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Temperature seasonality and soil phosphorus availability shape ginseng quality via regulating ginsenoside contents

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Abstract

The accumulation of secondary metabolites in *Panax ginseng* Meyer (*P. ginseng*) exhibits significant geographical variation, normally due to environmental factors. The current study aimed at elucidating the key environmental factors modulating the accumulation of secondary metabolites in *P. ginseng*. Plant and the associated soil samples were collected from ten geographical locations within the latitudinalrange of 27.09°*N* − 42.39°N and longitudinal range of 99.28°E − 128.19°E. 12 secondary metabolites in *P. ginseng* toots were measured. And the correlation between secondary metabolites with a series of soil properties and 7 climatic factors were investigated through Pearson's correlation, mantel test, random forest and pathway analysis. The results revealed that climatic factors were stronger drivers of *ginseng* secondary metabolite profile than soil nutrients. Specifically, temperature seasonality (TS) and soil available phosphorus (AP) were the most effective environments to have significantly and positively influence on the secondary metabolites of *ginseng*. This findings contribute to identifying optimal cultivation areas for *P. ginseng*, and hopefully establishing methods for interfering/shaping microclimate for cultivating high-quality *P. ginseng*.

Keywords *Panax* gingseng, Ginsenoside, Environmental factors, Secondary metabolite

Introduction

Panax ginseng Meyer (*P. ginseng*) of the Araliaceae family is an important economic crop. The roots and rhizome of *P. ginseng* are known as ginseng, which is widely used in Asia and Europe as a functional food, e.g. in soups and healthy tea, because it boosts vital energy [1]. Ginseng is believed to benefit the spleen and lungs, calm the mind, and improve intelligence. Modern studies show it treats metabolic syndromes, cardiovascular disease, and has antitumor, antifatigue, antidepressant, and anti-inflammatory effects [2, 3]. Ginsenosides was one of the most important group of compounds related to pharmacological effects of ginseng, such as Rb1 [4], Rb2 [5], Rb3 [6], Rd2 [7] etc., making these compounds more attentioned when evaluating the quality of *P. ginseng* raw products.

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Wild *P. ginseng*, once widespread in Russia, China, and Korea, now only survives in Jilin, Heilongjiang, and parts of Primorsky due to overexploitation and environmental changes [8]. And previous research shows that the quality of *P. ginseng* varies depending on the place of origin, demonstrating that environment have a significant impact on the distribution and ginsenoside of ginseng populations. The ginsenoside of *P. ginseng* vary significantly between different plantations, which is closely related to the physicochemical properties of the local soil [9], and the climate factors. For example, contents of Fe, available phosphorus, available potassium, total nitrogen, organic maters, and soil pH could promote the increase of ginsenoside content [10, 11], with Fe particularly proven to upregulate proteins involved in the ginsenoside synthesis pathway [12]. Climatic factors not only affect the migration of ginseng distribution areas but also influence ginsenoside accumulation. For example, low-temperature stimulation can promote the accumulation of ginsenosides [13]. Zhu et al. also found in field experiments that temperature and precipitation affect the accumulation of ginsenosides [14]. The health-promoting efficacy of *P. ginseng* have been shown to be influenced by environmental factors such as photosynthetically active radiation (PAR), soil water potential [15]. However, research on the relationship between ginseng habitats and its quality has been limited to its natural distribution in northeast China, parts of Russia and Korea [8], and studies on a larger scale are lacking.

In this study, we investigated the climatic and soil factors that potentially affect the ginsenosides content of *P. ginseng* in a larger geographical scale. The aim of this study was to evaluate: (1) the differences in ginsenosides contents of *P. ginseng* from different geo-origins, (2) the correlation between ginsenosides contents and climate and soil factors, and (3) the positive mechanisms how environments affect ginsenosides properties of *P. ginseng* in a raletive larger scale. We conducted correlation analyses between the concentration of ginsenoside compounds, climatic factors and physicochemical properties of the soil. The results will provide guidance for the identification of optimal locations for the cultivation of *P. ginseng* and the establishment of appropriate soil treatment systems or soil administrating regimes.

Methods

Collection and preparation of plant and soil samples

We collected five-year-old *P. ginseng* samples and their associated soil samples from 10 locations in three provinces in China. Samples were identified as *P. ginseng* by Prof. Guo Lanping (National Resource Center for Chinese Materia Medica), and voucher specimens were deposited in the National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences (Beijing). These locations ranged in elevation from 182 to 2861 m, as shown in Fig. 1 and Table S1. Each *P. ginseng* plant was excavated together with the surrounding soil 10 cm in diameter and 20 cm deep using a hand shovel. The soil was then carefully removed from the plant. Both the plants and the soil were collected, labelled accordingly, placed on ice, and quickly transported to the laboratory. And 9 plants and soil samples was collected from each location. After the *P. ginseng* samples were collected, they were thoroughly washed, The parameters of the freeze dryer is acuum gauge 8.0 Pa, trap temperature −60 ℃, and dried for 72 h, pulverised with a MM400 ball mill, and stored in a refrigerator at 4 °C for later use. And the soil samples were air-dried for subsequent analyses.

Reagents and chemicals

Methanol (chromatographic grade, UN1230) and acetonitrile (chromatographic grade, UN1648) were purchased from Merck KGaA, Germany. Ultrapure water was produced in-house using a Thermo Barnstead Gen Pure UV/UF system from Thermo Fisher Scientific, USA. The samples were filtered through 0.22 μm hydrophobic PTFE syringe filters (Millipore, USA).

