



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, China² Yunnan Key Laboratory of *Panax Notoginseng*, Kunming, China³ Laboratory of Sustainable Utilization of *Panax Notoginseng* Resources State Administration of Traditional Chinese Medicine, Kunming, 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	Tanfang Town, Yanshan County	1,200	20	Matang Town, Wenshan County	1,200	30	Yuezhe Town, Qiubei County	1,200

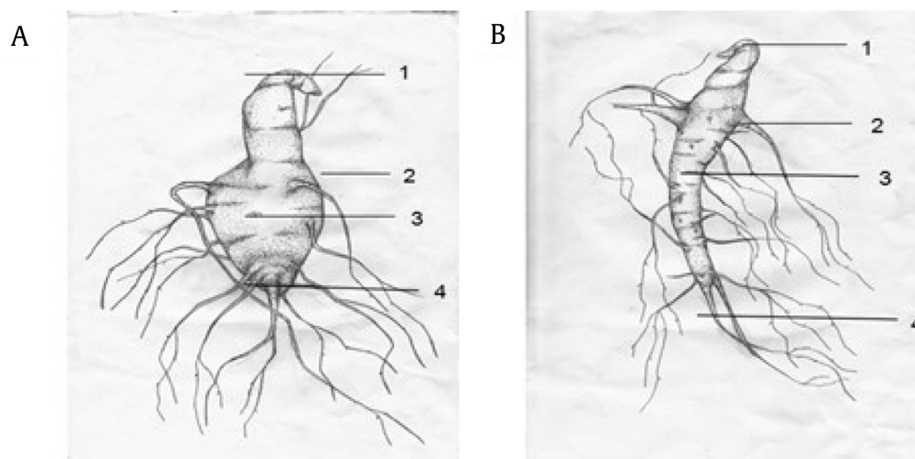


Fig. 1. Appearance of *Panax notoginseng* seedlings. (A) Group seedling. (B) Long seedling. 1, 2, 3, and 4 refer to the rhizome, lateral root, root tuber, and fibrous root of *P. notoginseng*, respectively.

Rb1 (MUST-13102301), Rd (MUST-13041212), Re (MUST-13041201), and notoginsenoside R1 (MUST-14022411) were purchased from Dawn of Nanjing Chemical Technology Co. Ltd. (Jiangsu, China).

2.4. Statistical analysis

Excel 2007 and statistical analysis software of SPSS21.0 (Statistical Program for Social Sciences, SPSS inc, Chicago) were used to analyze the data and plot acquired data, including data normalization, correlation analysis, principal component analysis (PCA), and *K*-means clustering analysis.

2.4.1. Data normalization

Normalization aims to remove the irregularity (abnormality) of data. The normalized values allow the comparison of corresponding normalized values for different datasets in a way that eliminates the effects of certain gross influences [11]. The normalized data are obtained and calculated according to the following equation:

$$P'_i = (P_i - P_{\min}) / (P_{\max} - P_{\min}),$$

where P'_i is the corresponding element value after normalizing; P_i is the initial element value; P_{\min} and P_{\max} are the minimum and maximum value of initial element, respectively.

2.4.2. Correlation analysis

Correlation analysis deals with the relationships among variables. The correlation coefficient is a measure of linear association between two variables. Values of the correlation coefficient are always between -1 and $+1$. A correlation coefficient of $+1$ indicates that two variables are perfectly related in a positive linear sense, a correlation coefficient of -1 indicates that two variables are perfectly related in a negative linear sense, and a correlation coefficient of 0 indicates that there is no linear relationship between the two variables [12]. In this study, Pearson correlation was used to describe the relationship between two continuous variables that are both normally distributed.

2.4.3. Principal component analysis

PCA is applied to extract sufficient relevant features presenting the major information of all indices of *P. notoginseng* seedlings. PCA is a chemometric, linear, unsupervised, and pattern recognition technique used for analyzing, classifying, and reducing the dimensionality of numerical datasets in a multivariate problem [13].

2.4.4. *K*-means clustering analysis

K-means analysis is one of the simplest unsupervised learning algorithms that solve the well-known clustering problem. The procedure follows a simple and easy way to classify a given data set through a certain number of clusters. The main idea is to define *K* centroids, one for each cluster. These centroids should be placed in a cunning way because different locations lead to different results. The algorithm is also significantly sensitive to the initial randomly selected cluster centers. The *K*-means algorithm can be run multiple times to reduce this effect. *K*-means is a simple algorithm that has been adapted to many problem domains, and it is a good candidate to utilize for randomly generated data points [14].

3. Results

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

3.1.1. Agronomic characters of *P. notoginseng* seedlings

The agronomic characters of seedlings were measured, including root length, root diameter, bud length, bud diameter, rootlets numbers, and fresh weight (Fig. 2 and Table 2). The results showed that all six agronomic indices presented normal distributions. Furthermore, the variation ranges of all measured indices were significant.

The size sequence of variation ranges was as follows: rootlet number > fresh weight > root diameter > bud diameter > root length > bud length. According to the result, the agronomic characters exhibited significant differences among *P. notoginseng* seedlings from different producing areas.

