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Variations in the morphological and chemical composition of the rhizomes of *Polygonatum* species based on a common garden experiment

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ABSTRACT

Polygonatum species have great potential in fighting chronic and hidden hunger. In this study, five *Polygonatum* species collected from different populations were cultivated in a common garden for 4 years. The species mainly differed in yield, saponin and polysaccharide contents, stem diameter, leaf width, inflorescence length, and floret inflorescence length. *P. cyrtonema* (PC) provides high-quality yield when planted in Zhejiang, with output as high as 7.5 tons per hectare and a promising breeding potential. Moreover, stem diameter can be used as an indicator of the harvest in the screening of varieties. In addition, the formation of plant genetic traits from different provenances is affected by the climatic factors of the origin. Furthermore, near-infrared spectroscopy combined with chemometrics for polysaccharide and saponin quantitation provides a rapid assessment of PC quality. Our findings provide a scientific basis for the development and sustainable utilization of PC as a high-yielding and high-quality forest crop.

Introduction

With increasing public health awareness, "medicine and food homology" and "food is medicine" have emerged as major international health trends (Downer et al., 2020). Epidemiological evidence shows that a phytochemical-rich diet helps prevent chronic diseases (Fahey & Kensler, 2021). Plants from the genus *Polygonatum* Mill. (Asparagaceae), commonly known as Solomon's Seal, have been used as food and medicine in Asia for over 2,000 years and as food during a famine in ancient China (Chen et al., 2021; Si & Zhu, 2020; Xia et al., 2021).

The *Polygonatum* genus comprises over 60 species worldwide (http://www.worldfloraonline.org, accessed 9 January 2023), with several species valued as significant medicinal resources (Chen et al., 2021). According to Chinese Pharmacopoeia (2020 version), the traditional Chinese herbs Huangjing and Yuzhu are derived from the dried rhizomes of the *Polygonatum* genus. Huangjing (Latin name for the medicine is Polygonati Rhizoma) is obtained from the rhizomes of *P. sibiricum* Red. (PS), *P. kingianum* Coll. et Hemsl. (PK), or *P. cyrtonema* Hua (PC) according to Chinese Pharmacopoeia and *P. filipes* Merr. (PF) according to the Processing Norms of Zhejiang province (Shi et al., 2023; Zhang et al., 2020). Yuzhu (Latin name: Polygonati Odorati Rhizoma), obtained from *P. odoratum* (Mill.) Druce (PO), is another legal medicinal material from the *Polygonatum* genus (Zhao et al., 2018). The five *Polygonatum* species mentioned above exhibit extensive morphological diversity, including alternative or whorled phyllotaxis, axillary inflorescences with multiple flowers or fruits, foliar epidermal traits (Ali et al., 2020), and pollen micro-morphological features (Ali et al., 2021). Furthermore, molecular phylogenetic analyses of the whole-plastome data support their identification (Floden & Schilling, 2018; Xia et al., 2021). However, these characteristics are seasonal, rendering it difficult

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for taxonomists to identify plants in the wild. Therefore, growing individuals from different populations in a common environment provide a suitable method for long-term observation (de Villemereuil et al., 2016).

Huangjing and Yuzhu have been used in traditional Chinese medicine to invigorate *qi* (life energy) and nourish *yin* (one of the qualities of *qi*) (Shi et al., 2023). Huangjing invigorates the spleen, moistens the lung, and benefits the kidneys, and Yuzhu relieves dryness, promotes fluid production, quenches thirst, and increases vitality (Li et al., 2021; Zhou et al., 2017). The government acknowledged the effectiveness of Huangjing and Yuzhu when the former Ministry of Health of China included them in the list of 86 items used as both food and medicine in 2002 (Si & Zhu, 2020). This recognition indicates that only the base species (PS, PC, PK, and PO) compliant with the Chinese Pharmacopoeia may be used as food in the production, processing, and sale of goods in China. Moreover, Huangjing has been included in the Chinese Academy of Engineering's Strategic Research and Consultation Project for 2022 (NO.2022XY140) as a forest crop grown in the forests without using farmland. Huangjing has enormous potential for use as food in combating chronic and hidden hunger (Chen et al., 2021).

Huangjing and Yuzhu contain diverse metabolites; polysaccharides and saponins are the main active components in the *Polygonatum* genus, which display anti-diabetic, anti-inflammatory, anti-tumor, and immune enhancement activities (Chai et al., 2021; Yelithao et al., 2019). The extensive research on the active ingredients of *Polygonatum* has led to a rapid increase in its utilization and market demand, rendering it difficult to rely solely on wild resources (Su et al., 2018). Artificial cultivation is a feasible solution to Huangjing's resource scarcity (Wang & Duan, 2018). However, germplasm confusion, lack of good varieties, the indiscriminate introduction of seedlings across regions, and insufficient utilization of medicinal sources in *Polygonatum* cultivation impede the development of this industry (Jiao, 2018; Li et al., 2021; Su et al., 2018).

Twenty endemic *Polygonatum* species are widely distributed in China owing to their high adaptability (Ali et al., 2020; Ali et al., 2021). *Polygonatum* is cultivated on over 600 ha of land in Zhejiang. *Polygonatum* production can be increased through cultivar improvement and efficient cultivation, which will alleviate poverty in underdeveloped mountainous regions (Si & Zhu, 2020). Germplasm resources are the basis for producing high-yielding and high-quality cultivars, as germplasm may include critical alleles for key traits (Akpertey et al., 2022; Han et al., 2022). Variations in germplasm resources can cause differences in Huangjing's biological, genetic, reproductive, sporulation, and pharmacologic characteristics, as well as chemical composition (Jiao, 2018).

Genetic variations due to long-term exposure to the growing environment affect plant morphology and metabolite accumulation (Liu & Zhang, 2007). Short-term changes in the growth environment cannot alter long-term genetic variations. Individuals of different populations have been introduced and planted in a common garden experiment to maintain their habitat consistency and eliminate environmental factors in phenotypic variations to investigate the effects of genetic factors on phenotypic variations separately (Ballentine & Greenberg, 2010). The performance of >70 % of metabolites in *Oryza sativa* varies significantly due to environmental factors (Chen et al., 2016). Therefore, a comprehensive evaluation of the germplasm resources of Huangjing and Yuzhu must be conducted. The assessment of *Polygonatum* plant germplasm resources provides a scientific basis for its sustainable utilization in the future. Combined with high-throughput phenotyping and hyperspectral data, common garden experiments help to understand how natural selection impacts the variations in plant species and guides plant breeding (VanWallendael et al., 2022).