Reference compounds of ginsenosides Rb1 (batch no. 19112451), Rb2 (batch no. 19092591), Rb3 (batch no. 19082903), Rc (batch no. 19111861), Rd (batch no. 19110713), Rd2 (batch no. 19102489), Re (batch no. 19092451), Rf (batch no. 19102798), Rg1 (batch no. 19122514), 20 S-Rg2 (batch no. 191102401), 20 S-Rh1 (batch no. 19101823), and Ro (batch no. 19111720) were all obtained from Shanghai YuanYe Biotechnology Co., Ltd., with a purity of ≥98%.

Sample and standard solutions preparation

Sample extraction was conducted following the protocols of Li et al. [16] with minor modifications: Approximately 0.15 g of *P. ginseng* powder was weighed (with the exact weight recorded as 'mg') and homogenized with 1.5 mL of 70% (V/V) methanol solution. The mixture was then subjected to ultrasonic extraction at 250 W, 40 kHz for 60 min. After cooling, add 70% (V/V) methanol to bring the volume back up to 1.5 mL. The mixture was then centrifuged at 13,000 rpm for 10 min. The supernatant was collected and filtered through a 0.22 μm microporous membrane and then diluted five fold with filtered 70% (V/V) methanol as the extracted sample solution.

For the preparation of the reference standard solutions: Accurately weigh an appropriate amount of each reference standard and dissolve it in methanol. Vortex to mix and prepare a 2 mg/mL stock solution. Dilute aliquots of this stock solution with methanol to obtain a range of concentrations for a mixed standard solution, which is then stored at 4 °C for later studies.

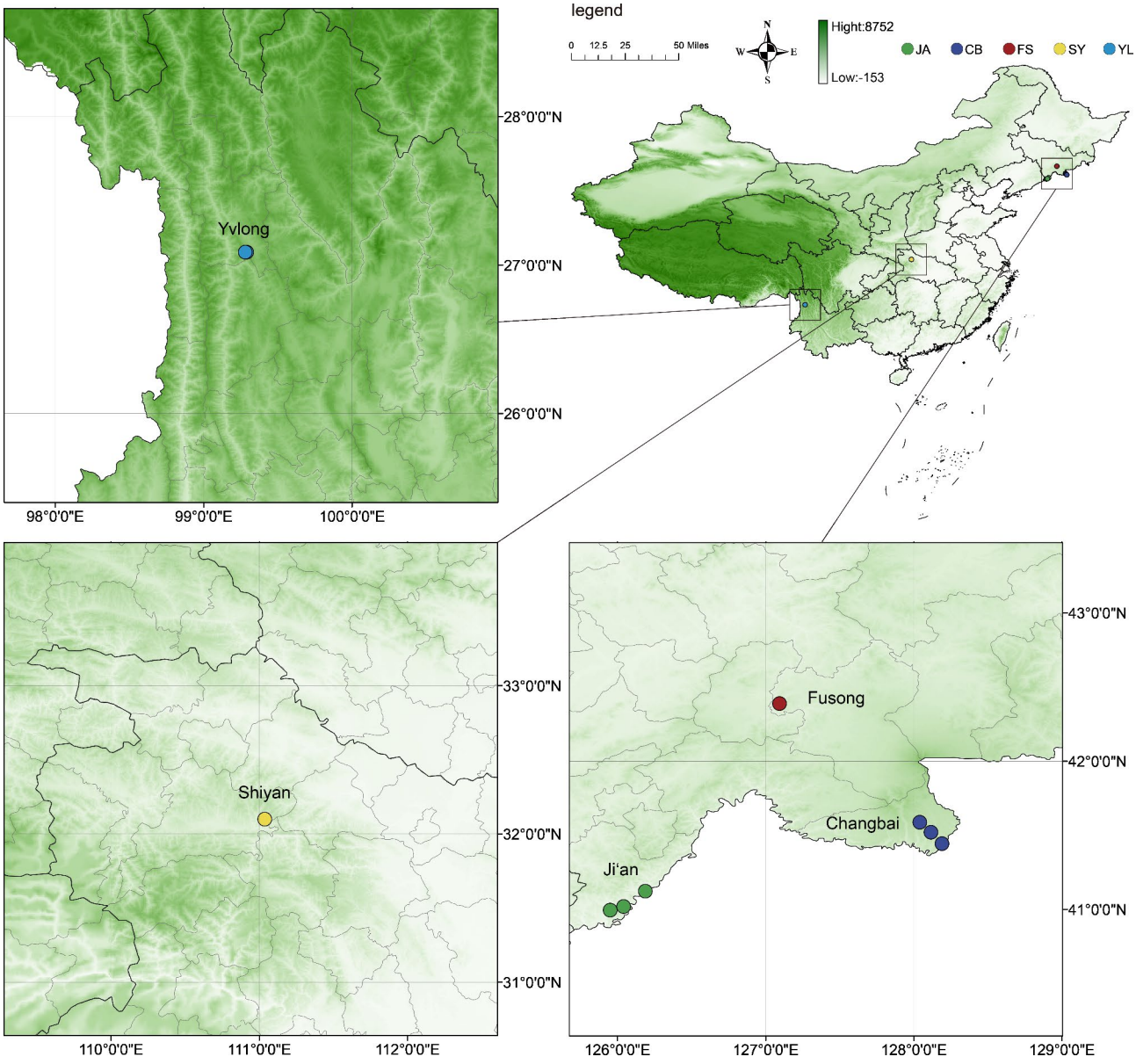

Fig. 1 Locations of sample collection

UPLC method

Chromatographic separation was performed using a Waters Acquity UPLC I-Class system (Waters Corp., Milford, MA, USA) equipped with a Waters ACQUITY UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm). The column temperature was maintained at 40 °C with a flow rate of 0.4 mL/min and an injection volume of 1 μL. The mobile phase consisted of 0.1% formic acid in acetonitrile (A) and 0.1% formic acid in water (B). The elution gradient was set as follows: 0–2.5 min, 28–31% A; 2.5–3 min, 31–35% A; 3–4.5 min, 35–36% A; 4.5–6 min, 36–37% A; 6–7 min, 37–50% A; 7–9 min, 50–98% A; 9–11 min, 98% A; 11–12 min, 98–28% A; 12–15 min, 28% A.