3.1.2. Correlation analysis for indices of *P. notoginseng* seedlings

The correlations among the root length, root diameter, bud length, bud diameter, rootlet number, and fresh weight of *P. notoginseng* seedlings were analyzed (Table 3). The fresh weight was significantly positively correlated with root length, root diameter, and bud diameter ($p < 0.01$), and presented a significantly positive correlation with bud length and rootlet number ($p < 0.05$). The result showed that the fresh weight of *P. notoginseng* was directly affected by root length, root diameter, bud length, bud diameter, and rootlet number. That is to say, with the increasing seedling root diameter, dormant bud diameter, and rootlet number, the plant fresh weight was increased. The influence of root diameter, root length, bud diameter, bud length, and rootlet number on fresh was gradually weakened. The root length was significantly

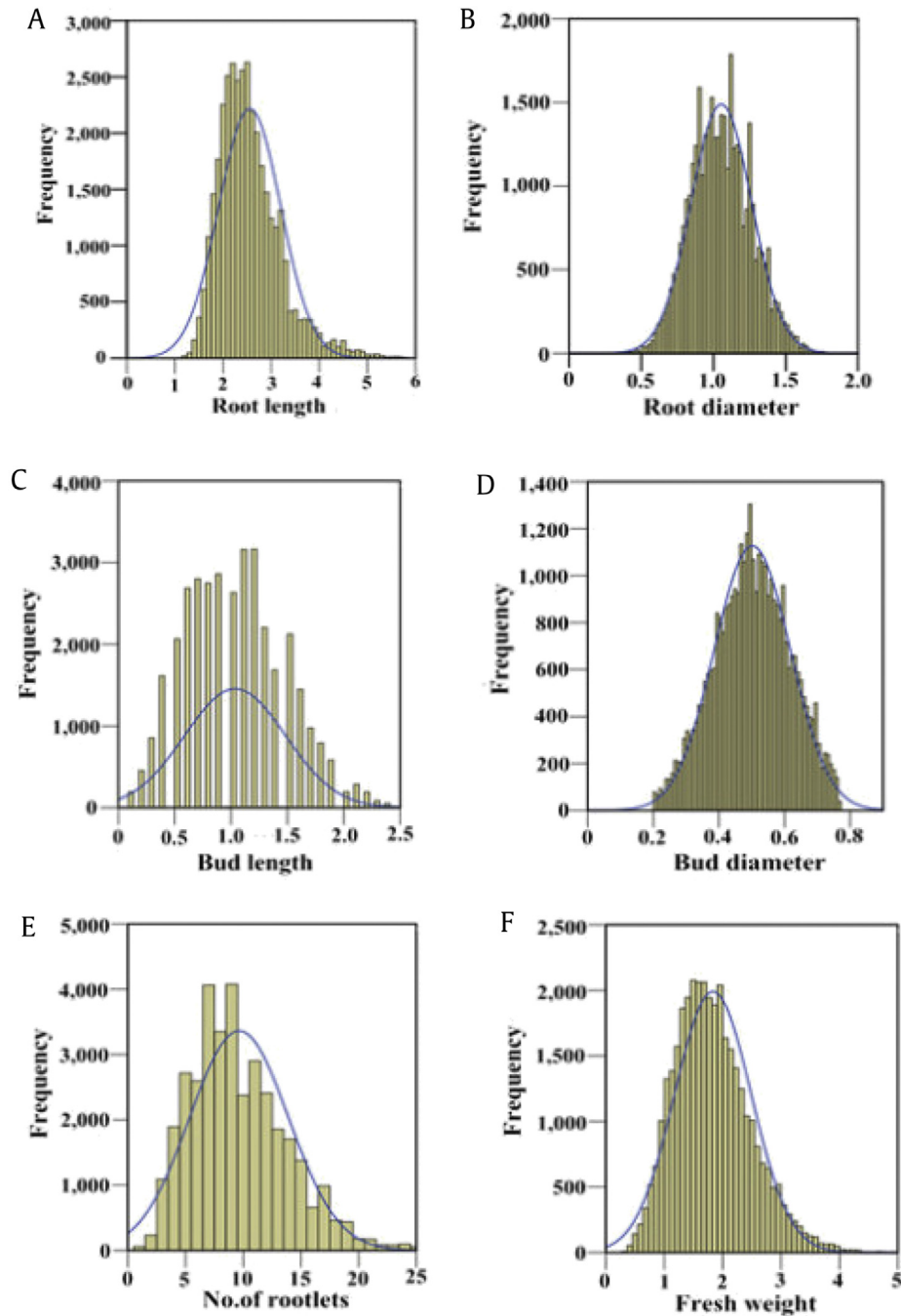


Fig. 2. A total of 36,000 *P. notoginseng* seedlings were collected from 30 producing areas, of which agronomic characters were measured. The normal distribution plots of *P. notoginseng* seedlings agronomic characters were made by SPSS21.0. (A) Root length. (B) Root diameter. (C) Bud length. (D) Bud diameter. (E) Number of rootlets. (F) Fresh weight.

negatively correlated with root diameter (-0.434), but presented significantly positive correlations with bud diameter (0.190) and rootlet number (0.073). This meant that the longer the root, the smaller the root diameter, which was consistent with the appearance of *P. notoginseng* seedlings (group seedlings were short and thick, as shown in Fig. 1A; long seedlings were thin and long, as shown in Fig. 1B). At the same time, the length of root was increased with the increase in bud diameter and rootlet number. The influences of root diameter, bud diameter, and rootlet number on the root length were gradually weakened. Root diameter was significantly positively correlated with bud diameter (0.441), and

presented significantly positive correlations with bud length (0.057) and rootlet number (0.084). This meant that the seedling root diameter was affected by bud diameter, bud length, and rootlet number. With the increase in dormant bud diameter, length, and rootlet number, the root diameter was increased. The influences of the indices above on root diameter were gradually weakened. Meanwhile, bud length and bud diameter were positively correlated with rootlet number, suggesting that bud length and bud diameter were affected by the rootlet number of seedlings. When rootlet number increased, the length and diameter of dormant bud were also increased.