Recently, near-infrared spectroscopy (NIRS) coupled with multivariate statistical analysis has been widely used to trace the origins of foods and herbs (Puleo et al., 2022; Zheng et al., 2022) and determine starch, fat, protein, and saponin contents in crops, such as rice and soybeans, for quality control (Bagchi et al., 2016; Berhow et al., 2020; Shi et al., 2022). Owing to its rapid and non-destructive sampling, simple operation, good stability, and high efficiency, NIRS has advanced in qualitative identification and quantitative analysis in the field of food and medicine, thus showing the broad application prospects of NIRS (Nagy et al., 2022; Nobari Moghaddam et al., 2022).

In this study, we screened high-yielding and good-quality species and provenance for cultivation and variety breeding in Zhejiang and surrounding areas. Further, we investigated the use of NIRS for rapid quality screening of medicinal compounds from the *Polygonatum* genus. Consequently, our findings may provide helpful information for future production and quality control practices related to the homology of food and medicinal herbs from the *Polygonatum* genus.

Materials and methods

Plant collection and identification

To investigate and evaluate the plant resources from the *Polygonatum* genus, 75 samples of five species, including PS, PK, PC, PF, and PO, were collected from 14 provinces and municipalities (Shaanxi, Henan, Shanxi, Jilin, Guizhou, Yunnan, Zhejiang, Anhui, Jiangxi, Fujian, Hubei, Hunan, Beijing, and Chongqing). At least 15 individual plants were collected from each population, locating their latitude, longitude, and elevation by GPS (Fig. 1A, C–G, Table S1). Nineteen bioclimatic layers (Bio1: Annual Mean Temperature, Bio2: Mean Diurnal Range, Bio3: Isothermality, Bio4: Temperature Seasonality, Bio5: Max Temperature of Warmest Month, Bio6: Min Temperature of Coldest Month, Bio7: Temperature Annual Range, Bio8: Mean Temperature of Wettest Quarter, Bio9: Mean Temperature of Driest Quarter, Bio10: Mean Temperature of Warmest Quarter, Bio11: Mean Temperature of Coldest Quarter, Bio12: Annual Precipitation, Bio13: Precipitation of Wettest Month, Bio14: Precipitation of Driest Month, Bio15: Precipitation Seasonality, Bio16: Precipitation of Wettest Quarter, Bio17: Precipitation of Driest Quarter, Bio18: Precipitation of Warmest Quarter, Bio19: Precipitation of Coldest Quarter) were obtained from WorldClim for the period 1970–2000 (https://www.worldclim.org/) (Hu et al., 2017) (Tables S2 and S3).

All samples were identified by Prof. Jinping Si of Zhejiang Agriculture and Forestry University (ZAFU) and Pan Li of Zhejiang University. The voucher specimens (No. ZJFC00021101–05) were preserved at the College of Forestry and Biotechnology, ZAFU, Zhejiang Province, China.

Sample acquisition and preparation

Plants were cultivated in the germplasm resource garden (common garden) in Lin'an, Zhejiang (119°26′'11″ E, 30°20′30″ N) (Liu et al., 2022) (Fig. 1B). Each plant was seeded with the first two nodes of the rhizome with buds. After measuring the aboveground parts, 4-year-old rhizomes were harvested in October 2020 (Fig. 1H–L). After sampling, the rhizomes were dried to constant weight, crushed, and passed through a 60-mesh sieve, and the resulting sample powder was stored in a desiccator.

Chemical and reagents

D-glucose of analytical grade and sarsasapogenin were purchased from Shanghai Yuanye Biotechnology Co. (Shanghai, China). Other reagents used were from Sinopharm Chemical Reagent Co. (Shanghai, China) and were analytically pure. Distilled water was filtered using a Milli-Q system (Millipore, Bedford, MA, USA). A SpectraMax 190 light absorption microplate reader (Molecular Devices, San Jose, CA, USA) and an Antaris II Fourier transform near infrared spectrometer (Thermo Scientific Inc., Madison, WI, USA) were used for the analyses.

