ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, 18(2) 2019, 171–191

www.acta.media.pl ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2019.2.16

ORIGINAL PAPER Accepted: 25.09.2018

EFFECT OF PROVENANCE AND WATER STRESS ON BIOMASS AND POLYPHYLLIN CONTENT IN THE MEDICINAL PLANT *Paris polyphylla* Smith var. *yunnanensis*

Xue-mei Wu[1, 2], Zhi-tian Zuo[2], Qing-zhi Zhang[1]✉, Yuan-zhong Wang[2]✉

[1] College of Traditional Chinese Medicine, Yunnan University of Traditional Chinese Medicine, Kunming 650500, China

[2] Institute of Medicinal Plants, Yunnan Academy of Agricultural Sciences, Kunming 650200, China

ABSTRACT

Water stress and provenance could affect the secondary metabolites synthesis and accumulation in herbs. Thus, this study explored the effect of soil water moisture and provenance on the growth of *Paris polyphylla* Smith var. *yunnanensis* (PPY). Three provenances (Jinping, Luquan and Weixi in Yunnan, China) of PPY samples were grown in different soil water moisture conditions [0.80, 0.70 and 0.50 field capacity (FC)] during Dec. 2015 to Sep. 2017. Results showed that the highest biomass weight was presented in 0.70 FC for Luquan and Weixi samples. Biomass weight for Jinping provenance presented a decreasing tendency with the decreased soil water moisture and the highest biomass were shown in 0.80 FC. However, quantitative analysis revealed that the total content of polyphyllin increased with decreasing the soil water moisture for Jinping and Weixi samples. The highest total content of polyphyllin in rhizome was inclined to show in Jinping samples, while the stem and leaf tissues were shown in Weixi samples. Additionally, results of ANOVA combined with PCA indicated that the difference among these three provenances were significant. Correlation analysis results revealed that 0.50 FC induced the competitive relationship occurrence for polyphyllin distribution. Thus, 0.70 FC was the most suitable soil-water condition for PPY growth. Besides, provenance collected from Jinping could consider as a good quality germplasm. Consequently, this study might provide a preliminary foundation for irrigation project formulated and provenance screened for PPY cultivation.

Key words: water stress, provenance, *Paris polyphylla* Smith var. *yunnanensis*, PCA, ANOVA, correlation analysis

INTRODUCTION

As the global warming intensified, drought was becoming a severe problem in many districts all over the world. Out of question, China is facing the increasing problems of water scarcity because of the rapid development of economy, urbanization, growing population and poor management of water resource [Jiang 2009]. Especially during 1999–2001 and 2009–2010, large-scale drought occurred in Yunnan Province, China, which brought out the dehydration and dried up the vegetation [Zhai et al. 2005, Liu et al. 2010, Pradhan et al. 2017]. As it is well-known, adequate and optimal water supply was usually one of the key pattern to sustain both normal vegetative growth and reproduction [Shao et al. 2008]. In recent years, many researches proved that drought stress could limit the plant growth and make further efforts to constrain the yield [Zhang et al. 2012, Layeghhaghighi et al. 2017]. Additionally, drought might induce the increasing/decre-

✉ boletus@126.com ynkzqz@126.com

asing of secondary metabolites synthesis and accumulation in herbs and even trigger many changes in gene expression [Bray 1997, Bray 2004, Chaves et al. 2003]. For instance, Wu et al. [2016] demonstrated that mild and moderate drought stress were beneficial for the accumulation of total flavonoids and total alkaloids in *Dendrobium moniliforme* in the early period time. Analogously, total saponins content was firstly increased and then decreased with the increasing water stress levels in both herbal medicine of *Stellaria dichotoma* L. var. *lanceolata* Bge and *Panax notogesing* [Zhang e al. 2017, Liao et al. 2017]. However, anthraquinones accumulation in medicinal herb *Cassia obtusifolia* L. was increased with weak and moderate drought stress and significantly decreased with the treatment of severe drought [Xue et al. 2018]. Thus, the effect of water stress was bidirectional and multiple for different herbal medicines.

Besides, provenance is one of the crucial part to affect the quality of herbal medicine as well. Various chemical profiles between different provenances might be caused by diverse environmental characteristics, such as temperature, radiation, soil chemical compositions, rainfall capacity etc. [Shao et al. 2017]. Zhang et al. [2014a] confirmed that the longitude and latitude were correlated with berberine content in samples of *Phellodendron amurense* significantly, and low longitude and latitude were more suitable for berberine accumulation than populations growing at high longitude and latitude. Li et al. [2015] measured 27 elements in samples of medicinal plant *Marsdenia tenacissima* and concluded that the highest concentration was present at different provenances among Sichuan, Guizhou, Guangxi and Yunnan samples. Zhao et al. [2014a] confirmed that the content of polyphyllin I and total content of polyphyllin in *Paris polyphylla* Smith var. *yunnanensis* (Franch.) Hand.-Mazz (PPY) samples were diverse among central, southwestern, southeastern and northwestern Yunnan, which might be affected by vertical climatic features, temperature, rainfall etc. Hence, the provenance was another important factor to affect herbal medicine quality. Therefore, exploring the chemical profiles differences among different provenances of herbal medicines were needed.

Paris genus plants were highly valued, which was mainly distributed in tropics and temperate regions, such as India, Bhutan, Laos, Myanmar, Nepal, Sikkim, Thailand et al. [Sharma et al. 2015]. This genus was precisely divided into 24 species and 22 species of them were confirmed distribution in China [Wu et al. 2017a]. PPY, as one of the representatives of this genus, was common and main resource of Paridis in China. It was mainly scattered in southwestern China, particularly in Yunnan, Guangxi and Sichuan Provinces [Yang et al. 2015]. Modern pharmacological researches uncovered the potential effectiveness of anti-Alzheimer's disease, antioxidant, anti-bacterial and anti-cancer (such as breast cancer, lung adenocarcinoma, cervical cancer etc.) [Lee et al. 2005, Yan et al. 2009, Tohda et al. 2012, Shen et al. 2014, Zhang et al. 2014b]. However, these latent diversity of pharmacological effects pushed the increasing demand of PPY and induced the wild products of this species on the edge of vulnerability [Sharma et al. 2015]. Hence, the artificial cultivation was carried out to meet the market demands and to protect the wild species escaped from extinction. However, the cultivation was subjected to a lack of scientific guidance. For instance, the provenance screening and irrigation were usually depended on experiments, which might conclude that the normal growth of cultivated plants has been affected [Li et al. 2008]. Furthermore, the ambiguous provenance might influence the clinical effects. Additionally, in previous study, few reports illustrated the effect of water stress on the PPY growth and secondary metabolites accumulation and the relationships among each other. Thus, exploring the metabolites variation in water stress condition and multi-provenance PPY samples was indispensable.

In the present study, a comprehensive analysis for PPY samples (85 batches) was carried out by high performance liquid chromatograph (HPLC) and Fourier transform mid-infrared spectroscopy (FT-MIR). All samples belonged to three provenances (Jinping, Luquan and Weixi) and submitted to three levels of water stress. Therefore, exploring the effect of water stress on PPY samples was a major justification in present study. Meanwhile, it might provide a preliminary foundation for provenance screening of PPY cultivation.

MATERIALS AND METHODS

Plant material habitat conditions and treatments

Plant materials. The mature and dry seed provenance source materials of this experiment were obtained from three different districts in Sept. 2011 (Tab. 1A). The seeds were identified as *P. polyphylla* Smith var. *yunnanensis* (Franch.) Hand.-Mazz by Professor Hang Jin (Institute of Medicinal Plants, Yunnan Academy of Agricultural Sciences). During Oct. 2012, these seeds were sown and cultivated in the same unit until three-year-old. To assure the representativeness, randomly selected thirty samples (similar thriving) from the same provenance and cultivated in three soil moisture level units were averaged, i.e. each factor dealt with 10 plants. The total number of plants was 90 (3 × 3 × 10). However, to a different degrees, a part of the provenance samples from Jinping died. Finally, the sample numbers of 0.80 field capacity (FC), 0.70 FC and 0.50 FC for Jinping samples were 8, 8 and 9, respectively.

Plant habitat conditions. This study was conducted in greenhouse and carried out during Dec. 2015 to Sept. 2017 at the resource nursery of Institute of Medicinal Plants of Yunnan Agricultural Academy China, which was located at 25°09'47.28"N and 104°59'59.98"E and the altitude 1294 m above sea level. Additionally, the average annual temperature and average annual precipitation were 15.5°C and 979 mm, respectively. The field cultivation experiments were conducted among samples separately. The area of each experimental unit was 4.2 m^2 (3.5 × 1.2 m) and each unit was covered by mixed soil with 30 cm depth. A soil moisture-meter Time Domain Reflectometry-3000 (TDR-3000) was embedded in the mixed soil to 15 cm depth to monitor the real-time moisture content. The physiochemical properties of soil in this experiment were characterized as sandy soil and humus and the percentages were 70 and 30%, respectively.

Water treatments. To evaluate and compare the impact of water on the biomass and accumulation of polyphyllin content, the factor of soil moisture was applied as follow: 0.80 FC (full irrigation), 0.70 FC (moderately water stress) and 0.50 FC (severe water stress). The maximum soil FC was determined by the initial soil moisture content. During the period of experiment, the soil moisture was timely added to the predetermined target value, which was detected by TDR-3000 every day. Under this condition, samples were dealt with rehydration before harvesting (24 h).

Reagents. The reference compounds of polyphyllin I, polyphyllin II and polyphyllin VII (PPI, PPII and PPVII, respectively) with 98% purity were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Chromatographic grade of acetonitrile and methanol were provided by Thermo Fisher Scientific (Massachusetts, USA) and the formic acid was purchased at DikmaPure (Beijing, China). The water for extraction and HPLC analysis was purified using a UPTL-II-40L system from Ulupure (Chengdu, China). The analytical grade methanol used for extraction was purchased from Tianjin Fengchuan Chemical Reagent Technologies Co., Ltd. (Tianjin, China).

Sample preparation and chromatographic conditions. Fresh samples were cleaned and washed with water and different tissues of rhizome, stem and leaf were dried at 50°C to constant weight by electric thermostatic drying oven (Experimental Instrument Factory, Shanghai, China). Following step was to weigh each dried tissue sample by electronic analytical balance (AR1140, Ohaus electronic analytical balance, Shanghai, China) and record the dry weight data. After that, each sample was disintegrated into powder by DFY-500 kibbler (Wenling City Forest Machinery, Zhejiang, China) and screened by 100-mesh stainless steel sieve. Powders were protected from light and stored at room temperature using labeled Ziploc bags until further analysis.

0.1000 ±0.0003 g of each sample powder was precisely weighted by electronic analytical balance (AR1140), powders of which were put into a cuvette and soaked with 2 mL 80% methanol, successively. The mixed suspension liquid was extracted by ultrasounds for 40 min at 150 W in the water bath at 30°C. Then the extracting solution was removed and cooled to the room temperature and filtered through a 0.22 μm membrane filter before injecting into the HPLC system for analysis.