Table 2
Statistical result of agronomic characters of *Panax notoginseng* seedlings

Index	Root length (cm)	Root diameter (cm)	Bud length (cm)	Bud diameter (cm)	Rootlet number (cm)	Fresh weight (g)
Effective value (N)	36,000	36,000	36,000	36,000	36,000	36,000
Maximum	6.3	1.99	2.9	0.767	32	5.94
Minimum	0.8	0.410	0.1	0.201	1	0.34
Average value	2.6	1.05	1.03	0.506	10	1.83
Standard deviation	0.64	0.42	0.15	0.20	4	0.76
Coefficient of variation (%)	25.7	39.8	14.3	38.7	44.3	41.2

Table 3
Correlation analysis of *Panax notoginseng* seedlings indices (N = 36,000)

Index	Fresh weight	Root length	Root diameter	Bud length	Bud diameter
Root length	0.759**				
Root diameter	0.994**	-0.434**			
Bud length	0.107*	0.072	0.057*		
Bud diameter	0.508**	0.190*	0.441**	0.057	
Rootlet number	0.106*	0.073*	0.084*	0.055*	0.067*

* $p < 0.05$; ** $p < 0.01$

3.1.3. PCA for indices of *P. notoginseng* seedlings

After all the data were normalized, PCA was carried out for the agronomic indices of *P. notoginseng* seedlings. Five indices—fresh weight (X_1), root diameter (X_2), root length (X_3), bud diameter (X_4), and bud length (X_5)—were initially selected by reducing the dimension. Next, the selected indices were used for the PCA. In general, the number of principal components was determined by the cumulative variance contribution rate (more than 85%). In this study, the cumulative variance contribution rate of main component 1 (F_1), main component 2 (F_2), main component 3 (F_3), and main component 4 (F_4) reached 98.18%. It indicated that the four main components could contain the major information of all indices.

The first four characteristic values were taken out, and we then calculated the corresponding characterized vectors. The expressions of each component were as follows:

$$F_1 = 0.999X_1 + 0.997X_2 + 0.989X_3 + 0.988X_4 + 0.068X_5$$

$$F_2 = -0.031X_1 + 0.077X_2 - 0.142X_3 + 0.151X_4 + 0.024X_5$$

$$F_3 = 0.009X_1 + 0.015X_2 + 0.043X_3 + 0.018X_4 - 0.041X_5$$

$$F_4 = 0.002X_1 + 0.005X_2 - 0.013X_3 + 0.014X_4 - 0.048X_5$$

The variance contribution rates of F_1 , F_2 , F_3 , and F_4 were 37.215, 26.586, 15.434, and 18.951, respectively. Because the variance contribution rate of F_1 was the highest (37.215), the first principal component contained the largest amount of information on *P. notoginseng* seedling quality. Therefore, priority should be given to F_1 in the analysis. In the expression of F_1 , the coefficients of components in descending order were as follows: $X_1 > X_2 > X_3 > X_4 > X_5$, of which the coefficients of the first four components were significantly higher than that of the fifth component. According to the result, the fresh weight, root diameter, root length, and bud diameter played the major role in the quality of seedlings.

3.1.4. K-means clustering analysis for indices of *P. notoginseng* seedlings

Indices including root length, root diameter, bud diameter, and rootlet number were chosen to preliminarily classify *P. notoginseng*

seedling grades. Through the K-means clustering analysis, all seedlings were classified into four categories with the final clustering centers acquired (Table 4).

The distances among the final cluster centers of four categories were calculated (Table 5). The distances in descending order were as follows: 9.908 (between the second category and the fourth category), 8.352 (between the first category and the fourth category), and 4.214 (between the third category and the fourth category). As the indices of seedlings in the fourth category for the productive practice were lower, they were classified as “culled” because of the inferior quality. According to the center distances between the fourth category and other categories, the second category, the first category, and the third category could be classified into the special grade, premium grade and standard grade among *P. notoginseng* seedlings, respectively.

Variance analysis for the fresh weight, root length, root diameter, bud diameter, and rootlet number was carried out. It was shown that there were significant differences among the fresh weight, root length, root diameter, bud diameter, and rootlet number of *P. notoginseng* seedlings ($p < 0.01$). Moreover, the F values of indices in descending order were fresh weight, root diameter, bud diameter, root length, and rootlet number. The current findings demonstrated that those character indices had a significant impact on the quality of *P. notoginseng* seedlings.

Based on the character indices of *P. notoginseng*, the seedlings were initially classified into four grades: special grade, premium grade, standard grade, and culled seedlings (Table 6). Because the size (including indices of root length, root diameter and bud length) of *P. notoginseng* seedlings was closely related to the fresh weight, and the rootlet number directly affected the vital energy of

Table 4
Final clustering center values of *Panax notoginseng* seedlings by K-means clustering analysis

Index	Final cluster center			
	First	Second	Third	Fourth
Fresh weight of per plant (g)	1.59	2.68	0.95	0.62
Root length (cm)	2.6	4.3	1.9	2.2
Root diameter (cm)	1.37	1.08	0.858	0.654
Bud diameter (cm)	0.504	0.629	0.332	0.236
Rootlet number	15	25	8	4
No. of clusters (N)	18,107	3,889	11,665	2,339
Percentage (%)	50.3	10.8	32.4	6.5