Measurement of soil physiochemical properties

The soil pH was determined using a CO_2-free water extraction-potentiometric method [17]. Soil organic matter (SOM) was quantified using the dichromate oxidation method with a heating plate [18]. Alkali-hydrolyzable nitrogen (TN) was measured using the 1 mol/L NaOH alkali dissolution gas diffusion method [19]. Available phosphorus (AP) was determined using a 0.5 mol/L sodium bicarbonate extraction-molybdenum antimony anticolorimetric method [20]. Available potassium (AK) was determined by 1 mol/L ammonium acetate extraction followed by atomic absorption spectrophotometry [21]. Available iron (Fe) was determined by extraction with DTPA solution and atomic absorption

spectrophotometry. Available boron (B) was determined by extraction with hot water and azomethine-H colorimetry [22]. Three technical replicates were performed for each measurement.

Acquisition of climate data

The selection of all climatic factors and the statistical approach for the (partial) Mantel tests in this study were based on the methodology of Liu et al. [23]. Climate data for all ten sampling locations, including mean annual temperature (MAT), temperature seasonality (coefficient of variation, TS), maximum temperature of the warmest month (MT), minimum temperature of the coldest month (MIT), annual temperature range (TR), mean annual precipitation (MAP), and precipitation seasonality (PS), were obtained from the WorldClim database (http://www.worldclim.org). Detailed climatic data can be found in Table S1 [24].

Statistical analyses

In this study, chemometric techniques were applied to evaluate the effects of environmental factors on the levels of secondary metabolites of *P. ginseng*. Our approach included the Kruskal-Wallis H test, principal component analysis (PCA) and hierarchical cluster analysis (HCA), complemented by multiple regression and path analyses, and Mantel tests to understand variable correlations. Due to the significant differences in size between the measurements of the variables, a log transformation was performed when calculating the Pearson correlation coefficients in order to ensure the normality of the data. The Kruskal-Wallis H-test and regression analyses were performed with SPSS. The principal component analyses (PCA) were performed with SIMCA 14.1. Hierarchical cluster analyses (HCA) were performed in R with the package "stats". Conduct path analysis using AMOS software. For more details, see the Supplementary Material. Data processing was performed with the library "Pandas" [25]. The graphical visualisation of the Spearman correlation coefficients was created with the libraries "Seaborn" and "Matplotlib" [26, 27]. The Pearson correlation coefficients were calculated and the linear relationships between the variables were evaluated using the "Pandas" library [25]. The significance of the correlation was displayed in a co-occurrence network created with "NetworkX" and "Matplotlib" [28]. For the analyses of correlations between different distance matrices derived from our dataset, Mantel tests used "scipy.spatial.distance" to calculate Euclidean and Bray-Curtis distances with respect to secondary metabolites, soil properties [29] and climatic factors. The Spearman rank correlation coefficients for these matrices were determined using "scipy.stats.spearmanr", with the partial Mantel test taking into account the influence of a third matrix using "sklearn.metrics.pairwise_distances" [30], with permutation tests used in both cases to check significance. R (v4.3.1) facilitated statistical modelling using random forest models to determine the predictive value of the environmental variables on the response metrics. Here, "randomForest" and "caret" were used for model development and validation [31, 32] and "rfUtilities" and "rfPermute" for significance testing of the predictors [33, 34]. The graphical visualisation was performed in R with "ggplot2" [35].

Results

Ginsenoside differentiation of *P. ginseng* and associated driving factors

A total of 12 metabolites were identified in *P. ginseng* from various origins. The current study began with a rigorous quantitative analysis of twelve ginsenoside compounds in these *P. ginseng* samples, combining these data with soil physicochemical properties. Using Shapiro-Wilk and Levene tests, we assessed the normality and homogeneity of variance in our data sets. As shown in Table S2, deviations from the normal distribution and homogeneity of variance required the use of non-parametric statistical approaches. Consequently, the Kruskal-Wallis H test, supplemented by Dunn's post-hoc test, was utilized to determine the statistical significance of the differences between the different groups, as shown in Fig. 2.

Considerable discrepancies were found in the physicochemical properties of the soil, with the acidic nature being a common feature of all samples. Notably, the pH of the soil in the JA group was identified as the highest. The concentration ranges for total nitrogen (TN), available phosphorus (AP) and available potassium (AK) were determined as 26.47-664.83 mg/kg, 14.30-81.98 mg/kg and 72.10–420.00 mg/kg, respectively, with soil organic matter (SOM) content ranging from 9.96 to 250.41 g/kg. A distinctive elevation in TN, AP and AK concentrations was observed in the CB group, whereas the YL group showed a significant increase in SOM content.