Table 1A. Provenance information for *P. polyphylla* Smith var. *yunnanensis* samples

No.	Description	Provenance	Latitude	Longitude	Elevation (m)	Average annual temperature (°C)	Average annual precipitation (mm)
1–10	0.80 FC	Jinping Honghe	22°47'08.31"N	105°00'00.18"E	1299	18.2°C	1600
11–20	0.70 FC						
21–30	0.50 FC						
31–40	0.80 FC	Weixi Diqing	27°7'35.48"N	110°13'35.56"E	1079	11.8°C	2700
41–50	0.70 FC						
51–60	0.50 FC						
61–68	0.80 FC	Luquan Kunming	25°33'48.31"N	105°00'00.03"E	1631	15.2°C	967
69–76	0.70 FC						
77–85	0.50 FC						

FC – field capacity

Table 1B. Method validation of polyphyllin VII, polyphyllin I and polyphyllin VII

Components	Stability (n = 7)		Intraday precision (n = 3)		Interday precision (n = 3 × 3)		Recovery (n = 3)		Equation	R^2	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
	Rt (%)	*Pa* (%)	*Rt* (%)	*Pa* (%)	*Rt* (%)	*Pa* (%)	Meamt (%)	RSD (%)				
Polyphyllin VII	0.09	1.19	0.10	4.52	0.05	2.40	94.21	3.26	Y = 1687X + 14.924	0.99	26.10	87.01
Polyphyllin I	0.03	2.05	0.10	2.57	0.16	4.30	94.89	2.14	Y = 4793.5 – 0.7049	0.99	11.37	37.91
Polyphyllin II	0.15	4.50	0.02	3.17	1.17	4.91	96.11	5.61	Y = 4418.2X + 33.059	0.99	13.05	43.50

Rt – retention time, *Pa* – peak area, R^2 – determination coefficient, LOD – limit of detection, LOQ – limit of quantitation

The quantitative analyses were carried out on Agilent 1260 infinity HPLC system, which was coupled with auto-sampler, diode array detector (DAD) and quaternary pump. Analyses were performed with an Agilent Intersil-C18 column (4.6 × 150 mm, 5 µm) (Japan GL Sciences Company). The mobile phase consisted of 0.5% formic acid aqueous solution (A) and acetonitrile (B) with a rate of 0.5 mL/min. The gradient elution procedure was set as follows: 0–10 min, 20–25% B; 10–19 min, 25–35% B; 19–29 min, 35–37% B; 29–31 min, 37–52% B; 31–36 min, 52–55% B; 36–48 min, 55–70% B; 48–54 min, 70–79% B; 54–55 min, 90–90% B and finally holding 20% B for 10 min. The column temperature was set at 32°C and the injection volume for each sample was 10 µL. The target compounds were detected at the wavelength of 203 nm.

FT-MIR spectra acquisition. A Nicolet 380 Fourier transform mid-infrared spectroscopy (Thermo Fisher Scientific Inc., Madison, USA) coupled with deuterated triglycine sulfate (DTGS) detector was used to obtain FT-MIR spectra. A horizontal attenuated total reflectance (ATR) sampling accessory equipped with diamond crystal was employed for the measurement. Approximately 0.1 g sample powder was placed on the sampling accessory (a metal O-ring) and regulated pressure tower around to a consistent pressure (134). Prior to analysis, an empty receiver was used to record the background spectrum for eliminating interferences of the laboratory air. Spectra were recorded in the range of 4000–550 cm^{-1} at a resolution of 4 cm^{-1} and each spectrum was scanned successively 16 times. Each sample was analyzed in triplicate and the averaged spectrum was used to data preprocess and analyze. Laboratory environment was maintained at constant temperature (25°C) and humidity (30%) in this study.

Data analysis. The initial chromatography data sets were transported out from Agilent station in the form of PDF files. Besides, FT-MIR spectra were transformed into .sp data sets using OMNIC 8.2 (Thermo Fisher Scientific, USA) for further processing. In the present study, each spectral variables were reduced from 1789 to 1323 by the treatment of excluding the spectral regions 4000–3700 cm^{-1} and 2400–1800 cm^{-1}. These regions did not provide relevant information but interference information, such as the absorbance of CO_2 and diamond crystal [Horn et al. 2018, Nunes et al. 2016]. Then the remaining variables coupled with number of samples were used to establish a two dimensional matrix, i.e. (1323 × 85). Then, processing methods, such as multivariant scattering correction (MSC), derivative and Savitzky-Golay (SG), were carried out by SIMAC-P^+ 13.0 successively [Escamilla et al. 2013, Li, Wang, Xu 2006, Li, Zhang, Wang 2017]. After pretreatment, the matrix consisted of 1295 × 85 data and these sets were chosen for the later chemometrics analysis.

One-way analysis of variance (ANOVA) was carried out to explore the chemical composition differences among samples from different tissues and provenances, attempting to explore the effect of soil moisture and provenance on polyphyllin content. It was analyzed by the package of "Multcomp" and 95% confidence intervals were assumed. Correlation analysis (CA) was used to study the relationship of both biomass and chemical compositions in different tissues, chemometrics analyses of which were created by the package of "Hmisc". Making a further insight into the provenance similarities and differences among Jinping, Weixi and Luquan, a visual method of principal component analysis (PCA) was chosen and it was executed by the package of "FactoMineR". In this study, the chemometrics analysis of ANOVA and CA included the biomass and polyphyllin content data matrices, and the PCA was used for the processed FT-MIR data sets. The analysis of ANOVA, CA, and PCA were performed by RStudio 3.4 software.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions and method validation. To achieve a satisfying separation of chemical compounds, some of HPLC parameters were adjusted on the base of official standards [Chinese Pharmacopoeia 2015]. In this study, acetonitrile was selected as the organic eluent as mobile phase system and 0.1% formic acid water solution as the water phase eluent, formic acid of which added for improvement of the peak shape of chromatogram during analyzing the samples. Additionally, gradient elution mode was optimized appropriately to separate

polyphyllin compounds completely. After optimization of chromatographic conditions, three target peaks were presented within 55 min when the column temperature was 32°C. The detection wavelength was set at 203 nm because of the characteristic absorption of polyphyllin compounds at this wavelength. Sample preparations were performed as in the former study, i.e. 80% methanol as the extraction solvent and following extraction conditions were selected: water-bath heated with ultra sonication extraction for 40 min [Yang et al. 2017].

The extraction solution stability was estimated by re-analyzing the same sample after 0, 4, 8, 12, 16, 20 and 24 h. Relative standard deviation (RSD %) of retention time (*Rt*) and peak area (*Pa*) show a better precision (RSD % < 4.50%) of the extraction stability. The *Rt* and *Pa* RSD % values of intra-day and inter-day are presented within an acceptable range (RSD % < 4.91%) and indicated that this method is reliable and efficient. Satisfactory recovery results reveal that this HPLC method is precise and accurate for PPI, PPII and PPVII analysis. Besides, the external standard method is used to calculate the content of above components in PPY samples. The calibration curves of these three components presented a good linearity ($R^2 > 0.99$). The range of detection limit (LODs) and quantification limit (LOQs) for PPI, PPII and PPVII are from 11.37–37.91 μg mL^{-1}, 13.05–43.50 μg mL^{-1} and 26.10–87.01 μg mL^{-1}, respectively. Results mentioned above are shown in Table 1B. All results showed that this HPLC method is satisfactory for PPY samples analysis.

The effect of soil moisture on variation of polyphyllin content

Quantification analysis. The optimized HPLC method was applied to quantify the content of PPI, PPII, PPVII and total polyphyllin content (TPP) in PPY samples. The polyphyllin content is summarized in Table 2A and the results are presented as means ± standard deviation (SD). Visually, PPVII and TPP in Jinping samples show the increasing tendency with the increasing water stress levels and the highest content is shown in 0.50 FC. The highest content of PPVII and PPII in rhizome and leaf tissues for Luquan samples are inclined to be present in 0.70 FC. Samples collected from Weixi reveal that the highest content of PPVII and TPP is in 0.50 FC. Compounds of PPI were not detected in stem tissues with the treatment of 0.50 FC in Weixi provenance samples. However, the highest content of this components was shown in rhizome tissues, which might be induced by nearly complete resistance in the extreme water stress condition [Verma and Shukla 2015]. The biomass of rhizome, stem and leaf tissues in Jinping samples shows a decreasing tendency with the increasing water stress levels, suggesting Jinping samples are unfit for cultivating in drought district. The biomass for Luquan and Weixi samples reveals the tendency of firstly increase and then decrease with the increasing water stress levels and the highest value are presented at 0.70 FC, which implied that the 0.70 FC might be the optimal condition for their planting.

One-way analysis of variance. One-way analysis of variance (ANOVA), as one of the classical analysis methods, was used to compare the significance level for different soil moisture factors on the content of polyphyllin in PPY samples, results of which are shown in Table 2. For rhizome parts, all samples presented insignificant difference among 0.80, 0.70 and 0.50 soil moisture. For the leaf and stem tissues, Weixi and Luquan samples were tending to show insignificant difference except from PPVII and TPP in Luquan samples, which implied that soil moisture had a significant effect on these two components synthesis and accumulation for Luquan samples. The polyphyllin content in stem and leaf tissues for Jinping samples revealed significant difference ($P < 0.05$) with the treatment of 0.80, 0.70 and 0.50 FC (except from PPI in leaf tissues), suggesting that Jinping provenance samples are sensitive to soil moisture and this may be influenced by genotype and endogenous hormones [Gharibi et al. 2016, Zhang et al. 2017]. Besides, biomass for Jinping and Weixi samples shows significant difference ($P < 0.05$) for different soil moisture, while the Luquan samples showed insignificant difference, suggesting Luquan samples may have a stable ability to overcome the water stress condition. ANOVA verified the results of quantification analysis, i.e. the soil moisture can affect the biomass and polyphyllin synthesis and accumulation, especially for Jinping provenance samples.

Table 2A. Effect of different field capacity on polyphyllin content (mean ± standard deviation)