Table 5
Distances among the final cluster centers of four categories

Index	Distance among final cluster centers			
	First	Second	Third	Fourth
First		5.252	3.904	8.352
Second	5.252		7.845	9.908
Third	3.904	7.845		4.214
Fourth	8.352	9.908	4.214	

Table 6
Preliminary grading standard of *Panax notoginseng* seedlings

Grade	Index				
	Fresh weight (g)	Root length (cm)	Root diameter (cm)	Bud diameter (cm)	Rootlet number
Special	≥2.68	≥4.3	1.08–1.29	≥0.629	≥25
Premium	1.59–2.68	2.6–4.3	1.08–1.37	0.504–0.629	15–25
Standard	0.95–1.59	1.9–2.6	0.858–1.08	0.332–0.504	8–15
Culled	≤0.95	≤1.9	≤0.858	≤0.332	≤8

P. notoginseng seedlings, the fresh weight and rootlet numbers were determined as the quality indices to simplify the final grading standard for the production guide.

3.2. Verification for grading standard of seedlings

3.2.1. Emergence rate and agronomic characters of seedlings

The emergence rate of different grades of *P. notoginseng* seedlings were recorded by planting them in plots for 60 days (Fig. 3). The result showed that the emergence rate of the special-grade seedlings was significantly higher than the rates of premium-grade ($p < 0.05$) and standard-grade seedlings ($p < 0.01$). Moreover, there was a significant difference between the emergence rate of premium-grade seedlings and that of standard-grade seedlings ($p < 0.05$).

The plant height, stem height, stem diameter, root length, root diameter, mid-leaf length, mid-leaf wide, rootlet number, and chlorophyll content were measured after *P. notoginseng* seedlings were transplanted for 60 days, 150 days, and 240 days (Table 7). The agronomic characters of seedlings of different grades in the same sampling period, and those of the same grade in different sampling periods both showed significant differences, and that with the improvement of seedling grade and delay in the transplanting time, the agronomic character values were increased. The agronomic characters of different grades of seedlings transplanted for 60 days were compared with those transplanted for 150 days; results showed that the average growth rates were 45.76% (for the special grade), 38.21% (for the premium grade), and 28.81% (for the standard grade). Meanwhile, the agronomic characters of different grades of seedlings transplanted for 150 days were compared with those transplanted for 240 days; results showed that the average growth rates were 13.39% (for the special grade), 12.41% (for the premium grade), and 9.67% (for the standard grade). We found that the growth rate of seedlings was increased as the seedlings' grades improved. Furthermore, the growth vigor of higher-grade seedlings was superior to that of lower-grade seedlings.

We also determined the fresh and dry weights of roots, stems, and leaves of *P. notoginseng* seedlings of different grades. The results showed that the sequence of the above indices was as follows: special-grade seedlings > premium-grade seedlings > standard-grade seedlings. Conversely, the fresh weight/dry weight (FW/DW) ratios in descending order were as follows: standard grade, premium grade, and special grade. Furthermore, with the delay in harvest time, the FW/DW ratios of roots, stems, and leaves also decreased (Fig. 4A). The relatively lower FW/DW ratio is often related to the higher proportion of dry matters (carbohydrates, nutrients, minerals, active constituents, proteins, etc.) in plants. Therefore, the lower the FW/DW ratio, the richer would be the nutrients or active constituents of plants [15,16]. This corresponded to the change in trend of the total biomass (the total dry weight of stems and leaves), in that along with the growing time, the total biomass of seedlings was increased (Fig. 4B). This suggested that the accumulations of dry matters of *P. notoginseng* seedlings in

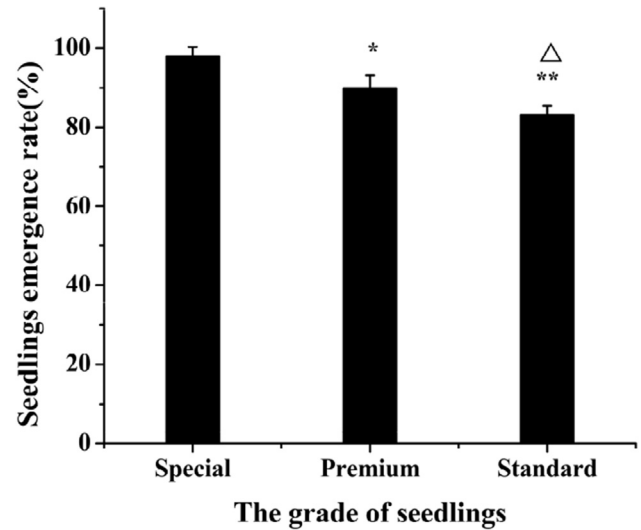


Fig. 3. Emergence rates of different grades of *Panax notoginseng* seedlings after transplantation of 60 days. The orthogonal design of $L_9 (3^4)$ was used in the plots. Twelve plots randomly permuted were set up. The graph shows the mean \pm standard deviation of at least three independent experiments. * $p < 0.05$ and ** $p < 0.01$, compared with the special grade; $\Delta p < 0.05$ and $\Delta\Delta p < 0.01$, compared with the premium grade.

descending order were as follows: special-grade seedlings, premium-grade seedlings, and standard-grade seedlings. In addition, the dry matters accumulated incrementally as time went by.