Within the analyzed ginsenoside spectrum, ginsenoside Re proved to be the most prevalent ginsenoside, whereas ginsenoside Rd2 was detected in the lowest concentration, with the CB group having the highest levels of ginsenoside Rb1. The analysis revealed no significant differences between the groups for ginsenosides Rg1, Rf, Rg2 and Rh1. To mitigate possible interference and avoid false-negative implications in the cluster analysis, these four ginsenoside components were excluded from further PCA. This analytical endeavor highlights the extensive environmental variations between sampling sites and facilitates the refinement of variables for subsequent analysis, ensuring a targeted and effective investigation.

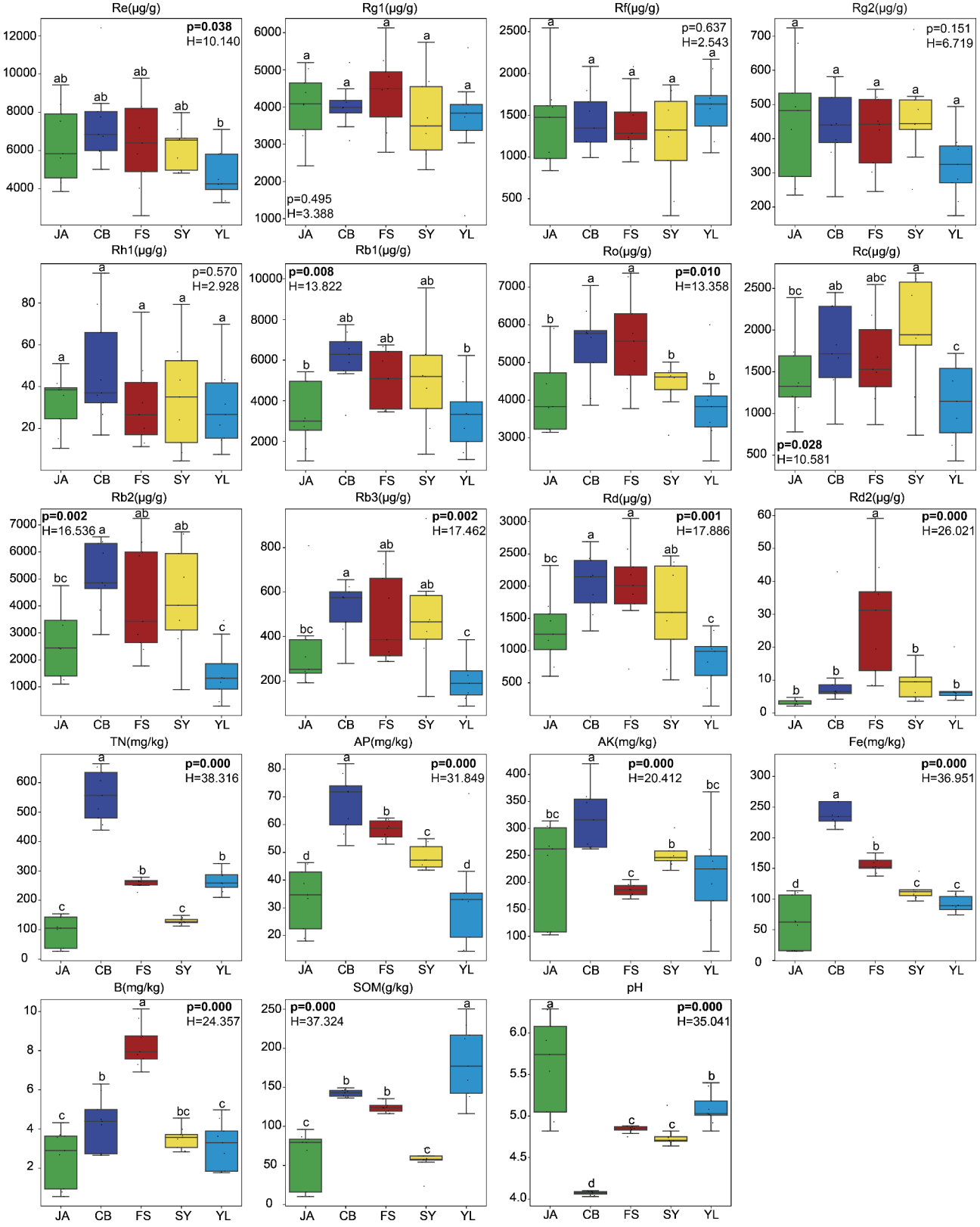

Fig. 2 Summary of the edaphic properties and Ginsenoside across the sampling groups from different sites in China. JA, Jian (Jilin); CB, Changbai (Jilin); FS, Fusong (Jilin); SY, Shiyan (Hubei); YL, Yulong (Yunnan); pH, soil pH; SOM, soil organic matter; TN, total nitrogen; AP, available phosphorus; AK, available potassium; Fe, iron; and B, boron. Different letters indicate significant differences within the same boxplot ($P < 0.05$; Kruskal-Wallis H Test, followed by Dunn's Test for post-hoc comparisons). Bold *P*-values indicate significant differences ($P < 0.05$) ($n = 9$)

Variation of ginsenoside content in *P. ginseng* cultivated under diverse environmental conditions

PCA is an effective tool for dimensionality reduction in data analysis, facilitating the preservation of original data variability through a transformed coordinate system. In our study of *P. ginseng*, PCA was strategically used to detect patterns within 8 significantly varying ginsenosides at different sampling sites, resulting in the derivation of 8 principal components that together comprise 100% of the variance, as shown in Table S5. The first 3 components, accounting for 91.400% of the total variance, enabled the construction of a three-dimensional score plot (Fig. 3a), visually dividing the samples into distinct clusters along the first 3 principal components and highlighting the effectiveness of the method in data clustering.