Specification	Part of plant	80 FC	70 FC	50 FC	80 FC	70 FC	50 FC	80 FC	70FC	50FC
PPVII	rhizome	2.13 ±0.67 ^{A}	2.39 ±2.87 ^{A}	3.20 ±1.50 ^{A}	2.24 ±0.96 ^{A}	5.59 ±8.23 ^{A}	1.74 ±0.59 ^{A}	3.18 ±3.49 ^{A}	3.05 ±0.72 ^{A}	2.01 ±1.41 ^{A}
	stem	1.67 ±0.99 ^{A}	1.84 ±1.80 ^{A}	4.89 ±1.95 ^{B}	3.88 ±2.33 ^{B}	*N*	2.52 ±1.67 ^{A}	6.49 ±2.29 ^{A}	4.69 ±2.87 ^{A}	7.21 ±2.20 ^{A}
	leaf	0.59 ±0.35 ^{A}	1.56 ±1.07 ^{AB}	2.89 ±2.76 ^{B}	2.18 ±0.86 ^{B}	1.66 ±0.65 ^{AB}	0.72 ±0.27 ^{A}	18.48 ±20.54 ^{A}	18.73 ±22.62 ^{A}	22.73 ±36.73 ^{A}
PPI	rhizome	1.59 ±3.00 ^{A}	1.19 ±3.98 ^{A}	1.61 ±3.32 ^{A}	0.75 ±0.50 ^{A}	0.66 ±0.41 ^{A}	1.77 ±3.09 ^{A}	0.65 ±0.41 ^{A}	0.86 ±0.53 ^{A}	3.34 ±8.05 ^{A}
	stem	0.26 ±0.08 ^{A}	0.33 ±0.13 ^{A}	0.37 ±0.08 ^{B}	0.44 ±0.12 ^{A}	0.44 ±0.08 ^{A}	0.40 ±0.05 ^{A}	0.29 ±0.16 ^{A}	0.28 ±0.09 ^{A}	*N*
	leaf	0.34 ±0.10 ^{A}	0.44 ±0.11 ^{A}	0.42 ±0.09 ^{A}	1.46 ±0.49 ^{A}	1.34 ±0.39 ^{A}	1.27 ±0.60 ^{A}	0.37 ±0.14 ^{A}	0.42 ±0.13 ^{A}	0.38 ±0.15 ^{A}
PPII	rhizome	2.51 ±1.18 ^{A}	2.24 ±0.85 ^{A}	1.87 ±0.69 ^{A}	0.74 ±0.99 ^{A}	1.22 ±1.75 ^{A}	0.52 ±0.66 ^{A}	1.00 ±1.32 ^{A}	0.94 ±0.92 ^{A}	1.27 ±1.98 ^{A}
	stem	*N*	*N*	*N*	0.19 ±0.21 ^{A}	0.32 ±0.49 ^{A}	0.83 ±1.00 ^{A}	*N*	*N*	*N*
	leaf	0.38 ±0.46 ^{A}	*N*	*N*	3.37 ±3.56 ^{A}	8.20 ±7.52 ^{A}	5.78 ±4.08 ^{A}	0.13 ±0.03 ^{A}	0.09 ±0.10 ^{A}	0.02 ±0.02 ^{A}
TPP	rhizome	6.23 ±3.02 ^{A}	6.54 ±5.19 ^{A}	6.68 ±3.78 ^{A}	4.11 ±1.28 ^{A}	7.94 ±9.75 ^{A}	4.28 ±3.95 ^{A}	4.83 ±3.39 ^{A}	4.85 ±1.57 ^{A}	6.63 ±11.20 ^{A}
	stem	1.78 ±1.01 ^{A}	2.04 ±1.76 ^{A}	5.23 ±1.94 ^{B}	4.45 ±2.28 ^{B}	0.76 ±0.46 ^{A}	3.10 ±2.59 ^{AB}	6.54 ±2.28 ^{A}	4.81 ±2.79 ^{A}	7.21 ±2.20 ^{A}
	leaf	0.96 ±0.54 ^{A}	1.96 ±1.14 ^{AB}	2.29 ±2.68 ^{B}	6.74 ±3.36 ^{A}	8.53 ±7.79 ^{A}	6.73 ±4.08 ^{A}	18.89 ±20.49 ^{A}	19.21 ±22.58 ^{A}	23.12 ±36.70 ^{A}
biomass	rhizome	2.47 ±0.72 ^{B}	2.30 ±0.86 ^{B}	1.53 ±0.41 ^{A}	3.00 ±1.56 ^{A}	3.64 ±1.49 ^{A}	2.74 ±1.27 ^{A}	3.84 ±0.99 ^{AB}	5.17 ±1.95 ^{B}	2.33 ±0.62 ^{A}
	stem	0.69 ±0.14 ^{B}	0.59 ±0.17 ^{B}	0.32 ±0.09 ^{A}	0.31 ±0.20 ^{A}	0.42 ±0.21 ^{A}	0.21 ±0.18 ^{A}	0.48 ±0.12 ^{AB}	0.85 ±0.53 ^{B}	0.31 ±0.21 ^{A}
	leaf	0.71 ±0.22 ^{B}	0.57 ±0.22 ^{B}	0.32 ±0.13 ^{A}	0.36 ±0.20 ^{A}	0.39 ±0.17 ^{A}	0.31 ±0.22 ^{A}	0.52 ±0.09 ^{B}	0.68 ±0.21 ^{C}	0.31 ±0.04 ^{A}

FC – field capacity, PPVII – polyphyllin VII, PPI – polyphyllin I, PPII – polyphyllin II and TPP – total content of polyphyllin, *N* – not detected in samples
Different letters of A, B and C within a row indicate significant differences for the same tissue and provenance among different field capacity treatment at $P < 0.05$

Table 2B. The characteristic wavenumber variables factors of the first 20 for each category with principal component analysis

No.	0.80 FC rhizome	0.80 FC stem	0.80 FC leaf	0.70 FC rhizome	0.70 FC stem	0.70 FC leaf	0.50 FC rhizome	0.50 FC stem	0.50 FC leaf
1	2873	872	818	607	661	648	637	656	606
2	2875	874	835	609	866	816	727	658	607
3	2877	885	1063	611	868	818	1374	660	609
4	2879	887	1065	613	883	1084	1375	665	833
5	2881	889	1066	1020	885	1086	1381	667	1055
6	2883	955	1082	1022	887	1088	1383	679	1057
7	2939	957	1084	1024	889	1090	1390	837	1059
8	2941	958	1086	1070	891	1092	1444	850	1084
9	2943	960	1088	1182	1003	1111	2835	964	1086
10	2956	1095	1090	1184	1005	1113	2858	1084	1088
11	2958	1452	1092	1186	1165	1115	2885	1086	1090
12	2970	1454	1093	1712	1167	2648	2887	1095	1092
13	2972	1456	1111	1714	1169	2650	2929	1097	1093
14	2974	1618	1113	1716	1171	2652	2949	1099	1429
15	2976	1620	1115	2669	1581	2667	2951	1101	1431
16	2978	1622	1425	2670	1583	2669	2952	1410	2667
17	2979	1624	1427	2673	1585	2671	2964	1462	2669
18	2981	1626	1429	2675	1587	2673	2965	1464	2671
19	2983	1628	1431	3001	1641	2675	2968	1724	2817
20	2985	1709	1433	3641	1643	2677	3016	3610	2819

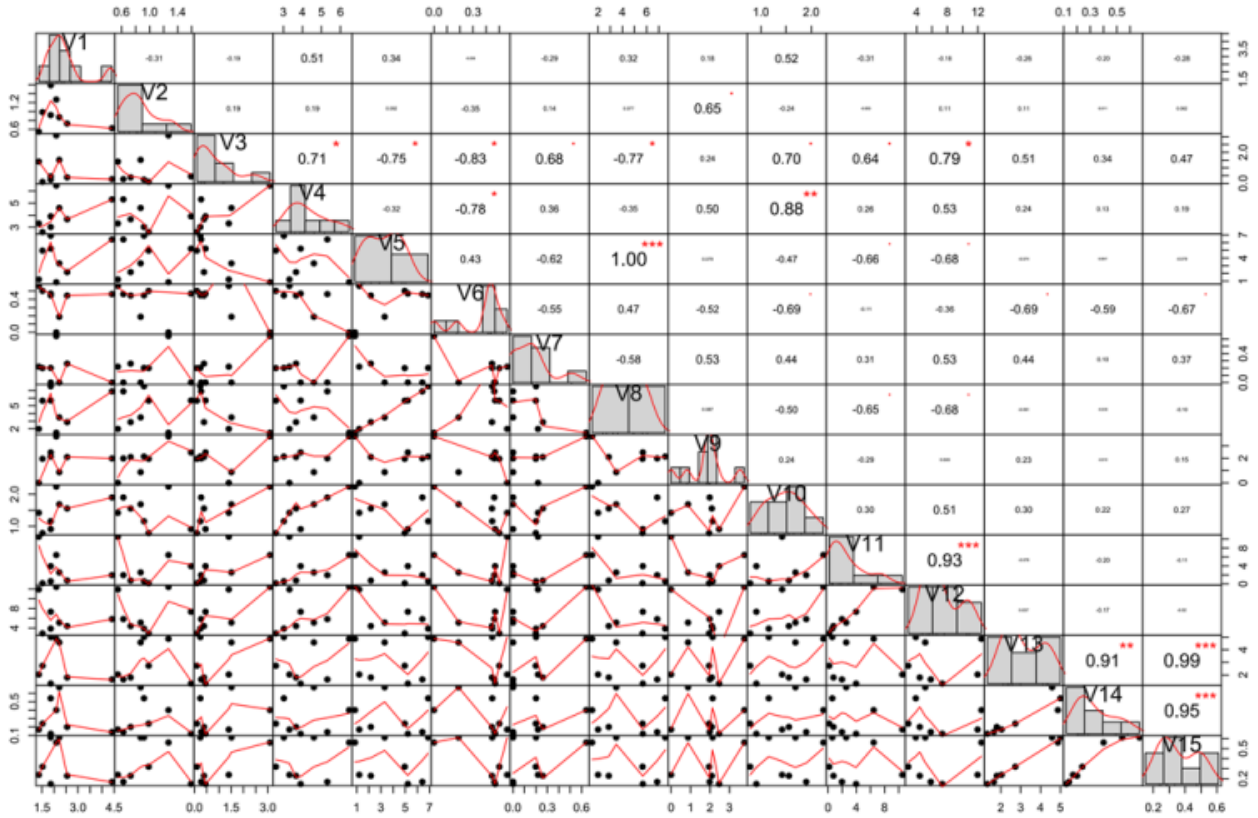
FC – field capacity

Correlation analysis among different tissues. In order to further investigate the relationship among these targets (PPVII, PPI, PPII and TPP in each tissue, separately, and the biomass of rhizome, stem and leaf tissues), Person's correlation analysis was employed to all data for different provenances under the conduction of multiple water stress, separately. Statistical evaluation shows a dramatic correlation. From the Figure 1A, Luquan samples treated with 0.80 FC appear a positive relationship ($P < 0.05$) between PPI in leaf and TPP in rhizome, results of which imply that the PPI synthesis (accumulation) more in the leaf is beneficial to the synthesis (accumulation) of polyphyllin in rhizome. For the treatment of 0.70 FC, Jinping samples display a significant positive relationship ($P < 0.01$) between PPVII in rhizome and the PPVII and TPP in stem tissues (Fig. 1B). Similarly, the polyphyllin in leaf of Luquan 0.70 FC samples reveals a positive relationship ($P < 0.05$) with the polyphyllin in stem tissues (Fig. 1C). Above analysis validated the relationship of polyphyllin transition among leaf, stem and rhizome parts, i.e. the polyphyllin is synthesized in leaf parts and remobilized to rhizomes for storing [Yu et al. 2009]. Besides, the biomass of rhizome and leaf presents a positive relationship ($P < 0.05$) with polyphyllin in rhizome parts for Jinping samples submitted with 0.70 FC (Fig. 1B), suggesting that higher weight of biomass is beneficial for polyphyllin synthesis and accumulation. For the treatment of 0.50 FC, a significant positive relationship ($P < 0.01$) among PPVII, PPI and PPII is shown in rhizome tissues of Weixi samples (Fig. 1D). Wang and Li [2018] confirmed that the PPI, PPII, PPV, PPVI, PPVII and gracillin in the rhizome parts revealed a significant positive relationship ($p < 0.01$), which was verified in this study. However, the correlations among targets are presented as unreadable in Jinping samples with 0.50 FC treatment. A negative correlation ($P < 0.05$) of polyphyllin content between aerial parts and rhizome are revealed (Fig. 1E). Additionally, the PPI between stem and rhizome also show negative correlation ($P < 0.05$). These contrasting relationship may occur because of the competitive relationship between diosgenin (PPI and PPII) and pennogenin saponins (PPVII). Huang et al. [2009] concluded that differences between diosgenin saponins and pennogenin saponins are sugar parts and the hydroxyl group at C-17 and the former are the precursors of pennogenine saponins. Besides, the severe lack of water situations may induce the diverse genetic responses [Borges et al. 2018]. Thus, the shift in soil moisture might lead to the comparative relationship occurred and PPY samples have to self-adjust to overcome the extreme environment. The biomass of rhizome, stem and leaf showed more or less positive correlation, which coincides with the growth rhythm and previous study [Zhao et al. 2014b].