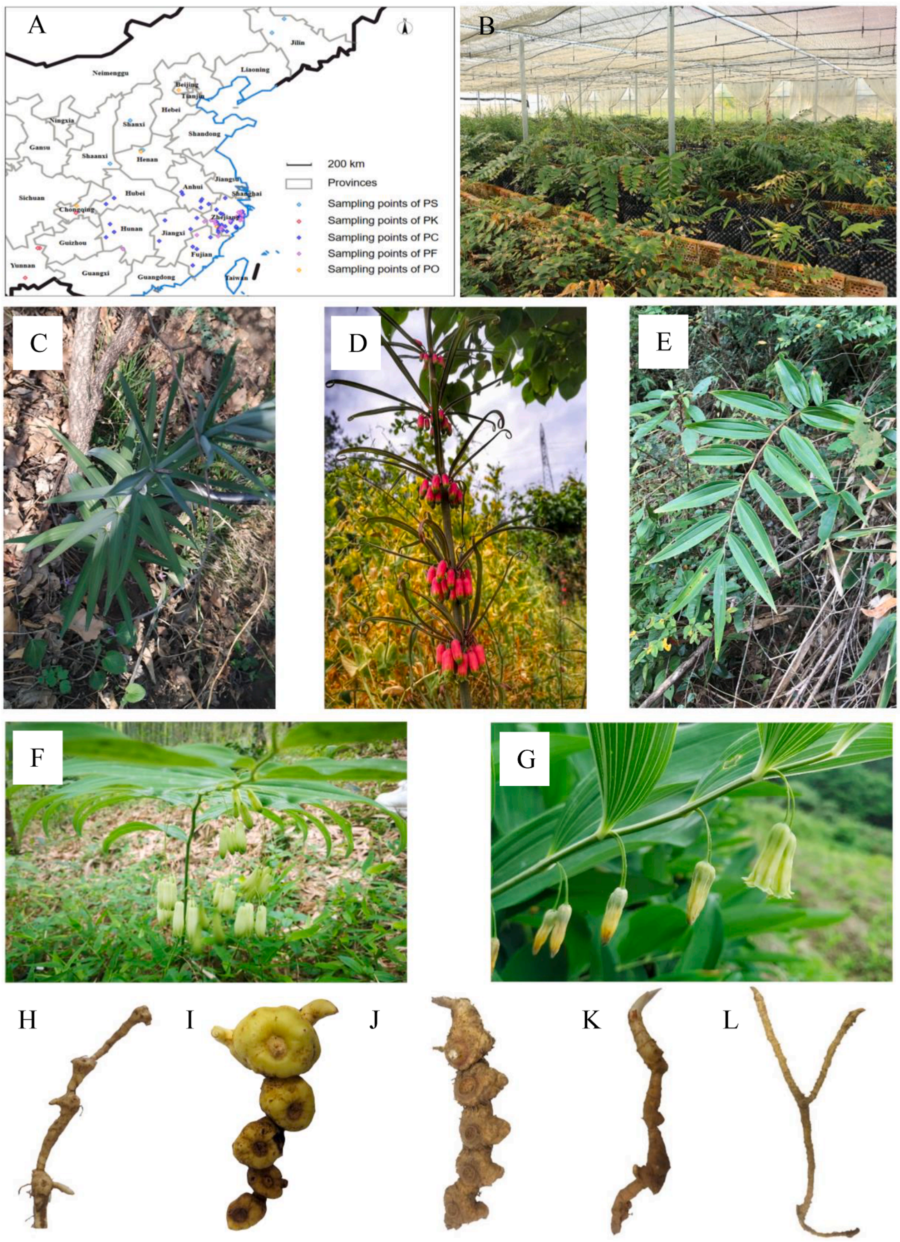

Fig. 1. The sampling distribution and morphology of five species from *Polygonatum* genus. (A) Sampling distribution of 5 different species from *Polygonatum* genus populations in map of China (partial); (B) the common garden in Zhejiang, China; (C) the aerial part of *Polygonatum sibircum* Red. (PS); (D) the aerial part of *P. kingianum* Coll. et Hemsl. (PK); (E) the aerial part of *P. cyrtonema* Hua (PC); (F) the aerial part of *P. filipes* Merr. (PF); (G) the aerial part of *P. odoratum* (Mill.) Druce (PO); (H) the rhizome morphology of PS; (I) the rhizome morphology of PK; (J) the rhizome morphology of PC; (K) the rhizome morphology of PF; (L) the rhizome morphology of PO. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Determination of agronomic qualities

Before crushing and testing the rhizome, the agronomic properties of the aerial parts, including the length and width of the rhizome's initial node and the fresh weight of the rhizome, were measured as these parameters are a direct representation of plant growth (Peng, 2018).

The samples (first two nodes with buds) were weighed before planting. After 4 years of cultivation, the stem length (X1) was measured with a straightedge, and the stem diameter (X2) was measured with a vernier caliper at near harvest. Three leaves were selected from each plant, and their length (X3) and width (X4) were measured with a straightedge. Three inflorescences were selected from each plant, and inflorescence length (X5) and floret inflorescence length (X7) were measured with a straightedge, and inflorescence diameter (X6) was measured with a vernier caliper. The fruit number of each infructescence (X8) was counted. After harvesting, the length (X9) and width (X10) of the rhizome's first node were measured with a straightedge. The rhizomes were weighed. For convenience, yield (X11) is expressed by the fresh weight of the rhizome, which was calculated using the following equation. The average of each indicator is calculated.

$$Freshweight = weightatharvest - weightatplanting$$

Determination of polysaccharide content

The polysaccharide content (X12) of Huangjing and Yuzhu was determined by anthrone-sulfuric acid colorimetry using the method of the Chinese Pharmacopoeia and previous work (Su et al., 2019). Each batch of sample solution was repeated thrice and calculated according to the anhydrous glucose standard curve.

Determination of saponin content

The saponin content (X13) was determined using vanillin-CH_3COOH-$HClO_4$ as the chromogenic reagent, as described in a previous study. Each batch of sample solution was repeated thrice and calculated according to the sarsasapogenin standard curve (Ye et al., 2017; Zhang et al., 2020).

Collection of NIR spectra

Due to the small sample size of Yuzhu, Huangjing was chosen to establish the NIR quantitative model. To acquire spectra, equal amounts of dried sample powder were collected using an integrating sphere diffuse reflectance sampling system with an Antaris II FT-NIR spectrometer (Thermo Fisher Scientific, Madison, WI, USA) and TQ Analyst spectral analysis software. To obtain reproducible results, all NIR spectra were recorded 64 times with background air as a blank standard. The spectra ranged from 10,000 cm^{-1} to 4,000 cm^{-1} with a spectral resolution of 8 cm^{-1}, and each sample was analyzed thrice at room temperature (about 25℃) to average the spectra for subsequent operations (Yu et al., 2022) (Fig. 4).

Spectral pretreatment

Unnecessary information in the spectra can introduce impurity variables and reduce model robustness; therefore, the spectra are usually preprocessed to reduce the impact caused by these variables (Wu et al., 2022). Commonly used spectral preprocessing methods, including Savitzky–Golay (SG) filtering, standard normal variate (SNV), multiplicative signal correction (MSC), and Savitzky–Golay first derivation (FD) and second derivation (SD), is used to remove or reduce unwanted variations in the NIR spectra. The effectiveness of these preprocessing methods is compared by building regression models of the original and preprocessed spectra in a full spectral range.

Outlier identification and design of sample sets

Outliers may lead to incorrect results in multivariate analysis and significantly reduce the quality of the model developed. Seven spectral outliers were rejected using principal component analysis (PCA) and martingale distance (Xiao et al., 2014; Zhu et al., 2021). Of the 64 spectral samples from Huangjing, 54 were randomly selected as the calibration set and the remaining 10 as the prediction set to generate the regression model.

Partial least squares (PLS) regression

PLS regression was used for model development (Shi et al., 2022; Xiao et al., 2014). The optimal number of factors for the PLS model was selected based on the variations in the residual variance. The model robustness was evaluated in terms of R^2 of calibration (Rcal) and prediction (Rp), root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP), and residual prediction deviation (RPD).

Statistical analysis

Statistical and factor analyses were performed using SPSS 22 (SPSS Inc., Chicago, IL, USA) software. Data are expressed as the mean ± standard deviation (SD) and statistically compared using F-test or one-way ANOVA (two-tailed analysis). $p < 0.05$ was considered a significant difference, and $p < 0.01$ was considered a highly significant difference. Orthogonal partial least squares-discriminant analysis (OPLS-DA) was performed using SIMCA-P software (version 14.1, Umetrics, Umea, Sweden). The data were plotted and analyzed using GraphPad Prism 8 and Origin 2021b. Raw NIR spectra and model building were performed using TQ Analyst spectral analysis software (Thermo Fisher Scientific). The spectra were preprocessed using Unscrambler X 10.4 chemometrics software (Camo Process, Oslo, Norway).

Results and discussion

Analysis of agronomic and quality traits

Discriminant analysis of Polygonatum species using the OPLS-DA model

The agronomic and quality traits (including X1–X13) of different *Polygonatum* species differed significantly (Table S4). OPLS-DA is often used as an effective method for sample classification (Kang et al., 2022). To study the agronomic and quality traits, 75 samples of five *Polygonatum* species were studied using 13 agronomic and compositional indicators as variables in a supervised OPLS-DA mode, and the corresponding models were obtained (Fig. 2A). The cumulative explanatory power parameters, R2X and R2Y, were 0.761 and 0.617, respectively, both being > 0.5, indicating that the model was stable and reliable with a strong predictive ability and could be used to distinguish different *Polygonatum* species. The OPLS-DA score plot revealed evident differences among the five *Polygonatum* species, with PC mainly concentrated on the left side of the x-axis and PF on the right side of the x-axis and lower side of the y-axis. The agronomic and quality traits of PC and PF were generally different from those of other *Polygonatum* species.