Despite the usefulness of PCA in revealing data structures, it lacks the functionality of a clustering algorithm. This limitation necessitated the integration of HCA, a method that groups entities based on their similarity or distance, to further stratify the data. Utilizing Ward's method with Euclidean distance as the similarity metric

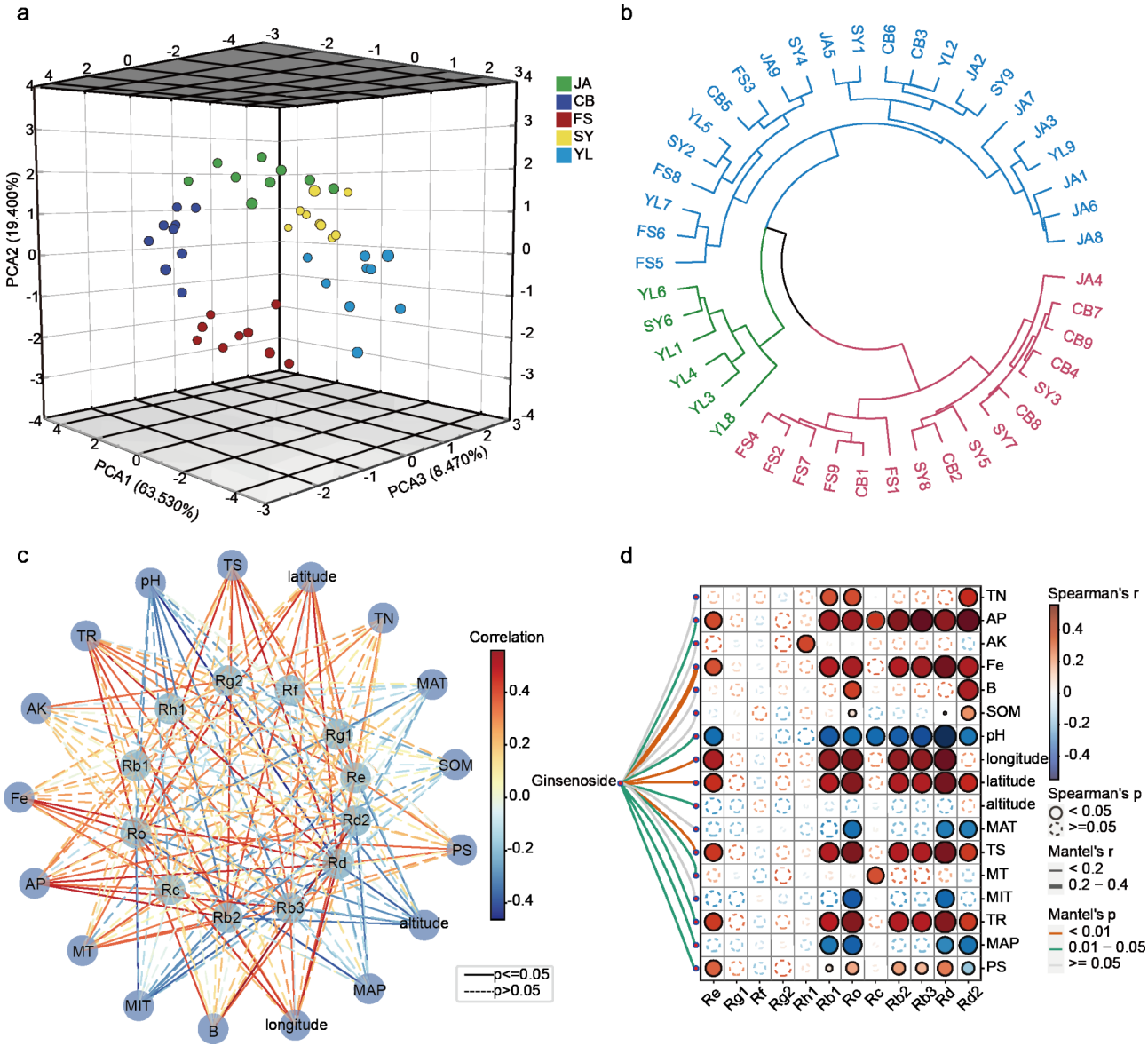

Fig. 3 (**a**) Unsupervised analysis of ginsenosides by PCA. (**b**) Dendrograms resulting from HCA of *P. ginseng* from different locations (k = 3). (**c**) Co-occurrence map of Pearson correlation coefficients between environmental factors and ginsenosides. The color stands for the strength of the correlation, solid lines indicate a $P \leq 0.05$ and dashed lines a $P > 0.05$. (**d**) Correlation analysis between soil properties, climatic characteristics and secondary metabolites of roots, determined using the Mantel test. The color of the line represents the significance of the differences (Mantel's P). The pattern of the dot represents the significance of the differences (Spearman's P). The size of the line and the dot represents the size of the correlation coefficients (Mantel's r and Spearman's r)

[31], we aimed to minimize the variance within clusters and thereby improve the homogeneity of samples within clusters. The preliminary PCA-guided clustering suggested a five-cluster model, which, after further examination using the Gap statistic [32], revealed a more refined scheme with three clusters to be optimal, combining superior clustering efficiency with minimized error (Table S6, Fig. 3b). In this model, the YL and JA samples in particular were divided into separate clusters, while the CB and FS samples were combined into a third cluster. In line with the findings of the HCA, the K-Means clustering analysis using the elbow method identified 3 as the optimal number of clusters, which corresponds to the result of the HCA (Fig. S2).