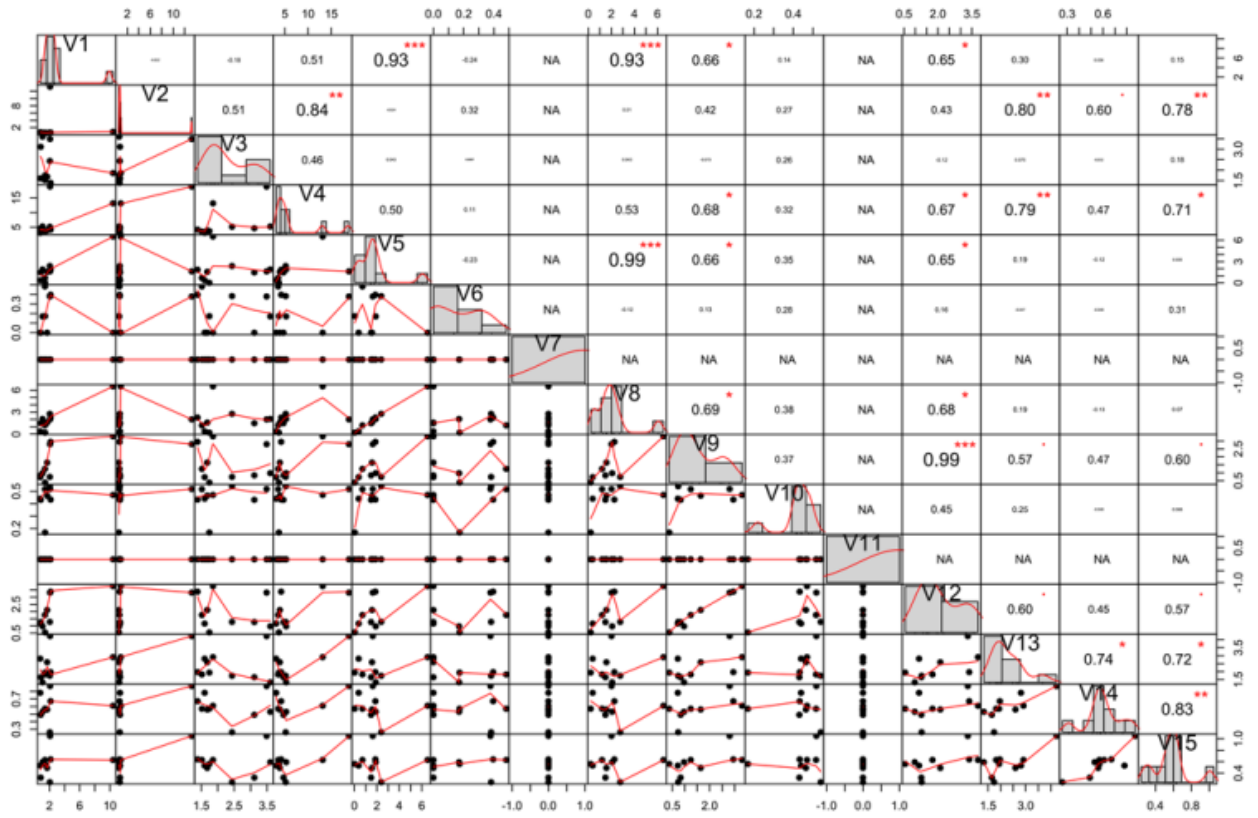
The provenance effect on variation polyphyllin content

Quantification analysis. Provenance is also the key pattern to influence the chemical profiles. From Table 3, it follows that the highest content of PPI and PPII in stem and leaf tissues is shown in Luquan samples in the treatment of 0.80, 0.70 and 0.50 FC. But the enriched content of PPVII and TPP in stem and leaf tissues is presented in Weixi samples. These interesting phenomena mean that the provenance (genetic information) might be the key to adjust the polyphyllin accumulation and transformation, or the soil moisture induced the genetic response diversely [Borges et al. 2017, Srivastava et al. 2018]. Additionally, the leaf tissues of Weixi samples are tending to accumulate more polyphyllin than rhizome and stem parts. Previous studies revealed that the leaf (chloroplasts) tissues for PPY samples might be the main place to synthesize steroid saponins, and then transmitted to rhizome storing through stem tissues [Feng et al. 2015, Liu et al. 2016]. The polyphyllin content in rhizome tissues show a dynamic changes. The highest content of PPII and TPP in rhizome is inclined to show in Jinping provenance samples. In the previous research, Zhao et al. [2014a] and Yang et al. [2017] confirmed that rhizome samples collected from southeast Yunnan accumulate more polyphyllin than other regions, which was in accord with the result of this study, i.e. Jinping (belonged to southeast Yunnan) samples own the highest TPP in rhizome tissues with the treatment of 0.80 and 0.50 FC. These results suggested that samples obtained from Jinping might be one of the potential high quality germplasm.