3.2.2. Photosynthesis indices of *P. notoginseng* seedlings

The photosynthesis indices of different grades of *P. notoginseng* seedlings in different growth stages are shown in Table 8. The net photosynthetic rate, transpiration rate, stomatal conductance, water use efficiency in leaf, and the intercellular CO_2 concentration of different grades of seedlings in the same period showed significant differences, of which the sequence was as follows: special-grade seedlings > premium-grade seedlings > standard-grade seedlings. Meanwhile, the intercellular CO_2 concentrations in descending order showed the following trend: special-grade seedlings, premium-grade seedlings, and standard-grade seedlings. The net photosynthetic rate, transpiration rate, stomatal conductance, and water use efficiency in leaf of the same grade of seedlings in different sampling periods were increased with the delay in transplanting time. Conversely, the intercellular CO_2 concentration was decreased with the delaying in transplanting time. It was also found that with the improvement of seedling grade, the vigor of seedlings was also increased.

3.2.3. Yields and saponins contents of *Notoginseng Radix*

The yields, size, and roots number per 500 g of *Notoginseng Radix* from different grades of seedlings are shown in Fig. 5. According to the result, the yields and size of *Notoginseng Radix* from seedlings in descending order were as follows: special-grade, premium-grade, and standard-grade seedlings. On the contrary, the roots number per 500 g of *Notoginseng Radix* from seedlings in descending order was as follows: standard grade, premium grade, and special grade of seedlings. Moreover, the contents of saponins in *Notoginseng Radix* from seedlings of different grades were determined (Fig. 6). It was shown that the contents of notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1 in *Notoginseng Radix* from seedlings of different grades in descending order were as follows: special grade, premium grade, and standard grade. The total content of saponins in *Notoginseng Radix* from different grades of seedlings also showed the same trend.

Table 7
Agronomic characters of different grades of *Panax notoginseng* seedlings at different growth stages

Time (d)	Grade	Plant height (cm)	Stem height (cm)	Stem diameter (cm)	Root length (cm)	Root diameter (cm)	Mid-leaf length (cm)	Mid-leaf width (cm)	Number of rootlets	Chlorophyll content (SPAD)
60	Special	20.9 ± 2.04	11.7 ± 1.19	0.372 ± 0.12	3.8 ± 1.19	1.19 ± 0.21	6.5 ± 1.53	2.3 ± 0.46	19 ± 3	29.9 ± 1.89
	Premium	20 ± 2.16	10.5 ± 1.16	0.359 ± 0.12	3.6 ± 1.03	1.13 ± 0.23	5.8 ± 1.41	2.2 ± 1.06	13 ± 3	28.7 ± 1.06
	Standard	18.3 ± 2.35	9.1 ± 1.28	0.323 ± 0.12	3.1 ± 1.72	1.01 ± 0.51	4.9 ± 1.66	2.1 ± 0.59	12 ± 3	26.8 ± 2.08
150	Special	24.8 ± 1.91	17.6 ± 2.08	0.406 ± 0.05	6.9 ± 0.67	1.65 ± 0.11	9.6 ± 0.52	3.5 ± 0.54	33 ± 4	41.8 ± 1.26
	Premium	22.9 ± 2.23	15.9 ± 4.30	0.389 ± 0.03	6.0 ± 0.48	1.31 ± 0.15	8.3 ± 0.50	3.0 ± 0.23	23 ± 4	37.5 ± 1.32
	Standard	19.7 ± 2.29	12.6 ± 1.94	0.366 ± 0.02	5.1 ± 1.53	1.27 ± 0.09	6.4 ± 1.01	2.5 ± 0.23	17 ± 2	31.7 ± 1.09
240	Special	26.3 ± 2.11	20.9 ± 2.02	0.449 ± 0.42	7.1 ± 0.81	1.74 ± 0.25	10.7 ± 0.45	4.1 ± 0.41	35 ± 5	59.4 ± 3.12
	Premium	24.1 ± 1.23	17.9 ± 1.52	0.408 ± 0.22	6.3 ± 0.46	1.45 ± 0.17	9.1 ± 0.39	3.5 ± 0.32	27 ± 4	48.8 ± 2.78
	Standard	20.9 ± 1.09	13.8 ± 1.26	0.379 ± 0.12	5.6 ± 0.61	1.31 ± 0.19	6.9 ± 0.52	2.9 ± 0.35	19 ± 3	37.8 ± 1.95

SPAD, Soil and Plant Analyzer Development

4. Discussion

Visual assessment of the growth vigor and general appearance is commonly used to evaluate the quality of *P. notoginseng* seedlings in current production processes. Because of the lack of scientific and systematic assessment criteria, the evaluation result is often subjective. Thus, the establishment of a grading standard for *P. notoginseng* seedlings could provide some specific character indices for the quality evaluation of seedlings in the trade and field management.

4.1. Determination of key agronomic characters for seedlings grading

Determining the key agronomic character indices for the grading of seedlings is the premise of establishing the standards [17]. Yu et al [18] found that the bulb diameter and rootlet numbers of *Anemarrhena asphodeloides* could be used as the grading indices of seedlings. Thus, they divided the seedlings into three grades, verified by the field cultivation. Tang et al [19] clarified that the seedling height of *Dendrobium nobile* seedlings was the main classification index. Moreover, the plant number of per clump of seedling was an important factor to the survival rate and the yield. Therefore, they eventually determined the seedling height and the plant number of per clump of seedling as the main grading indices of *D. nobile* seedlings, and classified the seedlings into three grades. Zhang et al [20] observed and recorded the plant height, branching numbers on the ground, inflorescence numbers, and other growth indices of *Salvia miltiorrhiza*, and classified the seedlings into three grades using the *K*-means clustering method.