Variable importance in the projection (VIP) is primarily used to explain the contribution of the variables to the model (Du et al., 2021; Kang et al., 2022). To screen the major indicators observed in the five *Polygonatum* species, the VIP was calculated in the OPLS-DA model, and 13 traits were ranked based on their VIP values (Fig. 2B). The component's contribution to the resulting differences increases with increasing VIP value. The VIP values of seven indicators, including yield, saponin content, polysaccharide content, leaf width, stem diameter, inflorescence length, and floret inflorescence length, were > 1.0, indicating the differences across different *Polygonatum* species.

Yield of Polygonatum species

The yield of Huangjing and Yuzhu cultivated in a common garden was not statistically different according to the comparison between the variability of the seven major indicators (Fig. 3A). This may be due to the large intraspecific variations. PC had the highest yielding provenances among the five species, 1.89 times the mean value of the remaining three Huangjing species. The yields of PC and PK were significantly higher than those of PS and PF; however, there was no significant difference between PC and PK and PS and PF. Huangjing is widely distributed in China. PS is mainly distributed in northern China, i.e., Heilongjiang, Jilin, Inner Mongolia, Shanxi, and Hebei. PK is mainly produced in Yunnan and Guizhou; however, its production has reduced after being introduced in Zhejiang due to environmental discomfort (Yang et al., 2022). Although PF originated from Zhejiang and its surrounding areas, owing to the plant characteristics, its rhizomes are thin and long, and its yield is not high (Zhao et al., 2018). PF has been used in some folk health products but is not worth further promoting.

Considering the four provenances with a fresh yield of >500 g/plant, numbered PC9 (Jingning, Zhejiang), PC63 (Pan'an, Zhejiang), PC26 (Xiushui, Jiangxi), and PC42 (Taining, Fujian), it is reasonable that the theoretical yield may reach 7.5 tons when 15,000 plants are cultivated per hectare in the forest. This result is consistent with the previous report that Huangjing can be cultivated in mountainous forest land without occupying farmland, which has great potential as food owing to its high yield and rich nutrients and functional substances (Si & Zhu, 2020).

Saponin and polysaccharide contents of Polygonatum species

There were significant differences in the saponin content among five *Polygonatum* species (Fig. 3B). The saponin content of PK was 10.54 %, significantly higher than PC, PS, and PF, and that of PC was 7.83 %, significantly higher than PS and PF. However, there was no significant difference between PS and PF. The polysaccharide content ranged from 25.30 % to 6.24 % in the *Polygonatum* species (Fig. 3C). The differences

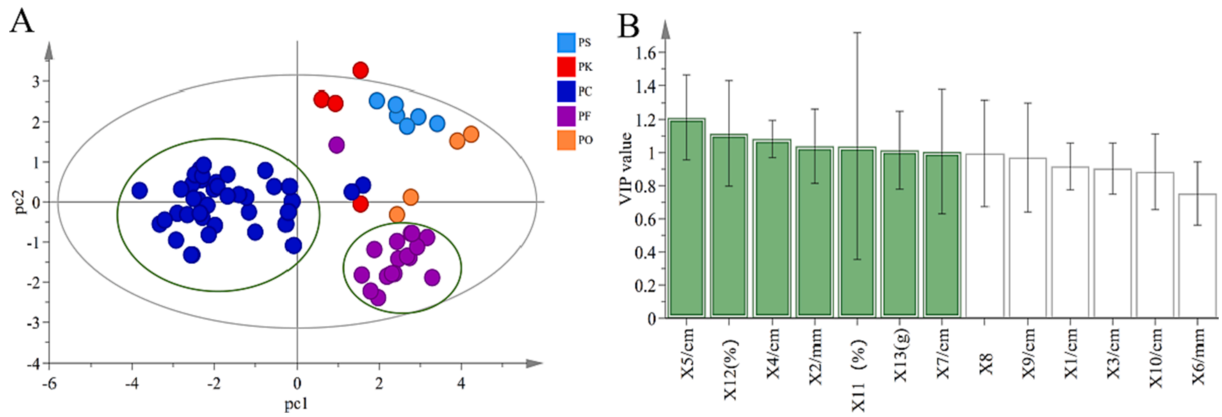

Fig. 2. The plants of 5 different species from *Polygonatum* genus of Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA). (A) Score plot. (B) Variable importance in the projection (VIP). Diagram: Green means the VIP value > 1. X1: Stem length/cm, X2: Stem diameter/mm, X3: Leaf length/cm, X4: Leaf width/cm, X5: Inflorescence length/cm, X6: Inflorescence diameter/mm, X7: Floret inflorescence length/cm, X8: Fruit number of each infructescence, X9: The length of rhizome first node/cm, X10: The width of rhizome first node/cm, X11: Yield per plant/g, X12: Polysaccharide content/%, X13: Saponin content/%. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in polysaccharide content were higher for Yuzhu than for Huangjing. The polysaccharide content of PF was significantly higher than that of PS and PK, and there was no significant difference between PS, PC, and PK.

The fluctuation range of saponin was 7.74 times, and that of polysaccharide was 4.05 times. The change of saponin is larger than that of polysaccharide, indicating that the polysaccharides accumulation is less affected by heredity than saponins. In the common garden experiment, the intraspecific differences in polysaccharide content were greater than the interspecific differences. A common garden experiment with *Isatis indigotica* Fort. demonstrated that the polysaccharide content of different provenances was relatively stable, and that of the same provenance in different growing areas was significantly different (Han et al., 2022). This indicates that polysaccharide accumulation as energy storage materials may be strongly related to ecological factors such as the cultivation environment. Saponin accumulation is also correlated with environmental factors; a change in elevation affects the total saponin accumulation in *Panax ginseng* (Ma et al., 2021).

Other agronomic traits of Polygonatum species

There was no significant difference in the leaf widths of Huangjing and Yuzhu. PC leaf width was significantly higher than PS and PK. PF leaf width was significantly higher than PS and PK, and there was no difference between PS and PK. The difference in stem diameter was significantly higher in Huangjing than in Yuzhu; it was significantly higher in PC than in PS, PK, and PF, while there was no significant difference among the three species. The stem diameter of PC was 1.55 times the mean value of the four species. Further, there was no significant difference in inflorescence lengths of Huangjing and Yuzhu. The inflorescence length of PF was significantly higher than that of PS, PK, and PC, and PC was significantly higher than that of PS, with no significant differences between PC and PK and PS and PK. There was no significant difference in floret inflorescence lengths of Huangjing and Yuzhu. The floret inflorescence length of PC was significantly higher than that of PS and PK, with no significant difference with PF. PF was significantly higher than PK and not significantly different from PS; there was no significant difference between PS and PK. The floret inflorescence length of PC was 1.45 times the mean value of the four species. Although there are evident agronomic trait variations among species, the variation range of aboveground PC and PF parameters after artificial cultivation is vast and easily misinterpreted. These differences should be considered during their production.