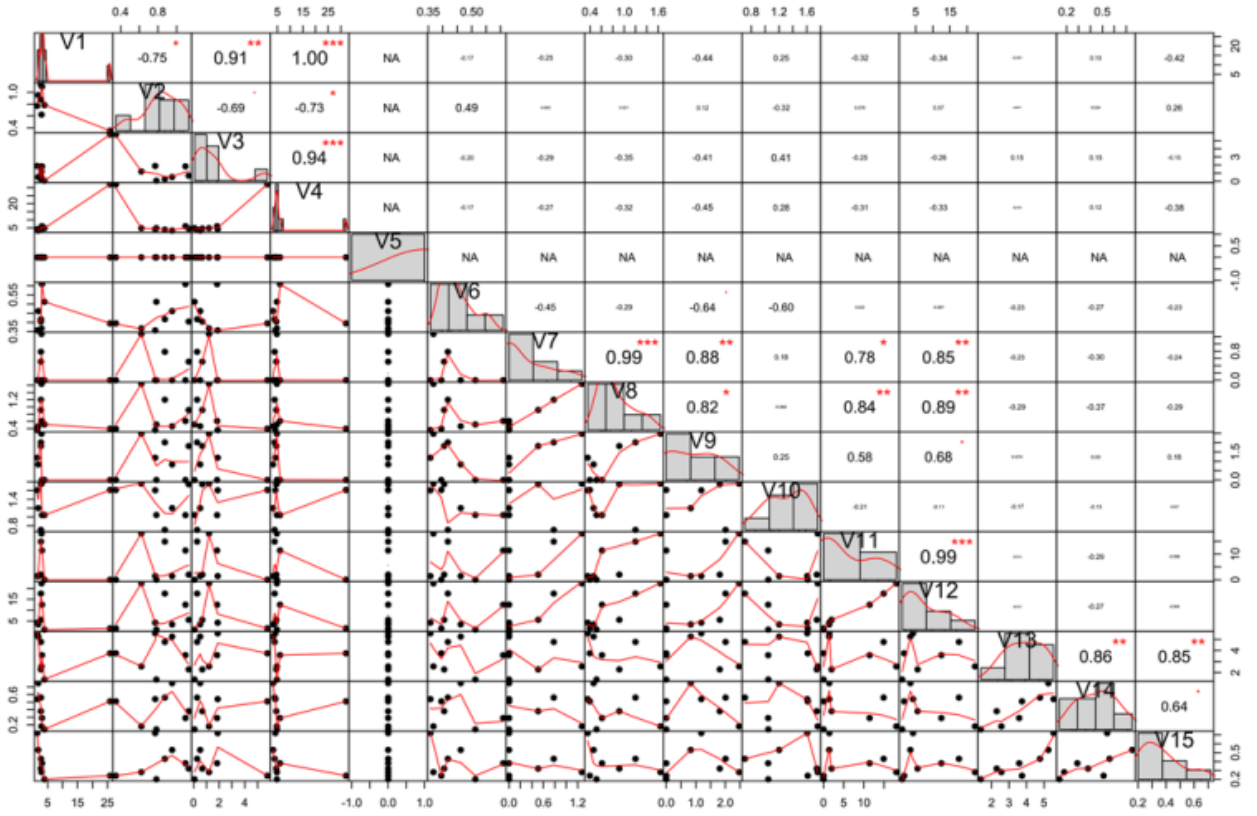
A


V1
V2
V3
V4
V5
V6
V7
V8
V9
V10
V11
V12
V13
V14
V15

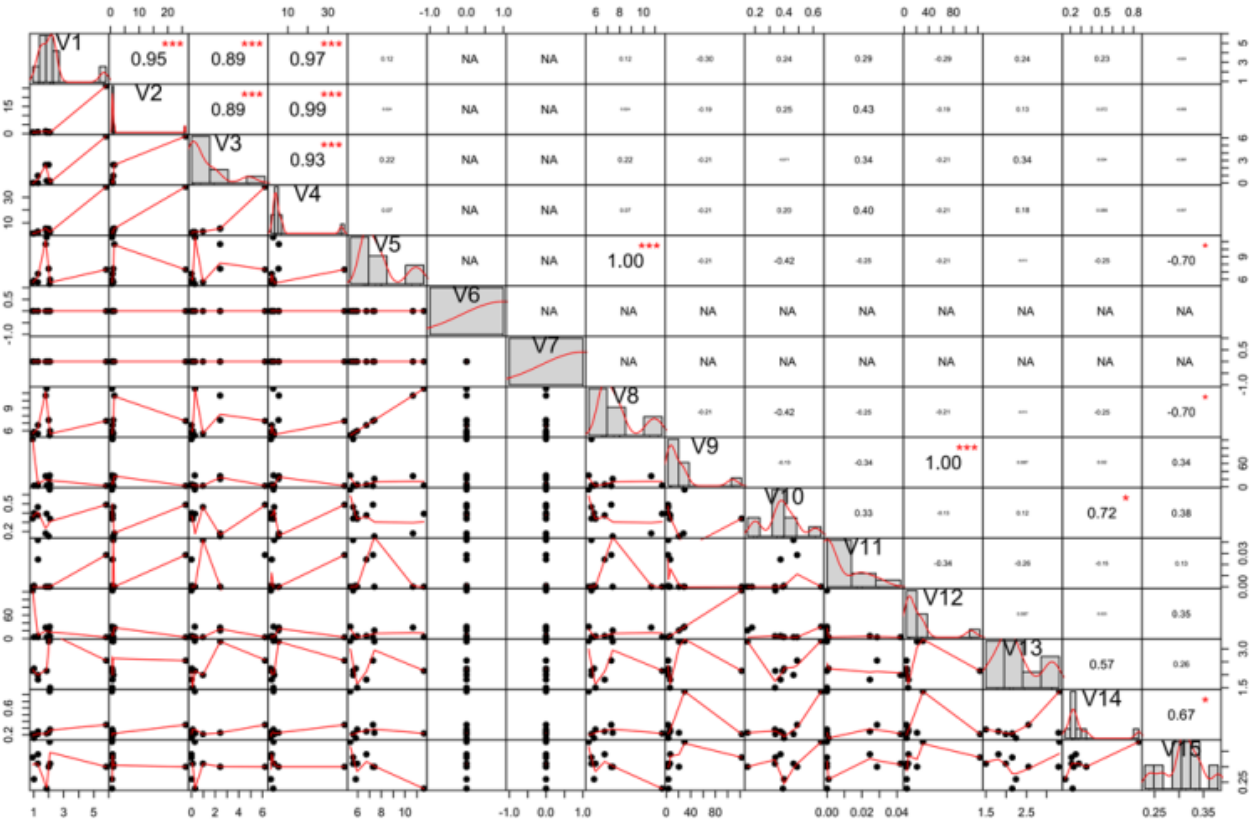
B


V1
V2
V3
V4
V5
V6
V7
V8
V9
V10
V11
V12
V13
V14
V15

C


V1
V2
V3
V4
V5
V6
V7
V8
V9
V10
V11
V12
V13
V14
V15

D


V1
V2
V3
V4
V5
V6
V7
V8
V9
V10
V11
V12
V13
V14
V15

E

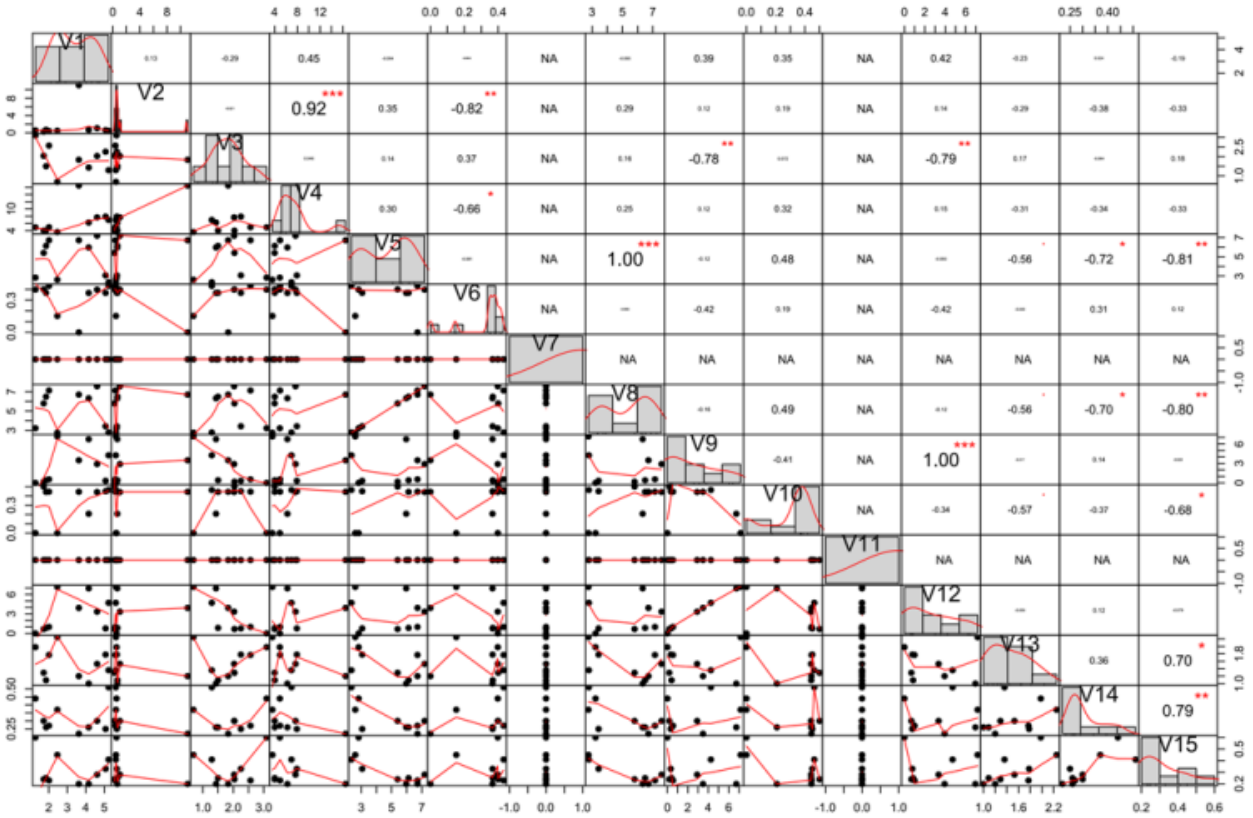


Fig. 1. Correlation among PPVII, PPI, PPII, TPP and biomass for each rhizome, stem and leaf tissue collected from same provenance with different field capacity treatment: 1A. 0.80 FC for Luquan samples; 1B. 0.70 FC for Jinping samples; 1C. 0.70 FC for Luquan samples; 1D. 0.50 FC for Weixi samples; 1E. 0.50 FC for Jinping samples

The abbreviations of V1–V12 represent the polyphyllin VII, polyphyllin I, polyphyllin II and total content of polyphyllin in rhizome, stem and leaf tissues, separately, and the V13–V15 represent the biomass of rhizome, stem and leaf, respectively. Note: * and ** represent significant correlation at $P < 0.05$ and $P < 0.01$, respectively

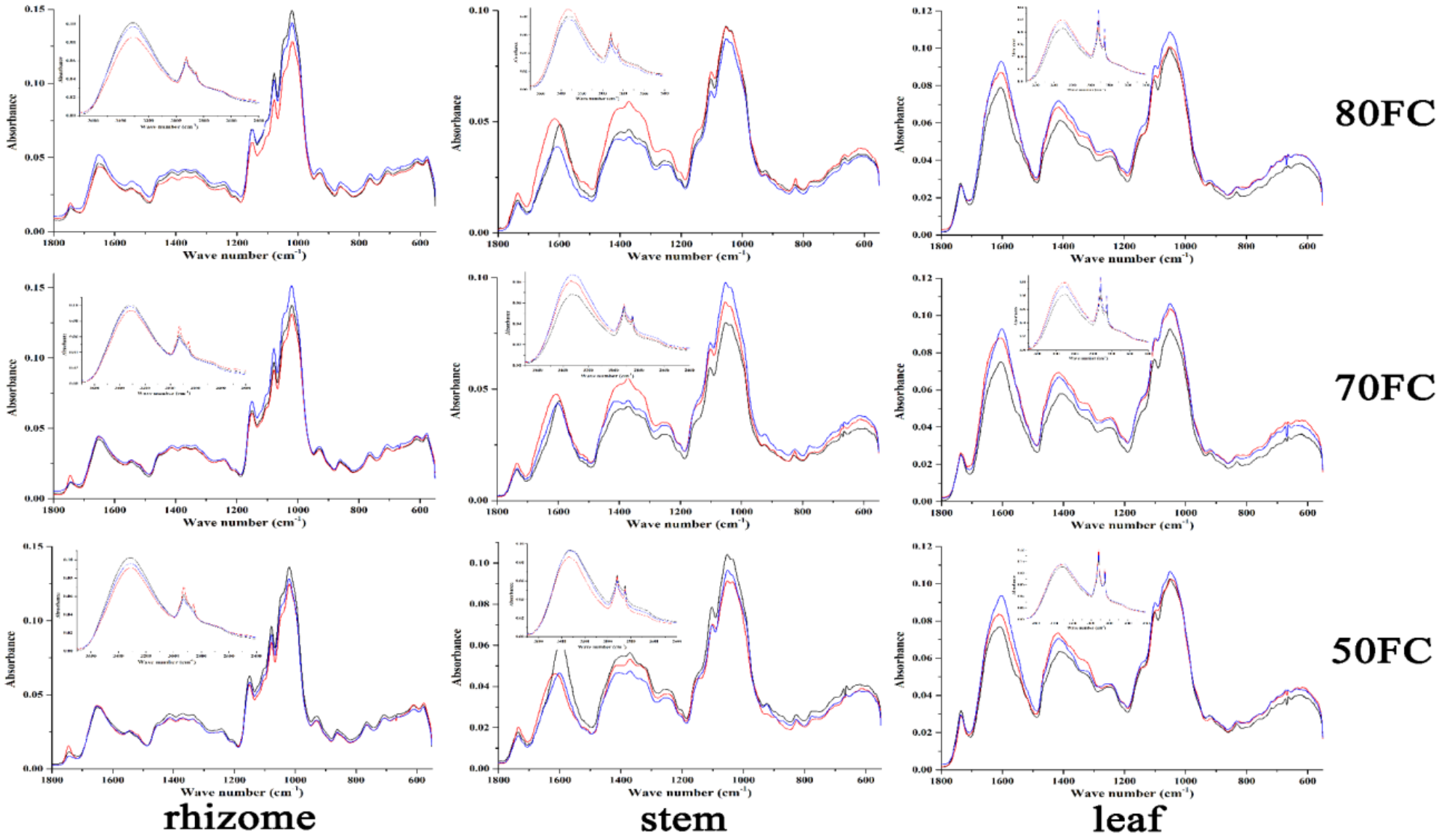


Fig. 1F. Raw FT-MIR spectra of *Paris polyphylla* Smith var. *yunnanensis* samples

Table 3. Effect of different provenance on polyphyllin content (mean ± standard deviation)

Speci-fication	Part of plant	0.80 FC			0.70 FC			0.50 FC		
		Jingping	Luquan	Weixi	Jingping	Luquan	Weixi	Jingping	Luquan	Weixi
PPVII	rhizome	2.13 ±0.67 [a]	2.24 ±0.96 [a]	3.18 ±3.49 [a]	2.39 ±2.87 [a]	5.59 ±8.23 [a]	3.05 ±0.72 [a]	3.20 ±1.50 [b]	1.74 ±0.59 [a]	2.01 ±1.41 [ab]
	stem	1.67 ±0.99 [a]	3.88 ±2.33 [a]	6.49 ±2.29 [a]	1.84 ±1.80 [a]	*N*	4.69 ±2.87 [b]	4.89 ±1.95 [a]	2.52 ±1.67 [a]	7.21 ±2.20 [b]
	leaf	0.59 ±0.35 [a]	2.18 ±0.86 [a]	18.48 ±20.54 [b]	1.56 ±1.07 [a]	1.66 ±0.65 [ab]	18.73 ±22.62 [b]	2.89 ±2.76 [a]	0.72 ±0.27 [a]	22.73 ±36.73 [a]
PPI	rhizome	1.59 ±3.00 [a]	0.75 ±0.50 [a]	0.65 ±0.41 [a]	1.19 ±3.98 [a]	0.66 ±0.41 [a]	0.86 ±0.53 [a]	1.61 ±3.32 [a]	1.77 ±3.09 [a]	3.34 ±8.05 [a]
	stem	0.26 ±0.08 [a]	0.44 ±0.12 [b]	0.29 ±0.16 [a]	0.33 ±.013 [a]	0.44 ±0.08 [b]	0.28 ±0.09 [a]	0.37 ±0.08 [a]	0.40 ±0.05 [a]	*N*
	leaf	0.34 ±0.10 [a]	1.46 ±0.49 [b]	0.37 ±0.14 [a]	0.44 ±0.11 [a]	1.34 ±0.39 [b]	0.42 ±0.13 [a]	0.42 ±0.09 [a]	1.27 ±0.60 [b]	0.38 ±0.15 [a]
PPII	rhizome	2.51 ±1.18 [b]	0.74 ±0.99 [a]	1.00 ±1.32 [a]	2.24 ±0.85 [a]	1.22 ±1.75 [a]	0.94 ±0.92 [a]	1.87 ±0.69 [a]	0.52 ±0.66 [a]	1.27 ±1.98 [a]
	stem	*N*	0.19 ±0.21	*N*	*N*	0.32 ±0.49	*N*	*N*	0.83 ±1.00	*N*
	leaf	0.38 ±0.46 [a]	3.37 ±3.56 [b]	0.13 ±0.03 [a]	*N*	8.20 ±7.52 [b]	0.09 ±0.10 [a]	*N*	5.78 ±4.08 [b]	0.02 ±0.02 [a]
TPP	rhizome	6.23 ±3.02 [a]	4.11 ±1.28 [a]	4.83 ±3.39 [a]	6.54 ±5.19 [a]	7.94 ±9.75 [a]	4.85 ±1.57 [a]	6.68 ±3.78 [a]	4.28 ±3.95 [a]	6.63 ±11.20 [a]
	stem	1.78 ±1.01 [a]	4.45 ±2.28 [b]	6.54 ±2.28 [b]	2.04 ±1.76 [a]	0.76 ±0.46 [a]	4.81 ±2.79 [b]	5.23 ±1.94 [ab]	3.10 ±2.59 [a]	7.21 ±2.20 [b]
	leaf	0.96 ±0.54 [a]	6.74 ±3.36 [ab]	18.89 ±20.49 [b]	1.96 ±1.14 [a]	8.53 ±7.79 [ab]	19.21 ±22.58 [b]	2.29 ±2.68 [a]	6.73 ±4.08 [a]	23.12 ±36.70 [a]
Biomass	rhizome	2.47 ±0.72 [a]	3.00 ±1.56 [ab]	3.84 ±0.99 [b]	2.30 ±0.86 [a]	3.64 ±1.49 [ab]	5.17 ±1.95 [b]	1.53 ±0.41 [a]	2.74 ±1.27 [b]	2.33 ±0.62 [ab]
	stem	0.69 ±0.14 [b]	0.31 ±0.20 [a]	0.48 ±0.12 [a]	0.59 ±0.17 [ab]	0.42 ±0.21 [a]	0.85 ±0.53 [b]	0.32 ±0.09 [a]	0.21 ±0.18 [a]	0.31 ±0.21 [a]
	leaf	0.71 ±0.22 [b]	0.36±0.20 [a]	0.52 ±0.09 [ab]	0.57 ±0.22 [ab]	0.39 ±0.17 [a]	0.68 ±0.21 [b]	0.32 ±0.13 [a]	0.31 ±0.22 [a]	0.31 ±0.04 [a]

FC – field capacity, PPVII – polyphyllin VII, PPI – polyphyllin I, PPII – polyphyllin II and TPP – total content of polyphyllin, *N* – not detected in samples
Different letters of a and b within a row indicate significant differences for the same tissue and field capacity among different provenance at $P < 0.05$

Besides, types or content of polyphyllin diversity among tissues might be caused by different course of plant growth [Kohara et al. 2005]. These results revealed that the multi-provenance may present different maturation stage at the same time, i.e. the steroid saponins synthesis and transferred phase were not exactly the same and the harvest time might not be exact congruent, which might be influenced by moisture, temperature, radiation etc.