In our study, we selected six agronomic character indices—fresh weight, root length, root diameter, bud diameter, bud length, and rootlet number—to carry on the study on the grading of *P. notoginseng* seedlings. The correlation analysis showed that the root length, root diameter, bud length, bud diameter, and rootlet number of *P. notoginseng* seedlings were significantly correlated with fresh weight. We preliminarily determined fresh weight, root length, root diameter, bud diameter, and bud length as the principal components affecting the quality of *P. notoginseng* seedlings. However, this was only a theoretical analysis result. Although the correlation of rootlet number with other indices was weak, the rootlets were inserted on main roots, of which root hairs sprouted from the rootlets so as to absorb nutrients and moisture. Therefore, the rootlet is an important guarantee for the survival and health of seedlings. In conclusion, the fresh weight, root length, root diameter, bud diameter, and rootlet number were determined as the key indices for the grading of *P. notoginseng* seedlings in the research.

4.2. Establishment of grading method for seedlings

At present, the system clustering method and the dynamic clustering method are widely used in the classification of various plant seedlings. However, the highly complex calculation process of the system clustering method prevents it from being considered the global optimum, which is not suitable for handling large amounts of data [21]. The dynamic clustering method is based on the idea of iterative method, and the *K*-means method is the most common dynamic clustering method at present [22]. When handling large amounts of data, the *K*-means method is given preference for its fast, efficient, and accurate features. The method

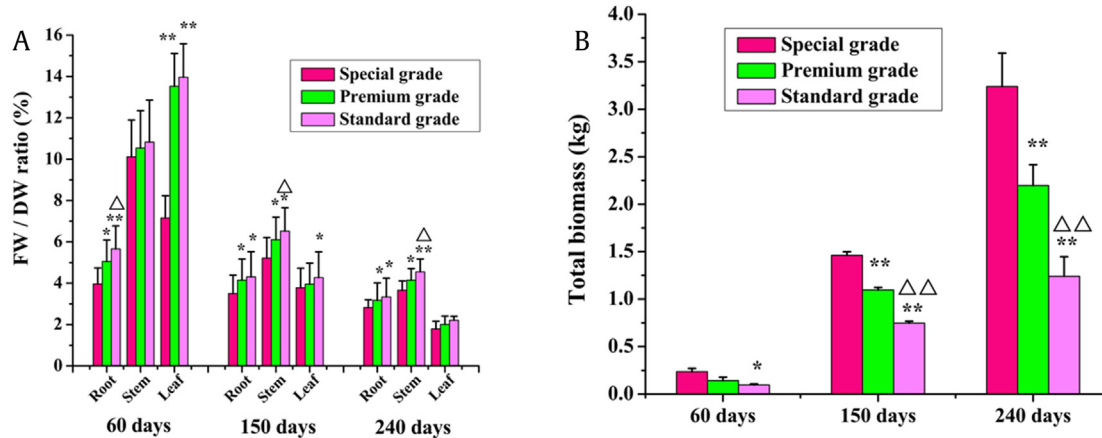


Fig. 4. Fresh weight/dry weight (FW/DW) ratios of *Panax notoginseng* seedlings of different grades at different growth stages. (A) Root, stem, and leaf. (B) Total biomass. Each graph shows the mean ± standard deviation of at least three independent experiments. **p* < 0.05 and ***p* < 0.01, compared with the premium grade; Δ *p* < 0.05 and ΔΔ *p* < 0.01, compared with the premium grade.

Table 8
Photosynthesis indices of *Panax notoginseng* seedlings in different grades and growth stages

Time (d)	Grade	Net photosynthetic rate [$\mu\text{mol}/(\text{m}^2 \text{ s})$]	Transpiration rate [$\text{mmol}/(\text{m}^2 \text{ s})$]	Stomatal conductance [$\text{mmol}/(\text{m}^2 \text{ s})$]	Intercellular CO_2 concentration ($\mu\text{L}/\text{L}$)	Water use efficiency in leaf
60	Special	3.265 ± 2.02	0.286 ± 0.35	13.06 ± 2.41	235.6 ± 9.33	11.42 ± 1.07
	Premium	2.243 ± 1.06	0.266 ± 0.32	12.98 ± 2.83	277.1 ± 10.8	8.43 ± 1.43
	Standard	1.996 ± 0.90	0.234 ± 0.29	12.78 ± 0.99	304.9 ± 13.19	8.52 ± 1.10
150	Special	5.941 ± 2.06	0.479 ± 0.39	19.74 ± 2.05	207.8 ± 8.12	12.41 ± 1.12
	Premium	3.892 ± 0.93	0.409 ± 0.18	19.32 ± 2.51	256.5 ± 12.8	9.52 ± 1.37
	Standard	2.269 ± 1.11	0.291 ± 0.11	18.98 ± 1.77	296.8 ± 13.86	7.79 ± 1.56
240	Special	6.911 ± 0.52	0.527 ± 0.06	19.71 ± 1.93	185.9 ± 11.08	13.11 ± 1.81
	Premium	4.227 ± 0.33	0.423 ± 0.13	18.82 ± 1.75	228.4 ± 15.90	9.99 ± 1.53
	Standard	2.655 ± 0.36	0.332 ± 0.03	18.57 ± 1.49	267.8 ± 18.56	8.01 ± 1.03