Thus, the common garden experiment results indicate that PC is more suitable for cultivation in Zhejiang and similar surrounding areas.

Diversity analysis of different provenances of P. cyrtonema

The agronomic and quality traits of 43 different provenances of PC were investigated to identify a suitable provenance for introducing PC for cultivation in Zhejiang (Table S4). To obtain stable and high yields in practical production and application, high-quality varieties with high-quality traits should be selected. The coefficient of variation is a measure of the variation range and degree of characters (Xu et al., 2021). The coefficients of variation of the agronomic and quality traits of *Polygonatum* species were, in descending order, total inflorescence diameter (42.93 %), fruit number of each infructescence (37.73 %), yield (34.62 %), leaf length (29.02 %), inflorescence length (28.42 %), polysaccharide content (27.55 %), stem length (25.04 %), saponin content (24.63 %), the width of rhizome first node (24.45 %), the length of rhizome first node (23.55 %), floret inflorescence length (21.33 %), stem diameter (18.30 %), and leaf width (14.57 %) (Table 1). The large variations in line with the high genetic diversity which related to wide geographical distribution of PC in China (Liu et al., 2020).

The yield of PC ranged from 104.83 g to 647.54 g fresh weight per plant, and polysaccharide and saponin contents ranged from 6.24 % to 20.31 % and 3.26 % to 11.82 %, respectively. These indicators were greatly influenced by provenance differences and showed great potential for PC variety breeding.

Analysis of different geographical provinces of P. cyrtonema

Forty-three different PC provenances from six geographical provinces were selected, and their agronomic and quality traits were compared (Fig. 1 and Table S5). The stem lengths of PC from the different germplasms ranged from 11.00 to 177.67 cm, with those from Hunan Province being the tallest (average length 158.47 cm), Hubei Province being the shortest (average length 103.35 cm), and Zhejiang, Anhui, Jiangxi, and Fujian Provinces having no significant differences. The stem diameter of different germplasms ranged from 6.47 to 14.17 cm, with that from Hunan Province being significantly higher than those from Anhui and Hubei. There was no significant difference in the stem diameters of the genotypes from the Hunan and Hubei Provinces and those from Zhejiang, Jiangxi, and Fujian. The leaf width of PC in different geographical provinces ranged from 4.05 to 8.80 cm, with that of plants from Hubei Province being significantly lower than those from Zhejiang, Anhui, Jiangxi, and Fujian Provinces. There was no difference between the leaf width of plants from Hubei and Hunan Provinces and those from Hunan Province and Zhejiang, Anhui, Jiangxi, and Fujian Provinces.

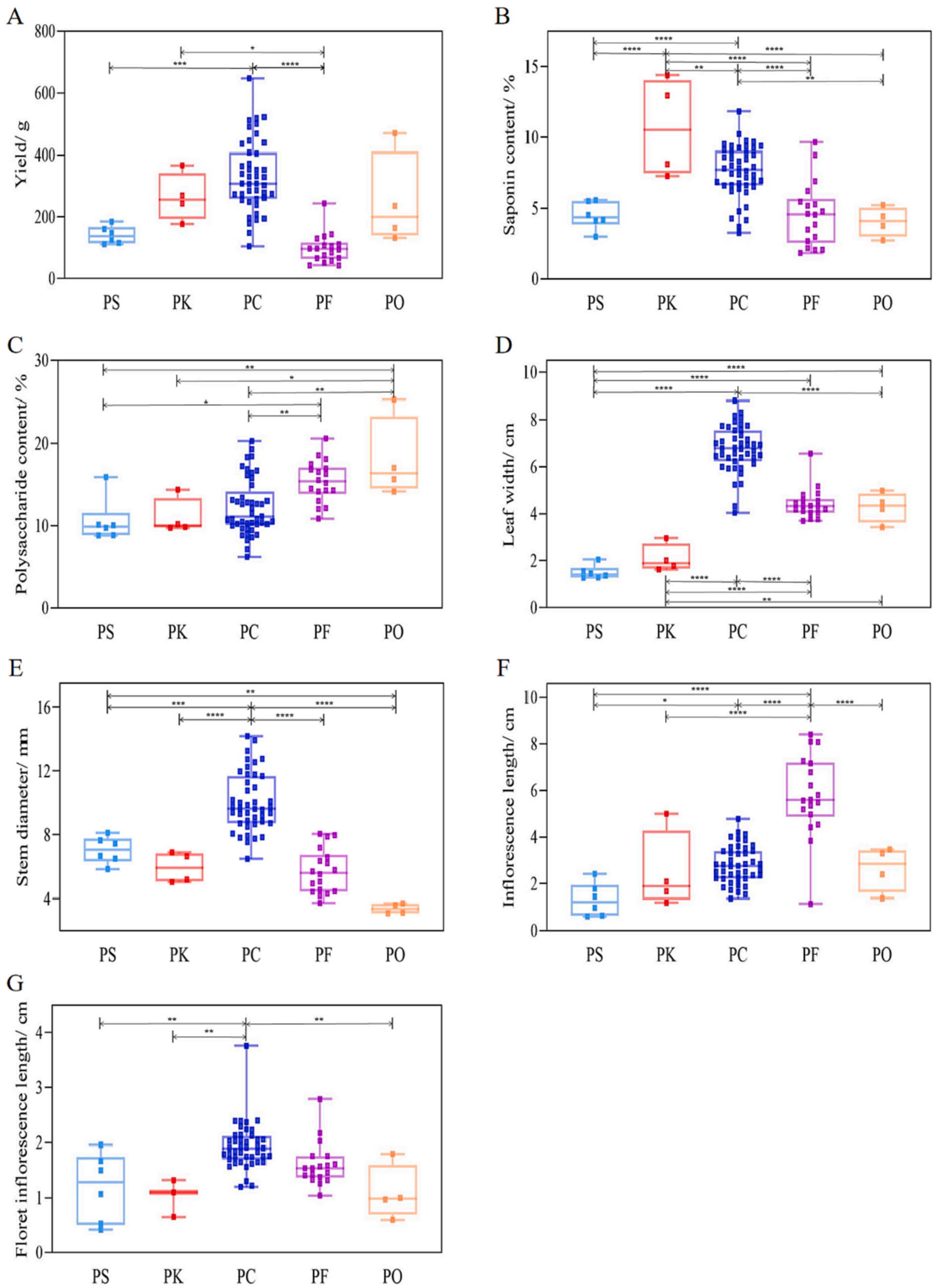

Fig. 3. Comparison of the variability of traits among 5 different species of *Polygonatum* genus. (A) Yield per plant, (B) Saponin content, (C) Polysaccharide content, (D) Leaf width, (E) Stem diameter, (F) Inflorescence length, (G) Floret inflorescence length (*: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$; ****: $P \leq 0.0001$).