The results of PCA and HCA show that *P. ginseng* from different countries of origin forms different clusters due to the different content of twelve metabolites, which illustrates the chemical differentiation of *P. ginseng* from different areas. In addition, the loadings of principal components are crucial for evaluating the contribution of each metabolite to the separation of the clusters. Notably, ginsenoside Rb2 with a loading of -0.541 on the first principal component (PC1) and ginsenoside Rd2 with a loading of 0.902 on the second principal component (PC2) were significant contributors to their respective axes. In addition, ginsenoside Rd with a high loading of -0.776 on the third principal component (PC3) emphasises the differential contribution of these saponins across different geographical origins, suggesting their potential as markers for origin discrimination.

Environmental determinants influencing the total content of twelve ginsenosides

Once the differences in ginsenoside content between the different groups of *P. ginseng* had been confirmed, further investigations were carried out to explore the possible causes of these differences. Using (partial) Mantel tests, we analyzed the influence of soil physicochemical properties and climatic factors on ginsenoside content (Table S7). A slight positive correlation was found between the ginsenoside content and both soil physicochemical properties of the soil and the climatic factors. However, when controlling for climatic factors, the relationship between ginsenoside content and soil physicochemical properties may no longer be significant. The co-occurrence matrix of Pearson correlation coefficients between environmental factors and ginsenoside content showed significant positive correlations with variables such as Fe, TS, longitude, MT, latitude, B, AP, PS and TR; conversely, MAP, pH, altitude and MIT showed significant negative correlations with various ginsenoside components (Fig. 3c). The analysis showed 66 coefficients with p-values less than 0.05, indicating significant correlations with ginsenoside content in *P. ginseng.* We focused on the top 50% of these coefficients. Specifically, in Pearson correlations, 15 were climatic, 9 geographic, and 9 edaphic; in Spearman correlations, 15 were climatic, 10 geographic, and 8 edaphic, all significantly correlated with ginsenoside content. Which is consistent with the (partial) Mantel test results. Similar trends were observed in the Spearman's rank correlation results (Fig. 3d).

The Mantel test results (Table S8) revealed significant positive correlations between AP, Fe and pH in soil physicochemical properties and ginsenoside content, while all other climatic factors except MAT and MIT showed significant positive correlations with ginsenoside content. PCA was employed to reduce the dimensionality of the matrix of ginsenoside content. The extracted PCA1 was subjected to multiple linear regression analysis with environmental factors identified as significantly different by Mantel tests, revealing significant correlations between AP and TS with ginsenoside content PCA1 (Table S9). To further clarify the effect of these two environmental factors on ginsenoside content, scatter plots were generated with ΔAP and ΔTS as x-axes and the Euclidean distance of ginsenoside content as y-axis (Fig. S3), which showed a positive correlation between AP and TS with ginsenoside content.

This analytical section, using (partial) Mantel tests, Mantel tests, and multiple linear regression analysis, confirmed the two most important environmental factors affecting the 12 measured ginsenosides: AP and TS.

The most important environmental factors influencing the individual categories of ginsenosides

To clarify the relationships between various environmental factors and the content of ginsenosides in *P. ginseng*, Random Forest was used to determine the environmental factors that significantly affect the content of each ginsenoside, where ginsenosides are the dependent variable and environmental factors are the independent variables. The results, as illustrated in Fig. 4, indicate that Rg2 was significantly affected by factors such as pH, AP, PS, and altitude; Rb2 by TS, MT, longitude, latitude, altitude, TR, and PS; Rd by pH and longitude; Rb3 by Fe, AP, longitude, and latitude; Ro by TR, TS, longitude, and latitude; Rb1 by latitude, TS, and TR; and Rd2 by AP, latitude, TR, B, PS, altitude, and TS.

To validate the random forest results and further investigate the influence of environmental factors on ginsenoside content, we performed path analysis to calculate both direct and indirect effects of these factors on secondary metabolites. This process involved two main steps: First, we conducted stepwise regression with ginsenosides as the dependent variable and environmental factors as independent variables (Table S10). This helped identify the dominant ecological factors for each ginsenoside component. Next, we calculated the direct