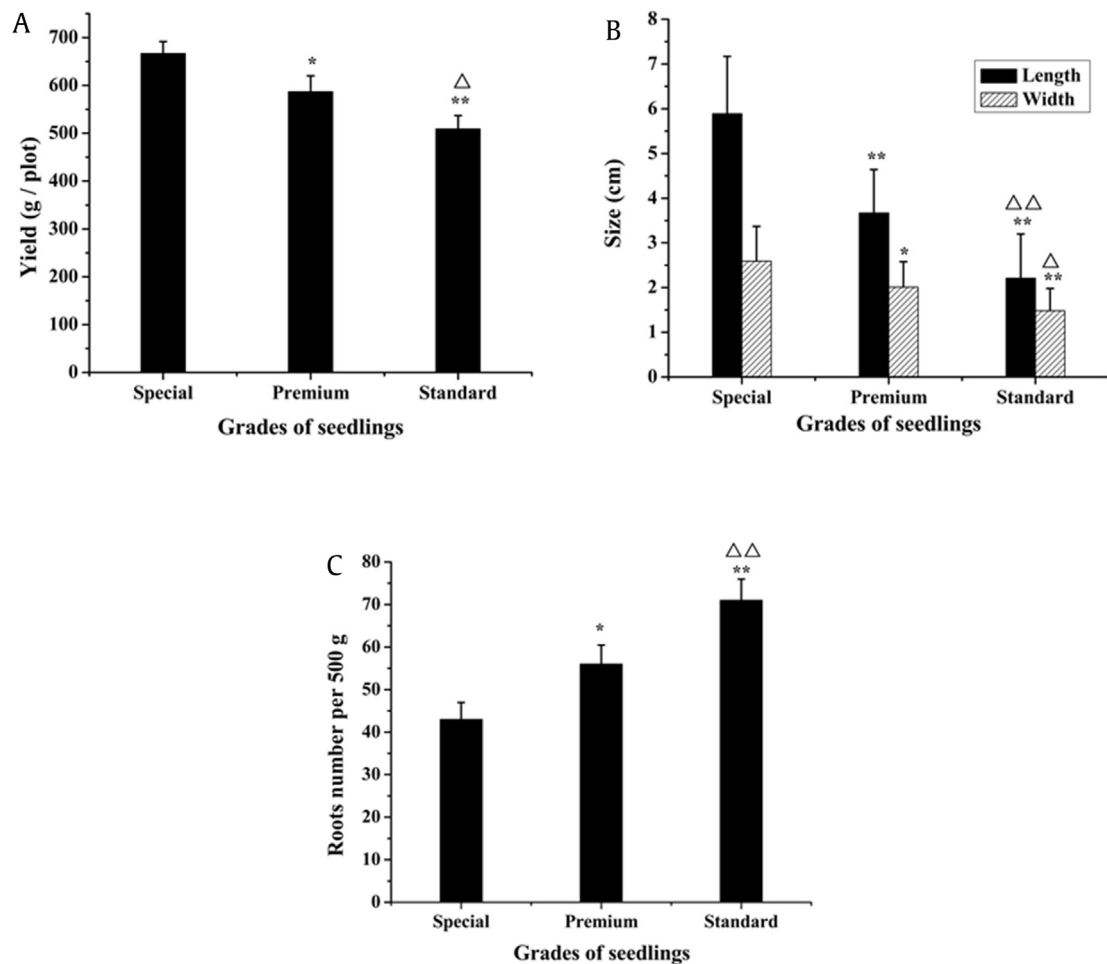


Fig. 5. The yields, size, and roots number per 500 g of Notoginseng Radix samples from *Panax notoginseng* seedlings of different grades collected from different plots in late April 2016. (A) Yields. (B) Size. (C) Number of roots per 500 g. Each graph shows the mean \pm standard deviation of at least three independent experiments. * $p < 0.05$ and ** $p < 0.01$, compared with the special grade; $\Delta p < 0.05$ and $\Delta\Delta p < 0.01$, compared with the premium grade.

had been used to successfully establish the grading standards for the seedlings of *Coptis chinensis* Franch., *D. nobile*, *S. miltiorrhiza*, etc. [17,19,20]. Therefore, we selected the K-means method to grade *P. notoginseng* seedlings in our study.

Although as many quality indices as possible would gain convincingness for the grading standard, practicability also needs to be considered in establishing process and further execution. During production, the fresh weight of root is usually an important index to reflect the seedling quality of herbal medicines [18]. In this

study, root length, root diameter, and bud diameter were positively correlated with the fresh weigh of the plant. Therefore, in order to facilitate the production application, fresh weight was determined as the representative index of other indices to simplify the quality standard. At the same time, the rootlet plays an essential role in many functions of the root such as the absorption of moisture, nutrients, and the survival rate during transplantation, and was therefore chosen as another quality index for *P. notoginseng* seedlings.