The average fruit number of each infructescence in different geographical provinces of PC ranged from 3.5 to 13.2, with that from Hunan Province being significantly higher than those from the remaining five provinces. There was no difference in the provenance in the remaining five provinces. The length of the rhizome first node of PC in different geographical provinces ranged from 1.79 to 7.24 cm, with those from Jiangxi Province being significantly higher than that from Hunan Province, and the plants from both provinces were not significantly different from those from the remaining four geographical provinces. The width of the rhizome first node of PC from different geographical provinces ranged from 1.68 to 5.36 cm, with those from Hunan Province being significantly higher than those from Anhui, Jiangxi, and Hubei Provinces. There was no significant difference between the plants from these provinces and those from Hunan, Zhejiang, and Fujian Provinces. The width of the rhizome first node of the plants from the Hubei Province was significantly lower than that of the Hunan, Zhejiang, and Jiangxi Provinces seeds.

The yield of PC in different geographical provinces ranged from 104.83 to 647.54 g. Although the yield difference among the four provinces was not statistically significant, the average yield from Zhejiang, Anhui, Jiangxi, and Fujian Provinces was relatively high. The polysaccharide content of PC in different geographical provinces ranged from 6.24 % to 20.31 %, with that from the plants in Hunan Province being significantly higher than that in Zhejiang, Anhui, Fujian, and Hubei, and there was no difference among the polysaccharide content from the plants in the four geographical provinces. Moreover, the leaf length of PC ranged from 9.3 to 47.37 cm, the inflorescence length ranged from 1.37 to 4.78 cm, the inflorescence diameter ranged from 0.53 to 4.29 cm, floret inflorescence length ranged from 1.20 to 3.76 cm, and saponin content ranged from 3.26 to 11.82 % among different

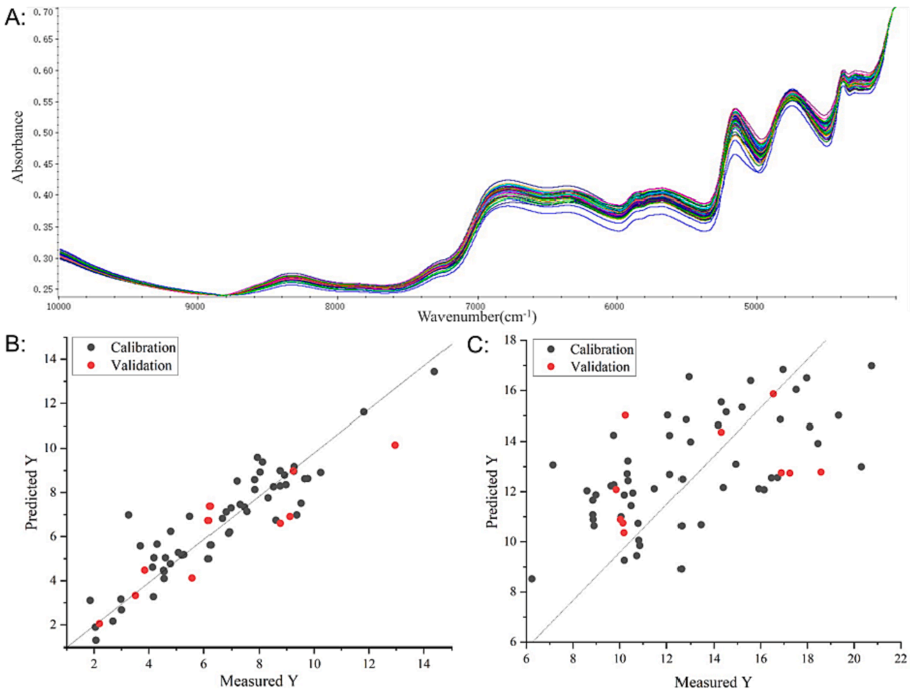

Fig. 4. The spectra and quantitative calibration model of Huangjing by Near-infrared (NIR) Spectroscopy. (A) All NIR spectra of samples of Huangjing; (B) The saponin predicted values versus measured values of samples of Huangjing by NIR; (C) The polysaccharide predicted values versus measured values of samples of Huangjing by NIR.

Table 1
Analysis of coefficient of variation of agronomic and quality traits of 43 different provenance of *P. cyrtonema*.

Traits	Min	Max	Average	SD	CV
Stem length/cm (X1)	11.00	177.67	124.92	31.28	25.04 %
Stem diameter/mm (X2)	6.47	14.17	10.03	1.84	18.30 %
Leaf length/cm (X3)	9.30	47.37	20.99	6.09	29.02 %
Leaf width/cm (X4)	4.05	8.80	6.76	0.98	14.57 %
Inflorescence length/cm (X5)	1.37	4.78	2.82	0.80	28.42 %
Inflorescence diameter/mm (X6)	0.00	4.29	1.53	0.66	42.93 %
Floret inflorescence length/cm (X7)	1.20	3.76	1.93	0.41	21.33 %
Fruit number of each infructescence (X8)	3.50	13.20	5.93	2.24	37.73 %
Length of rhizome first node/cm (X9)	1.79	7.24	4.72	1.11	23.55 %
Width of rhizome first node/cm (X10)	1.68	5.36	3.36	0.82	24.45 %
Yield per plant/g (X11)	104.83	647.54	332.71	115.20	34.62 %
Polysaccharide content/% (X12)	6.24	20.31	12.35	3.40	27.55 %
Saponin content/% (X13)	3.26	11.82	7.54	1.86	24.63 %

(Note: Min, minimum; Max, maximum; SD, standard deviation; CV, coefficient of variation).

provenances. None of the five indicators were significantly different among different geographical provinces. This result indicates that leaf length, inflorescence length, inflorescence diameter, floret inflorescence length, and saponin content are less influenced by environmental factors in different geographical provinces and more likely to be determined by themselves. The performance of traits of PC in six geographical provinces analyzed using the systematic clustering method (Figure S1) showed that the PC from six geographical provinces could be classified into three taxa. Zhejiang, Fujian, and Anhui provenances have good agronomic and quality traits with high similarity. Jiangxi and Hunan provenances are similar, and Hubei provenances are a separate class. The genetic variations in PC were closely related to mountains (Liu et al., 2020; Xia et al., 2022). The Tianmu Mountains separate Anhui and Zhejiang, whereas the Donggong Mountains separate Zhejiang and Fujian, explaining the similarity between the provenances of these three provinces.