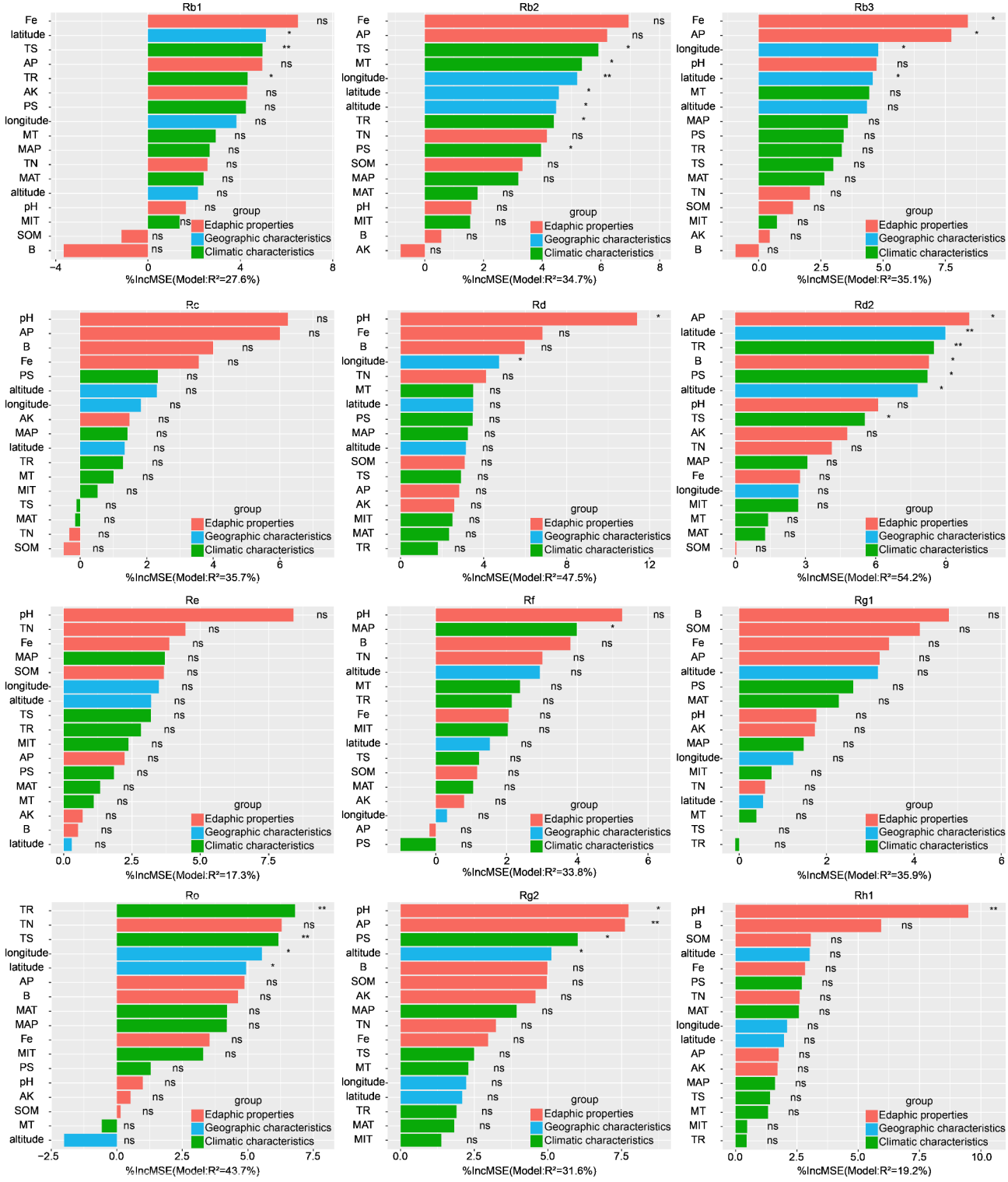

Fig. 4 Random forest mean predictor importance of environmental characteristics (climatic and soil) and geographical factors for ginsenoside content. "%IncMSE" stands for "Percentage Increase in Mean Squared Error" ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns, not significant. The fitting degree (R^2) of the random forest model is indicated

and indirect effects of these identified ecological factors on ginsenosides, as shown in Table 1. And the results indicated that AP had a significant direct effect on Rg2, while TN had a significant indirect effect. PS and TS had significant direct and indirect effects on Rb2. TS had a significant direct effect on Rd, whereas pH had a significant indirect effect. AP and MT had significant direct and indirect effects on Rb3. TR and latitude significantly affected Ro both directly and indirectly. AP and MT also had significant direct and indirect effects on Rc. TS and altitude significantly influenced Rb1 both directly and indirectly. B had a significant direct effect on Rd2, while AK and AP had significant indirect effects.

Discussion

Chemotypes of *P. ginseng* from different habitats

In the current study, soil properties and climatic variables had a significant effect on the concentration of secondary metabolites in *P. ginseng*, which was similar with a previous study that statses the importance of microenvironment in shaping active compounents of *P. ginseng* [36]. *P. ginseng* from different origins were different from each other in secondary metabolites according to PCA and a further HCA and K-mean indicates that they could be clustered as 3 chemotypes. The differences in chemical characteristics could be affected by the environmental differences. Such environmentally induced chemotypic differentiation is also uncovered in *Rh. tanguticum*, which exhibits significant variations in secondary metabolitesbetween canopy-covered and open habitats [37]. Similar reports exist in the current research on ginseng [38], our study innovatively expands the distribution range of ginseng in its natural habitat, collecting samples from a wider area and covering different climates and altitudes to the best of our ability. This provides scientific reference for predicting high-quality ginseng cultivation sites in the context of global climate change.

The complex interplay of environmental factors correlate with secondary metabolites in *P. ginseng*

Partial Mantel's tests was employed to decipher the independent effect of soil properties or climates on secondary metabolite concentrations and their interaction with climatic factors [23]. In the current study climate and

Table 1 Path analysis between environmental factors and secondary metabolites of *P. ginseng*