One-way analysis of variance. For rhizome parts treated with 0.80 FC, difference of polyphyllin content (PPVII, PPI, PPII and TPP) among Jinping, Luquan and Weixi provenances are insignificant except for PPII, which presents significant differences ($P < 0.05$) among these three provenances. Meanwhile, all polyphyllin content in aerial parts shows significant difference ($P < 0.05$) among these three provenances more or less, no matter in moderately irrigation or water stress treatment, which implies that the polyphyllin synthesis and accumulation among these three provenances is different and may be induced by gene differential expression [Farmer and Schilling 2002]. Xu et al. [2017] revealed that the expression of two squalene epoxidase genes presented significant differences in stem and leaf, and the squalene epoxidase 1 was most pronounced in the leaf tissues. Liu et al. [2011] investigated the expression difference of genes HMGCoA reductase (CL6) and squalene epoxidase (CL16) in root, stem and leaf of *Paris* genus, result of which indicated that CL6 and CL16 were expressed highest in root and the lowest in leaf tissues. In the treatment of 0.70 FC, rhizome tissues also show insignificant difference. The results of sever water stress (0.50 FC) treatment display significant differences ($P < 0.05$) among Jinping, Luquan and Weixi provenances for PPVII compounds in rhizome, which suggests that the rhizome tissues of PPY may show different stress response in somewhat under 0.50 FC treatment. In the previous report, Wen [2017] confirmed that the sunflower showed different expression genes under drought stress and screened the drought-resistant provenance on this basis. The biomass of rhizome, stem and leaf among these three provenances presented significant difference ($P < 0.05$) except for stem and leaf samples obtained from Luquan and Weixi under severe water stress (0.50 FC), which implies that the Luquan and Weixi samples were insensitive to soil moisture variation and might induce the interaction of environmental factors and alternative expression of genes. Besides, the biomass is significantly different among these three provenance, implying the provenance may be one of the factors to affect the yield.

Principal components analysis. Principal components analysis (PCA) is a standard, unsupervised and visualized multivariate data analysis method, which can reduce the dimensionality and retains the data variation as much as possible to compare the similarities and differences among samples [Qi et al. 2017]. In this study, PCA was created to build the discrimination model to further insight the similarities and differences among Jinping, Luquan and Weixi provenances. The FT-MIR initial spectra were preprocessed with MSC, second derivative and 15 gap of smoothing of SG successively. From Figure 1, it can be seen that the absorbance intensity makes a significant difference among Jinping, Luquan and Weixi provenances. Additionally, the diversity peak shapes are presented in the preprocessed spectra (Fig. 2). Above analysis indicated that the difference among these three provenance is significantly different. Making a further insight, the PCA loadings plots (Fig. 3) reveal that no matter the treatment of full irrigation or water stress condition, each tissue can be distinguished into three categories according to the provenance except from the rhizome that is treated with 0.50 FC, which implies that the provenance may be the key pattern to influence the chemical profiles in 80 FC and 70 FC. Additionally, the loadings plots showing the treatment of 50 FC for rhizome tissues are inadequate to distinguish samples, implying that the chemical profiles among these three provenances are tending to be similar with the condition of severe water stress.

Furthermore, the characteristic variable factors of the first 20 for each category are picked out to conjecture the components probably, inferring the components of which presented high contribution to the category [Pérez-Arribas et al. 2017]. The FT-MIR electromagnetic spectrum region from 3700–2400 cm^{-1} and 1800–550 cm^{-1} could be partitioned into

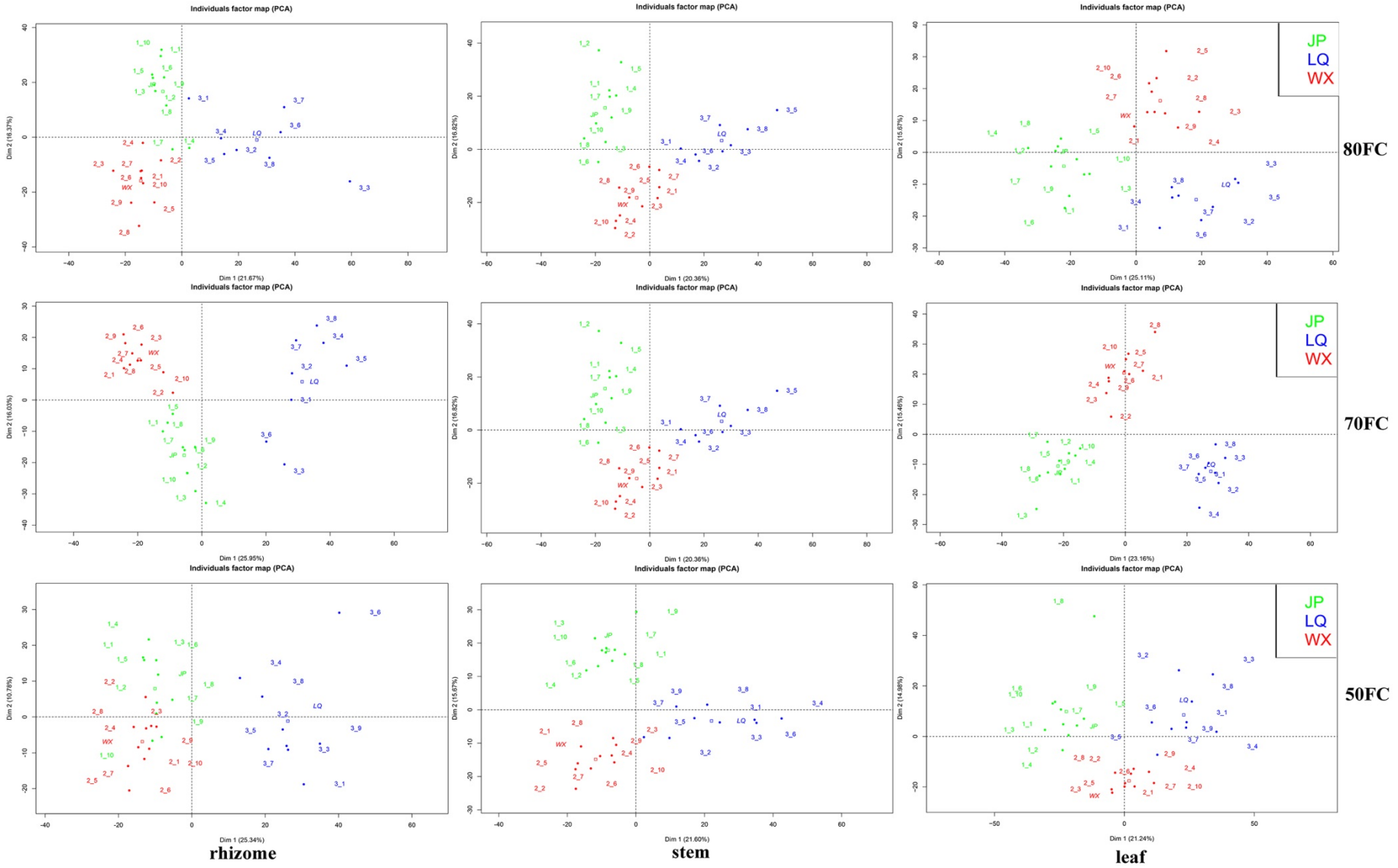


Fig. 2. Principal component analysis scores plot for the first and second dimensions for the same tissue among Jinping, Luquan and Weixi districts under the same field capacity treatment of *P. polyphylla* Smith var. *yunnanensis* samples