Comprehensive analysis of different provenances of P. cyrtonema

Factor analysis is currently the primary technique for product quality and authenticity evaluation (Kuang et al., 2022). The factor analysis of 13 agronomic and quality traits of 43 PC provenances shows that the cumulative contribution of the first five principal components was 69.52 % (Table S6), indicating that these principal component factors contained information on agronomic and quality traits of PC. Hence, the first five principal components could be used for their comprehensive evaluation. Because the factor loadings were not sufficiently clear, the maximum variance method was used to rotate the common factors, and the rotated factor loading matrix was obtained (Table S7). The contribution of the first principal factor was 17.43 %, with an eigenvalue of 2.27, which was mainly determined by two agronomic traits, the fruit number of each infructescence and stem length, with positive loadings. The contribution of the second principal factor was 14.63 %, with an eigenvalue of 1.90, which was mainly determined by the two rhizome first node traits, the length, and width of the rhizome first node, with positive loadings. The third main factor contributed 13.43 % with an eigenvalue of 1.75, which was mainly determined by the two yield factors, yield and stem diameter, with positive loadings. The fourth main factor contributed 12.32 % with an eigenvalue of 1.60, which was mainly determined by two appearance factors, leaf length and stem length, with positive loadings. The fifth principal factor contributed 11.71 % with an eigenvalue of 1.52 and was mainly determined by two composition factors, saponin content and small inflorescence length, with positive loading values.

The comprehensive analysis of PC from different provenances using five main factors was used to establish a comprehensive evaluation data model. The model was used to calculate the integrated scores of agronomic and quality traits of different PC provenances, and the results were ranked by the integrated scores for excellence (Table S8).

Cluster analysis of different provenances of P. cyrtonema

The combined scores of the 43 provenances for the performance traits were clustered and analyzed using the systematic clustering method (Figure S2). The results indicated that the specific relationship pattern between PC quality and their provenance was unclear. This finding is similar to the previous conclusion that 25 batches of PC samples were not different based on HPLC fingerprint. Most components did not significantly correlate with longitude, latitude, altitude, and other environmental factors (Zhou et al., 2021). Plants often adapt to the environment by changing their genotype to change their characters over a long period. Therefore, the characters reflected due to the short-term change in the growth environment are still determined by their original adaptive environment.

Correlation analysis of different provenances of P. cyrtonema

China's rich ecological climate and geographical conditions have led to the situation that there are many places of origin of Chinese herbal medicine, and PC is one of them (Che et al., 2022). Blind introduction and expansion may lead to a sharp decline in the quality and yield, so it is necessary to study the relationship between the suitability of Chinese medicinal materials and environmental factors (Chen et al., 2006). Detailed and accurate geographical and ecological attribute data of medicinal materials are the basis for the accuracy of analysis results (Chen et al., 2006; Shen et al., 2019). In this study, 19 climatic factors, including temperature and precipitation, during different seasons were discussed (Hu et al., 2017). The correlation analysis of agronomic and quality traits of different PC provenances with their geographical location, temperature, and precipitation factors is shown in Figure S3. The stem length was positively correlated with the annual mean temperature, the maximum temperature of the warmest month, the minimum temperature of the coldest month, the mean temperature of the driest quarter, the mean temperature of the warmest quarter, the mean temperature of the coldest quarter, stem diameter, leaf length, leaf width, inflorescence length, fruit number of each infructescence, the width of rhizome first node, and saponin content. This finding is similar to that of the *Epimedium sagittatum* study, in which stem length was positively correlated with main stem diameter, leaf length, and leaf width (Luo et al., 2022).

Stem diameter was positively correlated with the annual mean temperature, minimum temperature of the coldest month, mean temperature of the driest quarter, mean temperature of the coldest quarter, leaf width, floret inflorescence length, fruit number of each infructescence, the width of rhizome first node, and yield. Previous studies have also shown the positive correlation between stem diameter and yield. The study on the agronomic and quality traits of *Tetrastigma hemsleyanum* from different provenances revealed that plant-type factors such as stem diameter are significantly related to its quality (Xu et al., 2021). The stem is the main harvesting organ of sweet sorghum, and the stem diameter critically affects its yield (Zhao et al., 2008). The high yield of northern japonica rice at the end of the 20th century is positively correlated with short and sturdy stems, long leaves, and large spikes (Chen et al., 2007).

Leaf length was negatively correlated with temperature seasonality and annual temperature range and positively correlated with annual mean temperature, isothermality, the maximum temperature of the warmest month, mean temperature of the driest quarter, mean temperature of the coldest quarter, precipitation of the wettest month, precipitation of the coldest quarter, the width of rhizome first node, and yield. Leaf width was negatively correlated with elevation and precipitation of the warmest quarter and positively correlated with the maximum temperature of the warmest month, mean temperature of the driest quarter, precipitation of the driest month, precipitation of the wettest quarter, precipitation of the coldest quarter, inflorescence diameter, and the length and width of rhizome first node. The leaf phenotypic traits of *Albizia odoratissima* from different provenances are strongly influenced by latitude, average annual temperature, and average annual sunlight (Gao et al., 2022).

Inflorescence length was negatively correlated with annual precipitation and precipitation of the coldest quarter and positively correlated with the fruit number of each infructescence. Inflorescence diameter was positively correlated with the fruit number of each infructescence. Floret inflorescence length was positively correlated with temperature seasonality and saponin content. The fruit number of each infructescence was positively correlated with the annual mean temperature, the maximum temperature of the warmest month, minimum temperature of the coldest month, and mean temperature of the warmest quarter.

The length of the rhizome first node was negatively correlated with elevation and mean temperature of the wettest quarter and positively correlated with temperature seasonality, the maximum temperature of the warmest month, the annual temperature range, and the mean temperature of the warmest quarter. The width of the rhizome first node was negatively correlated with elevation and temperature annual range and positively correlated with the annual mean temperature, the maximum temperature of the warmest month, minimum temperature of the coldest month, and mean temperatures of the driest, warmest, and coldest quarters. Temperature is the primary factor affecting the distribution and quality of *Panax ginseng* and *P. quinquefolium*, whose roots are also used in medicine (Dong, 2011).

The yield was positively correlated with the mean diurnal range, mean temperature of the driest quarter, annual precipitation, and precipitation of the wettest and driest months and driest, wettest, and coldest quarters.

Overall, the agronomic traits of different provenances of *P. cyrtonema* were related to the location and temperature, whereas the quality traits were strongly related to the precipitation of the provenance environment. Temperature and rainfall affect the accumulation of effective substances in *Salvia officinalis* L., thus affecting its efficacy (Generalić Mekinić et al., 2019). Precipitation affects the accumulation of chemical substances in raw coffee beans, thus affecting their flavor quality (Mendes et al., 2022). Annual precipitation can improve secondary metabolite contents in *Ziziphus jujuba* Mill. (Wang et al., 2022).