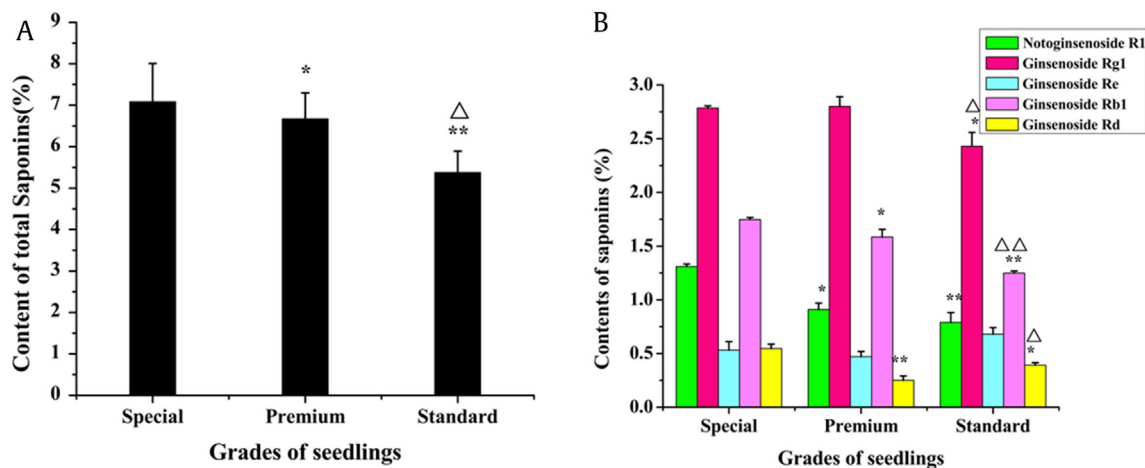


Fig. 6. Content of total saponins and contents of saponins of Notoginseng Radix samples from *P. notoginseng* seedlings of different grades planted for 2 years. (A) Total saponins. (B) Contents of saponins. Each graph shows the mean \pm standard deviation of at least three independent experiments. * $p < 0.05$ and ** $p < 0.01$, compared with the special grade; $\Delta p < 0.05$ and $\Delta\Delta p < 0.01$, compared with the premium grade.

4.3. Verification of grading standard of seedlings

The emergence rate and agronomic characters are important indices to verify the quality of seedlings, which indicate the plant potential to tolerate soil salinity under field conditions [23,24]. They represent the combined genetic and environmental effects on the growth of seedlings, of which the improvement is associated with better capabilities in the balance of nutrients, tolerance of salinity, accumulation of organic metabolites, etc. According to the result, the emergence rate and values of agronomic characters of special-grade seedlings were higher than those of premium-grade seedlings, and both had higher values than standard-grade seedlings. Therefore, the grading prior to planting could be beneficial for obtaining seedlings of superior quality.

Photosynthetic efficiency could be defined as the efficiency of converting light energy into chemical energy, which is an essential quality index because it incorporates the absorption cross section and the actual amount of light absorbed by plants [25,26]. Photosynthetic efficiency can be expressed in different ways: net photosynthetic rate (the total rate of photosynthetic CO_2 fixation minus the rate of loss of CO_2 during respiration), stomatal conductance (the rate of passage of CO_2 entering, or water vapor exiting through the stomata of a leaf), water use efficiency (the ratio of water used in plant metabolism to water lost by the plant through transpiration), and so on. In our study, the net photosynthetic rate, transpiration rate, stomatal conductance, water use efficiency in leaf, and intercellular CO_2 concentration were determined to represent the photosynthetic efficiency of seedlings. After transplanting different grades of *P. notoginseng* seedlings, there were significant differences in the photosynthesis indices of seedlings at different growth stages. It indicated that, along with the increase in grades of seedlings, values of photosynthesis indices were also increased, which were consistent with the agronomic characters.

Notoginseng Radix is the medicinal part of *P. notoginseng*, which soared to great importance during Qing dynasty time—the master herbalist Li Shizhen referred to it as Jinbuhuan (Not Even Exchange for Gold), a status it has maintained until today. Achieving high yields of medicinal parts is the vital purpose of establishing a standard of *P. notoginseng* seedlings. By measuring the yields and size of Notoginseng Radix, we found that in terms of the two indices, the following trend (in descending order) occurred: special-grade seedlings, premium-grade seedlings, and standard-

grade seedlings. Moreover, we also determined the roots number per 500 g, which is a traditional quality index to grade Notoginseng Radix in the market circulation: the lower the roots number per 500 g, the better is the quality of Notoginseng Radix [27]. Based on the results, along with the increase in seedling grades, roots number per 500 g was decreased, meaning that the quality of Notoginseng Radix was improved from the viewpoint of traditional evaluation.

The contents of five saponins were determined to verify the quality of Notoginseng Radix from different grades of seedlings. Among them, notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1 are index constituents for the qualitative and quantitative analysis required in the current quality standard. Recent studies reported that these saponins had multiple pharmacological activities including antioxidation, anti-inflammation, anticoagulation, anticancer, and cardioprotective effects, which could be associated with the efficacies of Notoginseng Radix such as treating blood stasis, bleeding, and some other blood disorders [28–30]. Although the contents of ginsenoside Rd and ginsenoside Re in Notoginseng Radix were irregular among different grades of seedlings, notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1, and the total of five saponins all showed increasing trends of contents along with the improvement in grades. It indicated that the grading of seedlings could be conducive to the production of medicinal parts of high quality. The grading standard of seedlings in the study was reasonable and feasible. Because notoginsenoside R1, ginsenoside Rb1, and total saponins showed significant differences ($p < 0.05$) in the contents among different grades of samples (Fig. 6), the three markers could be determined as indices to differentiate seedlings in the grading standard.

5. Conclusion

P. notoginseng seedlings were classified into the special grade, premium grade, standard grade, and culled samples by the K-means clustering method in the study. In order to verify the reasonability of the standard, the emergence rate, agronomic characters, and photosynthetic efficiency of seedlings after transplantation were investigated, as well as the yields and contents of saponins of medicinal parts from those seedlings. The results showed that the values of those indices were increased along with the improvement of the grades. The grading standard of

P. notoginseng seedlings could provide some references for the normalized planting and management of the medicinal herb.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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