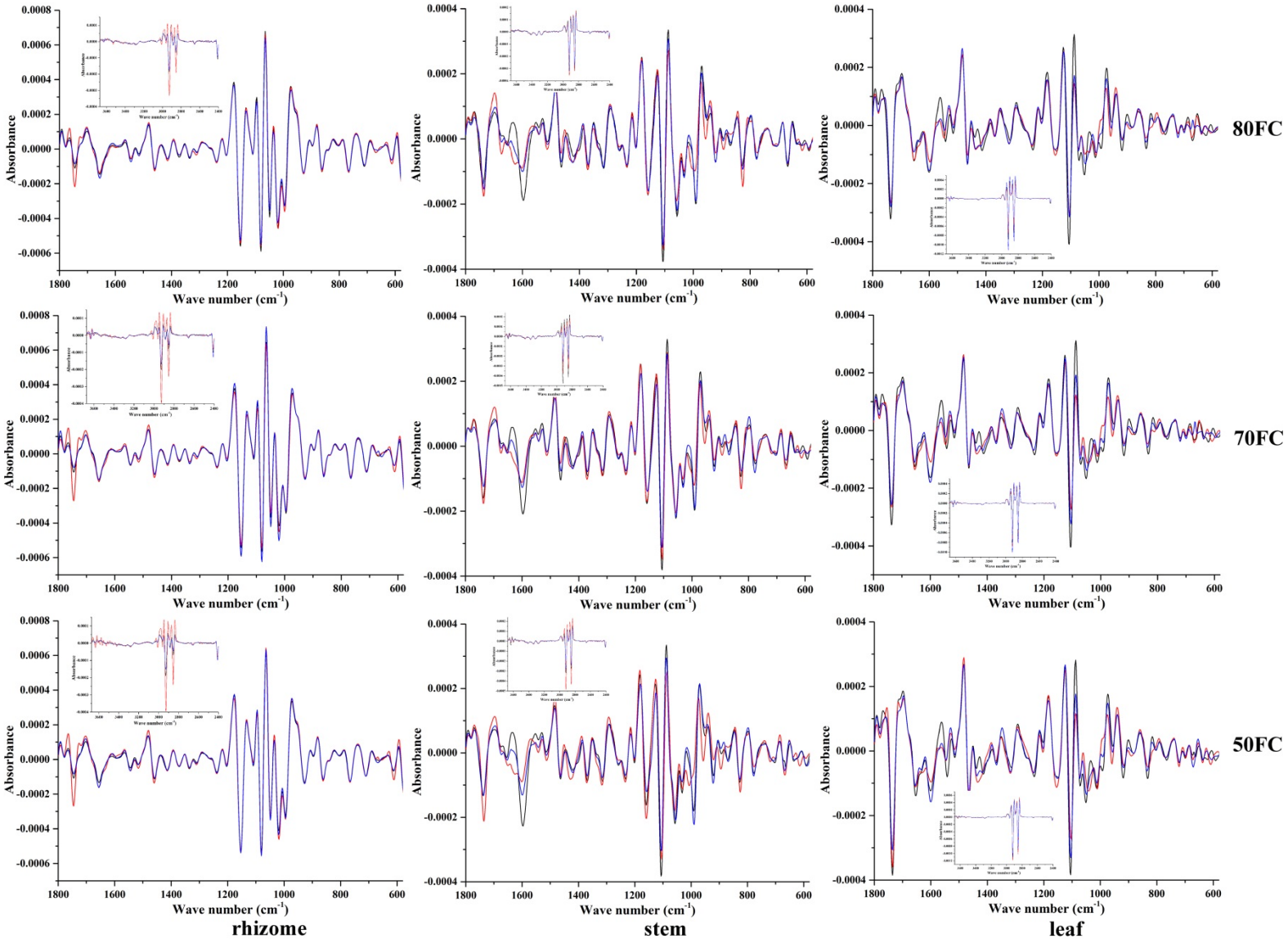


Fig. 3. Pretreated of FT-MIR spectra

four sub-regions: the X-H stretching region (3700–2500 cm^{-1}) and the X represented carbon (C) in most cases, and the triple bond region from 2500–2400 cm^{-1}, the double bond region (1800–1500 cm^{-1}) and the fingerprint regions (1500–550 cm^{-1}) [Karoui et al. 2010]. From the Table 2B, the fingerprint regions and X-H stretching regions show more contribution to the category. Detailed assignment of FT-MIR wavenumber is presented in Table 4. The X-H regions results shown in this study are mainly distributed in 0.50 FC conditions among rhizome, stem and leaf tissues, which suggests that with the decrease of water supply, aliphatic compounds in different provenances of PPY samples show similar variation tendency. The contribution of double bond region is less for the category in this study and the difference among these three provenances is mainly shown in stem parts, which suggested that the constituents of such flavones in this tissue are differentiable. The fingerprint regions present the highest contribution to the category among different provenances and the main constituents may be assigned to starches, saccharides and saponins, which suggested that these components among Jinping, Weixi and Luquan were significantly different and contributed high contribution to the PCA category.

Table 4. Assignments of characteristic variable factors of the first 20 in the macroscopic FT-MIR spectra for PPY samples

Wave numer (cm^{-1})	Primary assignment	Possible compound	References
3641–3016	O-H	hydroxy compound	Gao 2016
2985–2817	*v* C-H (CH_2, CH_3)	lipids, carbohydrates	Zhu and Tan 2015, Chen et al. 2017, Rohaeti et al. 2015
2677–2648	*δ* C=C	aliphatics	Dushyant et al. 2014
1724–1709	*v* C=O (carbonyl or aldehyde)	saturated aliphatic esters	Zhu and Tan 2015, Sun et al. 2010, Li at al. 2013
1643–1641	*v* C=O (Amide I)	proteins, alkaloids	Türker-Kaya and Huck 2017
1628–1618	*v* C=C, *v* C=O	aromatic ring, flavonoids	Ashokkumar and Ramaswamy 2014, Petrakis and Polissiou 2017
1587–1581	*v* C-N	lignins, alkaloids	Türker-Kaya and Huck 2017
1464–1410	*v* C-H (CH2, CH3), *δ* O-H	polysaccharides, carboxylic acids	Zhu and Tan 2015, Li et al. 2013, Türker-Kaya and Huck 2017
1390–1374	*v* C-H	polysaccharides, celluloses	Türker-Kaya and Huck 2017, Petrakis and Polissiou 2017
1186–1182	*v* C-O	saponins	Wang and Li 2018
1171–1101	*v* C-O-C	cutins	Türker-Kaya and Huck 2017
1099–1003	*v* C=O, *δ* O-H	starchs, glycosides, saponins	Türker-Kaya, Huck 2017, Lu et al. 2008, Wu et al. 2017b, Zhu et al. 2014, Chen et al. 2014
964–960	*δ* C=C (saccharide ring)	starches, glycosides, saponins	Xu et al. 2015
891–850	*δ* C=C (saccharide ring)	starches, saponins	Türker-Kaya and Huck 2017, Xu et al. 2010, Lu et al. 2008, Sekkal et al. 1995
837–727	benzene ring skeletal vibration	saccharides, saponins	Wu et al. 2017a, Chen et al. 2017, Lu et al. 2008, Zhu et al. 2014
679–606	*v* C-H	aromatic hydrocarbones, alkynes	Ray et al. 2013, Theivandran et al. 2015

CONCLUSIONS

The complexity of habitats and provenances caused the significant variation of chemical profiles in PPY samples. In the present study, three soil moisture levels and three provenances were conducted to explore the chemical profile difference of PPY samples. Polyphyllin content and biomass were affected by soil moisture, especially for stem and leaf tissues. Polyphyllin content combined with biomass revealed that the 0.70 FC might be considered the optimal irrigation level. Provenance collected from Jinping showed the highest polyphyllin content in rhizome and might serve as the high quality germplasm. Provenance obtained from Weixi presented the highest TPP content in stem and leaf, which suggested that the steroid saponins synthesis and transfer period might be different among multi-provenance and this needs further research. This study provided a basic reference for optimal irrigation volume and provenance screening in the field of PPY cultivation.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (grant number 81460584).

CONFLICT OF INTEREST

No conflict of interest exist in this submission manuscript. And this manuscript is approved by all of authors for publication.

REFERENCES

Ashokkumar, R., Ramaswamy, M., 2014. Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants. Int. J. Curr. Microbiol. Appl. Sci., 3, 395–406.

Bray, E.A., 1997. Plant responses to water deficit. Trends Plant Sci., 2, 48–54.

Bray, E.A., 2004. Genes commonly regulated by water-deficit stress in *Arabidopsis thaliana*. J. Exp. Bot., 55, 2331–2341.

Borges, C.V., Minatel, I.O., Gomez-Gomez, H.A., Lima, G.P.P., 2017. Medicinal plants: influence of environmental factors on the content of secondary metabolites. In: medicinal plants and environmental challenges, Ghorbanpour, M., Varma, A. (eds.). Springer, Cham, 259–277.

Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Understanding plant responses to drought-from genes to the whole plant. Funct. Plant Biol. 30, 239–264.

Chen, J.B., Sun, S.Q., Ma, F., Zhou, Q., 2014. Vibrational microspectroscopic identification of powdered traditional medicines: chemical micromorphology of *Poria* observed by infrared and Raman microspectroscopy. Spectrochim. Acta, Part A, 128, 629–637.

Chen, J., Guo, B., Yan, R., Sun, S., Zhou, Q., 2017. Rapid and automatic chemical identification of the medicinal flower buds of *Lonicera* plants by the benchtop and hand-held Fourier transform infrared spectroscopy. Spectrochim. Acta, Part A, 182, 81–86.

Dushyant, S., Kumar, S., Maurya, A.K., Meena, K.R., 2014. Efficacy of organic manures on growth, yield and biomolecules of stevia (*Stevia rebaudiana* Bertoni). J. Crop. Weed., 10, 107–110.

Escamilla, M.N., Sanz, F.R., Li, H., Schönbichler, S.A., Yang, B., Bonn, G.K., 2013. Rapid determination of baicalin and total baicalein content in *Scutellariae* radix by ATR-IR and NIR spectroscopy. Talanta, 114, 304–310.

Farmer, S.B., Schilling, E.E., 2002. Phylogenetic analyses of trilliaceae based on morphological and molecular data. Syst. Bot., 27, 674–692.

Feng, L.L., Zhang, L., Li,, H.F., Zhang, C.G., 2015 Quality evaluation of *Paris polyphylla* var. *yunnansis* and accumulation low analysis of its steroidal saponins. Chin. J. Exp. Tradit. Med. Formulate, 21, 41–45.

Gao, S.S., 2016 Identification method of Chinese medicine decoction Piece by Mid-Infrared spectroscopy, pp. 15.

Gharibi, S., Saeidi, G., Goli, S.A.H., 2016 Effect of drought stress on total phenolic, lipid peroxidation, and antioxidant activity of achillea species. Appl. Biochem. Biotechnol., 178, 796–809.

Horn, B., Esslinger, S., Pfister,, M., Fauhl-Hassek, C., Riedl, J., 2018. Non-targeted detection of paprika adulteration using mid-infrared spectroscopy and one-class classification-Is it data preprocessing that makes the performance. Food Chem., 257, 112–119.

Huang, X.X., Gao, W.Y., Man, S.L., Zhao, Z.Y., 2009 Advances in studies on saponins in plants of *Paris* L.

and their biosynthetic approach. Chin. Tradit. Herb Drugs., 3, 483–489.

Jiang, Y., 2009. China's water scarcity. J. Environ. Manage., 90, 3185–3196.

Karoui, R., Downey, G., Blecker, C., 2010. Mid-infrared spectroscopy coupled with chemometrics: A tool for the analysis of intact food systems and the exploration of their molecular structure-quality relationships – A review. Chem. Rev., 110, 6144–6168.

Kohara, A., Nakajima, C., Hashimoto, K., Ikenaga, T., Tanaka, H., Shoyama, Y., 2005. A novel glucosyltransferase involved in steroid saponin biosynthesis in *Solanum aculeatissimum*. Plant Mol. Biol., 57, 225–239.

Layeghhaghighi, M., Asil, M.H., Abbaszadeh, B., Sefidkon, F., Matinizadeh, M., 2017. Mycorrhiza effect on yield and component yield of *Nepeta pogonosperma* under water deficit conditions. Adv. Bio. Res., 8, 174–179.

Lee, M.S., Chan, J.Y.W., Kong, S.K., Yu, B., Eng-Choon, V.O., Nai-Ching, H.W., 2005. Effects of polyphyllin D, a steroidal saponin in *Paris polyphylla*, in growth inhibition of human breast cancer cells and in xenograft. Cancer Biol. Ther., 4, 1248–1254.

Li, N., Wang, Y., Xu, K., 2006. Fast discrimination of traditional Chinese medicine according to geographical origins with FTIR spectroscopy and advanced pattern recognition techniques. Opt. Express., 14, 7630–7635.

Li, S.P., Yang, L.Y., Yang, B., Gu, A.N., Yan, S.W., Li, L.Y., 2008. Study on high propagation efficiency and high yield cultivation of *Paris polyphlla* var. *yunnansis*. Southwest China J. Agricultureal. Sci., 4, 956–959.

Li, Y.Q., Kong, D.X., Wu, H., 2013. Analysis and evaluation of essential oil components of cinnamon barks using GC-MS and FTIR spectroscopy. Ind. Crops Prod., 41, 269–278.

Li, C., Guo, Q.S., Yang, S.C., Zheng, K.Y., Li, W.P., Meng, Z.G., 2015. Determination of multiple elements in samples of the medicinal plant *Marsdenia tenacissima* and estimation of geographic origin via pattern recognition techniques. J. Nat. Med., 69, 55–62.

Li, Y., Zhang, J.Y., Wang, Y.Z., 2017. FT-MIR and NIR spectral data fusion: a synergetic strategy for the geographical traceability of *Panax notoginseng*. Anal. Bioanal. Chem., 1, 91–103.

Liao, P., Liu, D., Xu, T.R., Yang, Y., Cui, X., 2017. Soil water stress attenuate the growth and development but enhance the saponin synthesis of *Panax notogesing* during flowering stage. Ind. Crops Prod., 108, 95–105.