Establishment of the quantitative calibration model using NIRS

Polysaccharide and saponin contents are important indicators for evaluating Huangjing quality (Zhang et al., 2019). Therefore, the quantitative determination of its polysaccharide and saponin contents is highly important for their use and quality evaluation. PF had the highest polysaccharide content of 15.52 ± 2.61 %, whereas PS, PK, and PC contained 10.59 ± 2.44 %, 11.05 ± 2.24 %, and 12.35 ± 3.40 % of polysaccharides, respectively. PK had the highest saponin content with 10.69 ± 3.51 %, whereas the saponin contents of PS, PC, and PF were 4.6 ± 1.03 %, 7.54 ± 1.86 %, and 4.54 ± 2.25 %, respectively (Table S4). At present, the traditional colorimetric method is more widely used to determine polysaccharide and saponin contents. Although its results are more realistic and reliable, the whole process is time-consuming and labor-intensive, rendering it unsuitable for determining the contents of large quantities. This disadvantage can be greatly mitigated by combining NIRS with chemometrics to establish a rapid quantitative technique (Xie et al., 2009).

Sixty-four Huangjing samples were randomly divided into calibration and prediction sets, their content ranges are shown in Table S9. First, the conditions were optimized using the calibration set to generate a prediction model, which was then validated using the prediction dataset. The spectra collected by NIRS are easily affected by solid powder particle size and homogeneity, subjecting the measured spectra to baseline shift and drift. In addition, the number of principal components remarkably influences model predictiveness and accuracy. An extremely high number of principal components will complicate the model, and overfitting will occur. An extremely low number of principal components will provide incomplete information and reduce the model's predictiveness (Zhou et al., 2018). Therefore, the spectral preprocessing method and the number of principal component factors for screening must be optimized to enhance the model accuracy by calculating the Rp, Rcal, RMSEC, RMSEP, and RPD values of the model. Higher Rp and Rcal and lower RMSEC and RMSEP values indicate better model performance (Zhu et al., 2021). The RPD value is usually used to evaluate the model performance comprehensively; models with RPD $<$ 1.4 indicate unstable performance, models with $1.7 < \text{RPD} < 2.4$ can be used for quantitative grading, and models with RPD $>$ 2.4 are excellent and reliable (Zhu et al., 2021).

Determination of the total saponin contents in Huangjing using NIRS

The model results obtained after different spectral preprocessing methods and PCA factor number screening indicate that the model results are better at full wavelength based on SNV with a PCA factor of 12, R^2 of 0.91812 and 0.91698, and RMSEC and RMSEP values of 1.04 and 1.48, respectively (Table 2). The RPD value was 2.25, indicating that this model has great potential for quantitatively grading Huangjing saponin content.

Moreover, the measurement and prediction results indicate that NIRS combined with chemometrics can effectively predict the saponin content of Huangjing (Fig. 4). This method can accurately predict isoflavone and saponin contents in ground soybean (Berhow et al., 2020), indicating that the combination of NIRS technology and chemometric method can be applied to the rapid non-destructive detection of Huangjing quality.

Determination polysaccharide contents of Huangjing by NIRS

Table S10 shows the model of the quantification of Huangjing polysaccharide content derived after various spectral preprocessing methods and PCA factor number screening. The model results were better at full wavelength based on SNV and PCA factor number 6, with R^2 of 0.61605 and 0.44498 and RMSEC and RMSEP values of 2.73 and 3.17, respectively. The RPD value was 1.15, indicating that this model can only be used for the preliminary screening of Huangjing polysaccharide content. The measured and predicted results are shown in Fig. 4. A previous study successfully established a quantitative detection model of *Schisandra chinensis* polysaccharide using NIRS and chemometrics, validating the feasibility of NIRS for the non-destructive detection of polysaccharides in medicinal products (Wu et al., 2022).

The method of NIRS with chemometrics was used to rapidly detect the content of saponins in Huangjing, which proved the feasibility of using NIRS for quantitative detection of chemical substances in Huangjing.

Conclusions

In this study, we analyzed the agronomic and quality traits of five *Polygonatum* species, a medicinal herb, using a common garden located in Hangzhou, Zhejiang. Significance testing and cluster analysis revealed that Huangjing differs from Yuzhu in both agronomic and quality traits. *P. cyrtonema* is more suitable for introduction and cultivation in Zhejiang Province and similar districts considering its yield and quality, and the germplasm resources in Zhejiang, Fujian, and Anhui are similar. In addition, correlation analysis revealed that the stem diameter is positively correlated with yield, which provided a basis for the preliminary assessment of the quality of Huangjing. Future research needs to conduct more extensive scale provenance experiments to provide a reference for the introduction and cultivation in more regions.

Moreover, an effective PLS regression model for the rapid determination of polysaccharide and saponin contents in Huangjing was established based on NIRS and chemometric analysis. Compared with the conventional chemical method, the NIRS model is more efficient and

Table 2
PLS regression models for total saponin prediction of Huangjing by NIR.

Structure of model	Parameters	Rcal	RMSEC	Rp	RMSECP	RPD
Spectral pretreatment methods	RAW	0.81253	1.53	0.78329	1.93	1.72
	MSC	0.84171	1.42	0.81915	1.86	1.79
	MSC + FD	0.96336	0.704	0.75194	2.16	1.54
	MSC + SD	0.99956	0.777	0.53837	2.74	1.21
	MSC + SG	0.8416	1.42	0.81915	1.86	1.79
	MSC + SG + FD	0.95073	0.814	0.75040	2.15	1.55
	MSC + SG + SD	0.99159	0.34	0.60975	2.56	1.30
	SNV	0.84293	1.42	0.81941	1.86	1.79
	SNV + FD	0.96324	0.705	0.75162	2.16	1.54
	SNV + SD	0.99956	0.0776	0.53921	2.74	1.21
	SNV + SG	0.84228	1.42	0.81941	1.86	1.79
	SNV + SG + FD	0.95052	0.816	0.74978	2.15	1.55
	SNV + SG + SD	0.99137	0.344	0.61365	2.55	1.30
Number of factors	1	0.70071	1.87	0.73789	2.30	1.45
	2	0.74514	1.75	0.74105	2.27	1.46
	3	0.76983	1.68	0.81720	1.91	1.74
	4	0.80998	1.54	0.78695	1.93	1.72
	5	0.82672	1.48	0.79091	1.94	1.71
	6	0.84293	1.42	0.81941	1.86	1.79
	7	0.86980	1.30	0.88218	1.62	2.05
	8	0.88008	1.25	0.87446	1.63	2.04
	9	0.89519	1.17	0.91394	1.39	2.39
	10	0.90074	1.14	0.92711	1.38	2.41
	11	0.90994	1.09	0.89214	1.57	2.12
	12	0.91812	1.04	0.91698	1.48	2.25
	13	0.93037	0.963	0.88052	1.75	1.90

less labor-intensive. The preliminary screening and evaluation of the quality of Huangjing by NIRS will remarkably benefit this industry and its further utilization such as the quality grading of Huangjing and the selection of raw materials for its products. The rapid detection of polysaccharide content in Huangjing needs to be improved.

Furthermore, the evaluation approaches of the PC germplasm resources explored in this study provide a scientific basis for breeding high-yielding and high-quality medicinal herbs. This will help protect and cultivate its germplasm resources while promoting the development of Huangjing as a forest crop and future food and industrial crop in China.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100585.

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