the contents of five saponins. The chromatographic column was Vision HT C₁₈ column (5 μm, 250 mm × 4.6 mm). Methyl alcohol (MeOH) and acetonitrile (MeCN) (HPLC grade) were purchased from Sigma-Aldrich, Inc. (USA). Ultrapure water was generated with an UPT-I-20T ultrapure water system (Chengdu Ultrapure Technology, Inc. Sichuang, China). Standard ginsenosides Rg1 (MUST-13041301),

Table 1
Information on *Panax notoginseng* seedlings collected from different regions

| No. | Producing area | Number of seedlings (plant) | No. | Producing area | Number of seedlings (plant) | No. | Producing area | Number of seedlings (plant) |
|-----|---------------------------------|-----------------------------|-----|--------------------------------|-----------------------------|-----|--------------------------------|-----------------------------|
| 1 | Xisa Town, Xichou County | 1,200 | 11 | Jiayi Town, Yanshan County | 1,200 | 21 | Dongshan Town, Wenshan County | 1,200 |
| 2 | Xinjie Town, Xichou County | 1,200 | 12 | Pingyuan Town, Yanshan County | 1,200 | 22 | Zhuilijie Town, Wenshan County | 1,200 |
| 3 | Mali Town, Malipo County | 1,200 | 13 | Jiangna Town, Yanshan County | 1,200 | 23 | Gumu Town, Wenshan County | 1,200 |
| 4 | Daping Town, Malipo County | 1,200 | 14 | Baga Town, Yanshan County | 1,200 | 24 | Mabai Town, Maguan County | 1,200 |
| 5 | Mazheng Town, Malipo County | 1,200 | 15 | Dehou Town, Wenshan County | 1,200 | 25 | Nanlao Town, Maguan County | 1,200 |
| 6 | Donggan Town, Malipo County | 1,200 | 16 | Pingba Town, Wenshan County | 1,200 | 26 | Xinhua Town, Funing County | 1,200 |
| 7 | Heizhiguo Town, Guangnan County | 1,200 | 17 | Panzhuhua Town, Wenshan County | 1,200 | 27 | Guichao Town, Funing County | 1,200 |
| 8 | Babao Town, Guangnan County | 1,200 | 18 | Kaihua Town, Wenshan County | 1,200 | 28 | Tianpeng Town, Funing County | 1,200 |
| 9 | Nanping Town, Guangnan County | 1,200 | 19 | Xigu Town, Wenshan County | 1,200 | 29 | Jinping Town, Qiubei County | 1,200 |
| 10 | Tanfeng Town, Yanshan County | 1,200 | 20 | Matang Town, Wenshan County | 1,200 | 30 | Yuezhe Town, Qiubei County | 1,200 |

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