Liu, Y., Zhao, E.R., Huang, W., Zhou, J.Q., Ju, J.H., 2010. Characteristic analysis of precipitation and temperature trend in Yunnan Province in recent 46 years. J. Catastrophol., 25, 39–44.

Liu, J., Liu, X.D., Jiang, X.M., Liao, L.Q., 2011. Differential expression of functional genes in *Paris* L. as revealed by quantitative competitive RT-PCR. China J. Mod. Med., 21, 2709–2714.

Liu, L.M., Zhao, Z.L., Han, D., Li, H.F., 2016. Investigation on accumulation of dry weight and active ingredients of *Paris polyphlla* var. *yunnanensis* with different growth years. Chin. J. Pharm., 47, 706–710.

Lu, G.H., Zhou, Q., Sun, S.Q., Leung, K.S.Y., Zhang, H., Zhao, Z.Z., 2008. Differentiation of Asian ginseng, American ginseng and Notoginseng by Fourier transform infrared spectroscopy combined with two-dimensional correlation infrared spectroscopy. J. Mol. Struct., 883, 91–98.

Nunes, K.M., Andrade, M.V.O., Santos Filho A.M., Lasmar, M.C., Sena, M.M., 2016. Detection and characterisation of frauds in bovine meat in natura by non-meat ingredient additions using data fusion of chemical parameters and ATR-FTIR spectroscopy. Food Chem., 205, 14–22.

Pérez-Arribas, L.V., León-González, M.E., Rosales--Conrado, N., 2017. Learning principal component analysis by using data from air quality networks. J. Chem. Educ., 94, 458–464.

Petrakis, E.A., Polissiou, M.G., 2017. Assessing saffron (*Crocus sativus* L.) adulteration with plant-derived adulterants by diffuse reflectance infrared Fourier transform spectroscopy coupled with chemometrics. Talanta, 162, 558–566.

Pradhan, N.S., Fu, Y., Zhang, L., Yang, Y., 2017. Farmers' perception of effective drought policy implementation: A case study of 2009–2010 drought in Yunnan province, China. Land Use Pol., 67, 48–56.

Qi, L.M., Zhang, J., Liu, H.G., Li, T., Wang, Y.Z., 2017. Fourier transform mid-infrared spectroscopy and chemometrics to identify and discriminate *Boletus edulis* and *Boletus tomentipes* mushrooms. Int. J. Food Prop., 20, S56–S68.

Ray, A., Gupta, S.D., Ghosh, S., 2013. Isolation and characterization of potent bioactive fraction with antioxidant and UV absorbing activity from *Aloe barbadensis* Miller gel. J. Plant Biochem. Biotechnol., 22, 483–487.

Rohaeti, E., Rafi, M., Syafitri, U.D., Heryanto, R., 2015. Fourier transform infrared spectroscopy combined with

chemometrics for discrimination of *Curcuma longa*, *Curcuma xanthorrhiza* and *Zingiber cassumunar*. Spectrochim. Acta, Part A, 137, 1244–1249.

Sekkal, M., Dincq, V., Legrand, P., Huvenne, J.P., 1995. Investigation of the glycosidic linkages in several oligosaccharides using FT-IR and FT Raman spectroscopies. J. Mol. Struct., 349, 349–352.

Shao, H.B., Chu, L.Y., Jaleel, C.A., Zhao, C.X., 2008. Water-deficit stress-induced anatomical changes in higher plants. C.R. Biol., 331, 215–225.

Shao, W., Li, Y., Diao, S., Jiang, J., Dong, R., 2017. Rapid classification of Chinese quince (*Chaenomeles speciosa* Nakai) fruit provenance by near-infrared spectroscopy and multivariate calibration. Anal. Bioanal. Chem., 409, 115–120.

Sharma, A., Kalita, P., Tag, H., 2015. Distribution and phytomedicinal aspects of *Paris polyphylla* Smith from the Eastern Himalayan Region: A review. Tang Humanitas Med., 5, 15–1.

Shen, S., Chen, D., Li, X., Li, T., Yuan, M., Zhou, Y., 2014. Optimization of extraction process and antioxidant activity of polysaccharides from leaves of *Paris polyphylla*. Carbohydr. Polym., 104, 80–86.

Srivastava, R., Rai, K.M., Srivastava, R., 2018. Plant biosynthetic engineering through transcription regulation: An insight into molecular mechanisms during environmental stress. In: Biosynthetic Technology and Environmental Challenges, Varjani, S., Binod, P., Kumar, S., Khare, S.K. (eds.). Springer, Singapore, 51–72.

Sun, S., Chen, J., Zhou, Q., Lu, G., Chan, K., 2010. Application of mid-infrared spectroscopy in the quality control of traditional Chinese medicines. Planta Med., 76, 1987–1996.

Chinese Pharmacopoeia. (2015). The State Pharmacopoeia Commission, Beijing, 260–261.

Theivandran, G., Mohamed Ibrahim, S., Murugan, M., 2015. Fourier transform infrared (FT-IR) spectroscopic analysis of *Spirulina fusiformis*. J. Med. Plants Stud., 3, 30–32.

Tohda, C., Urano, T., Umezaki, M., Nemere, I., Kuboyama, T., 2012. Diosgenin is an exogenous activator of 1,25D_3-MARRS/Pdia3/ERp57 and improves Alzheimer's disease pathologies in 5XFAD mice. Sci. Rep., 2, 535.

Türker-Kaya, S., Huck, C.W., 2017. A review of mid-infrared and near-infrared imaging: principles, concepts and applications in plant tissue analysis. Molecules, 22, 168.

Verma, N., Shukla, S., 2015. Impact of various factors responsible for fluctuation in plant secondary metabolites. J. Appl. Res. Med. Aromat. Plants, 2, 105–113.

Wang, Y.Z., Li, P., 2018. Effect of cultivation years on saponins in *Paris polyphylla* var. *yunnanensis* using ultra-high liquid chromatography-tandem mass spectrometry and Fourier transform infrared spectroscopy. Plant Growth Regul., 84, 373–381.

Wen R., 2017. Analysis of differential expression genes by cDNA-AFLP approach during drought stress in sunflower seed germination. Inner Mongolia Agricultural University, pp. 3.

Wu, X., Yuan, J., Luo, A., Chen, Y., Fan, Y., 2016. Drought stress and re-watering increase secondary metabolites and enzyme activity in dendrobium moniliforme. Ind. Crops Prod., 94, 385–393.

Wu, Z., Zhang, J., Xu, F.R., Wang, Y.Z., Zhang, J.Y., 2017a. Rapid and simple determination of polyphyllin I, II, VI, and VII in different harvest times of cultivated *Paris polyphylla* Smith var. *yunnanensis* (Franch.) Hand.-Mazz by UPLC-MS/MS and FT-IR. J. Nat. Med., 71, 139–147.

Wu, Z., Zhao, Y.L., Zhang, J., Wang, Y.Z., 2017b. Quality assessment of *Gentiana rigescens* from different geographical origins using FT-IR spectroscopy combined with HPLC. Molecules, 22, 1238.

Xu, R., Sun, S.Q., Xin, S.G., Zhu, W.C., Shi, Y., Liu, T.N., Chen, J., 2010. Qualitative and semi-quantitative analysis of *Cistanche deserticola* by mid-infrared spectroscopy. J. Infrared. Millimeter. Waves, 29, 325–328.

Xu, C.H., Chen, J.B., Zhou, Q., Sun, S.Q., 2015. Classification and identification of TCM by macro-interpretation based on FT-IR combined with 2DCOS-IR. Biomed. Spectrosc. Imaging, 4, 139–158.

Xu, Y., Zhao, S., Dong, X., Ye, X., 2017. Cloning and expression of squalene epoxidase from *Paris polyphylla* var. *yunnanensis*. Chin. Tradit. Herb Drugs, 48, 1839–1844.

Xue, J., Zhou, S., Wang, W., Huo, L., Zhang, L., Fang, X., 2018. Water availability effects on plant growth, seed yield, seed quality in *Cassia obtusifolia* L., a medicinal plant. Agric. Manage Water Qual. 195, 104–113.

Yang, Y., Yang, S.C., Zhao, J., Udikeri, S., Liu, T., 2015. Microbial diversity in *Paris polyphylla* var. *yunnanensis* rhizomes of varying ages. Genet. Mol. Res., 14, 17612–17621.

Yang, Y.G., Jin, H., Zhang, J., Zhang, J.Y., Wang, Y.Z., 2017. Quantitative evaluation and discrimination of

wild *Paris polyphylla* var. *yunnanensis* (Franch.) Hand.-Mazz from three regions of Yunnan Province using UHPLC-UV-MS and UV spectroscopy couple with partial least squares discriminant analysis. J. Nat. Med., 71, 148–157.

Yan, L.L., Zhang, Y.J., Gao, W.Y., Man, S.L., Wang, Y., 2009. *In vitro* and *in vivo* anticancer activity of steroid saponins of *Paris polyphylla* var. *yunnanensis*. Exp. Oncol., 31, 27–32.

Yu, K., Wang, Y., Wei, J., Ma, Q., Yu, D., Li, J., 2009. Improving rhizome yield and quality of *Paris polyphylla* through gibberellic acid-induced retardation of senescence of aerial parts. Plant Signaling Behav., 4, 413–415.

Zhai, P., Zhang, X., Wan, H., Pan, X., 2005. Trends in total precipitation and frequency of daily precipitation extremes over China. J. Clim., 18, 1096–1108.

Zhang, L.H., Shao, H.B., Ye, G.F., Lin, Y.M., 2012. Effects of fertilization and drought stress on tannin biosynthesis of *Casuarina equisetifolia* seedlings branchlets. Acta. Physiol. Plant., 34, 1639–1649.

Zhang, Y., Xu, L., Qiu, J., Sun, M., Xia, C., Zhou, Z., 2014a. Provenance variations in berberine content of *Phellodendron amurense*, a rare and endangered medicinal plant grown in Northeast China. Scand. J. Forest Res., 29, 725–733.

Zhang, W., Zhang, D., Ma, X., Liu, Z., Li, F., Wu, D., 2014b. *Paris* saponin VII suppressed the growth of human cervical cancer Hela cells. Eur. J. Med. Res., 19, 41.

Zhang, W., Cao, Z., Xie, Z., Lang, D., Zhou, L., Chu, Y., 2017. Effect of water stress on roots biomass and secondary metabolites in the medicinal plant *Stellaria dichotoma* L. var. *lanceolata* Bge. Sci. Hortic., 224, 280–285.

Zhao, Y., Zhang, J., Yuan, T., Shen, T., Li, W., Yang, S., 2014a. Discrimination of wild *Paris* based on near infrared spectroscopy and high performance liquid chromatography combined with multivariate analysis. PloS one, 9, e89100.

Zhao, T.Z., Wang, B.Q., Ma, Q., Fan, Q.L., 2014b. Study on best harvesting time of *Paris polyphylla* var. *yunnanensis*. Chin. Wild Plant Resour., 33, 61–63 + 66.

Zhu, Y., Xu, C.H., Huang, J., Li, G.Y., Liu, X.H., Sun, S.Q., Wang, J.H., 2014. Rapid discrimination of cultivated *Codonopsis lanceolata* in different ages by FT-IR and 2DCOS-IR. J. Mol. Struct., 106, 272–279.

Zhu, Y., Tan, A.T.L., 2015. Discrimination of wild-grown and cultivated *Ganoderma lucidum* by Fourier transform infrared spectroscopy and chemometric methods. Am. J. Anal. Chem., 6, 480–491.