Items	Fcators	Direct path coefficients	Indirect path coefficient			
Rg2			Total	→AP	→TN	
	AP	0.288***	0.097		0.097***	
	TN	-0.008	0.338	0.338		
Rb2			Total	→PS	→TS	
	PS	0.148***	0.001		0.123***	
	TS	0.337***	0.001	0.279***		
Rd			Total	→TS	→pH	
	TS	0.301***	-0.091		-0.091***	
	pH	-0.095	0.000	0.000		
Rb3			Total	→AP	→MT	
	AP	0.281***	0.000		0.000***	
	MT	0.259***	0.037	0.037***		
Ro			Total	→TR	→latitude	
	TR	0.135***	0.134		0.134***	
	latitude	0.123**	0.123	0.123**		
Rc			Total	→AP	→MT	
	AP	0.125**	0.018		0.018**	
	MT	0.211***	0.030	0.030***		
Rb1			Total	→Fe	→TS	→altitude
	Fe	0.009	-0.003		-0.003	0.000
	TS	0.095**	0.000	0.000**		0.000**
	altitude	0.130***		0.35***	0.054***	
Rd2			Total	→B	→AK	→AP
	B	0.316***	0.166		0.086***	0.080***
	AK	0.015	0.011	0.005		0.006
	AP	0.017		0.006	0.006	

Note: pH: soil pH; SOM: soil organic matter; TN: total nitrogen; AP: available phosphorus; AK: available potassium; Fe: iron; and B: boron; MAT: Mean Annual Temperature; TS: Temperature Seasonality; MT: Maximum Temperature of Warmest Month; MIT: Minimum Temperature of Coldest Month; TR: Temperature Annual Range; MAP: Mean Annual Precipitation; PS: Precipitation Seasonality. The symbol "→" represents the causal path in path analysis: indicating the indirect effect of the variable following the "→" on the Items variable via the Factors variable

soil were found to have joint influences on secondary metabolite content in *P. ginseng*, with climate likely being the stronger driving factor. This was also supported by the following Pearson's correlation and Spearman's rank correlation. Soil properties was also thought to have a media role, in which climate affect secondary metabolites of *P. ginseng* through soil microbial community and soil properties [39]. Similar results have been reported for different species within the genus *Panax*, such as *P. notoginseng* [40] and *P. quinquefolius* [41], indicating that climatic factors significantly influence ginsenoside content. This emphasises the broad applicability of environmental influences on ginsenoside profiles throughout the genus. The Mantel test, Pearson's and Spearman's correlations indicate that both low and high temperatures, such as MT, MIT, and TR, promote ginsenoside accumulation in ginseng. This may be due to ginsenosides' antioxidant activity protecting ginseng cells from oxidative stress and free radical damage under extreme temperatures [42]. Thus, ginsenosides likely act as a defense mechanism, helping ginseng withstand various stresses, which fit the theory that stressed environments was suitable for producing plant medicines with high active compounds [43]. The negative correlation between pH and ginsenoside content is also an interesting result, indicating that more acidic environments lead to greater ginsenoside accumulation. This could be caused by the upregulated ginsenosides' synthesis to resist acid stress [44]. However, prvious studies has also revealed continuous ginseng cropping acidifies soil [45], and the allelopathic effect of ginsenosides may also causing regional death of cultivated ginseng and continuous cropping obstacles [46].

Key factors influencing secondary metabolites in *P. ginseng*

The Mantel test identifies AP and TS as key environmental factors significantly influencing ginsenoside levels. Using PCA for dimensionality reduction, a multiple linear regression analysis reveals their positive impact on PCA1 of the ginsenoside data. Further analysis with path and random forest models shows AP's direct effect on Rg2 and Rb3 and its indirect effect on Rd2.

Previous studies have shown that phosphorus can affect ginsenoside content in *Panax quinquefolius* and *P. ginseng*, which is consistent with the results of the current study [47, 48]. Phosphorus is also a crucial element for plant growth and has been shown to influence the synthesis of various secondary metabolites in different plant genera [49], for example, glycoside content in Stevia rebaudiana Bertoni and flavonoids in maize [50, 51]. This could be due to the fact that phosphorus conditions could influence different gene expressions in the synthesis of regulatory secondary metabolites [52]. In contrast, TS showed significant direct and indirect effects on Rb2, but only direct effects on Rb1. This suggests that TS may be a key factor affecting the quality of *P. ginseng*. The ginsenoside content of *Panax quinquefolius*, a close relative of ginseng [53], is also significantly affected by TS [54]. An influence of TS on the secondary metabolite content of *Salvia miltiorrhiza* and *Pilocarpus pennatifolius* (Rutaceae) was also found [55, 56]. Previous studies have shown that TS is crucial for the geographical distribution of plants and is essential for plant growth [57]. This indicator shows a predominantly positive correlation with ginsenoside content, suggesting that more significant seasonal temperature variations may be beneficial for the quality of ginseng.

Conclusions

This study employs a combination of methods including PCA, HCA, Mantel tests, path analysis and Random Forest to investigate the correlations between environmental factors and ginsenoside content in cultivated *P. ginseng*. The results indicate that climatic characteristics have a more significant influence on ginsenoside content than edaphic properties. Of all the factors analyzed, AP and TS exert the greatest influence on ginsenoside content. In particular, TS shows a significant correlation with the primary active compounds of *P. ginseng*, suggesting that it may be a key determinant in the formation of *P. ginseng* quality. Our research provides scientific reference for predicting high-quality ginseng cultivation sites.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05518-2.

Supplementary Material 1

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Author contributions

Dehua Wu: Conceptualization, Methodology, Writing original draft preparation. Feng Xiong: Methodology, original draft preparation. Siqi Liu: Writing review. Jitong Zhu: Writing review. Dan Zhao: Writing review. Jian Yang: Writing review. Chuanzhi Kang: Writing review & editing. Hongyang Wang: Formal analysis. Wenqi Ma: Resources. Lanping Guo: Supervision, Project administration, Writing review & editing. All authors read and approved the final manuscript.

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Data availability

The datasets generated for this study are available on request to the corresponding authors.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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