Morphological and Chemical Variation of *Prunella vulgaris* Populations from Different Locations in China

LIAO Li[1, 2], LIU Li[1], GUO Qiao-sheng[1*], WANG Zhi-yong[2], CHEN Yu-hang[1]

1. Institute of Chinese Medicinal Materials, Nanjing Agricultural University, Nanjing 210059, China
2. Agronomy College, Hainan University, Danzhou 571737, China

Abstract: **Objective** To investigate the variation of chemical characteristics with environmental factors and establish a relationship between morphological characters and chemical composition of *Prunella vulgaris* collected in different areas of China. **Methods** Twelve phenotypic traits and three chemical compositions were assessed in 28 populations of *P. vulgaris* collected from different locations in China. **Results** The variability ranges observed at phenotypic and chemical levels were polymorphic. According to the morphological traits, 28 populations of *P. vulgaris* could be grouped into six clusters, and two morpho-types could be clearly distinguished. Perceptible differences could be discerned in the plant height, leaf length, corolla length, calyx length, fruiting spikes length, and maturity period. Based on three kinds of components including ursolic acid, total flavonoids, and total polysaccharides, all populations could be identified as four types. Cluster IV showing high content of ursolic acid, total flavonoids, and total polysaccharides could be utilized to develop superior derivatives. **Conclusion** The variation of chemical characteristics is influenced by the genetic and environmental factors, such as soil, climate, longitude, and altitude. It provides a solid basis for efficiently evaluating qualities and establishing good agricultural practices for *P. vulgaris*.

Key words: morphology; polymorphic variation; populations; *Prunella vulgaris*
DOI: 10.3969/j.issn.1674-6384.2010.04.008

Introduction

Prunella vulgaris L. is an annual to perennial hemicryptophyte herb. It is an important traditional antipyretic Chinese medicine (Chang and But, 1987). It was widely used for high blood pressure, human tuberculosis, thyroid gland swell, jaundice, infectious hepatitis, bacillary dysentery, hypertension, and cancer in the clinical treatment (Liu, Song, and Xu, 2003; Sun *et al*, 2003; Psotova, 2003; Lee *et al*, 1998; Zhang, Zhang, and Wang, 2005). It is also very popularly used as food with not only a wide range of clinical applications and has a high edible value, which had been recorded in China ancient books of *Bencao Yanyi*, *Compendium of Materia Medica*, *Food Materia Medica*, and *Save the Shortage of Chinese Materia Medica*. At present, it is used with high frequency in vegetables, soup, herbal tea, and other forms.

For the last few decades, the studies of *P. vulgaris* have focused on chemistry, pharmacology, and other aspects. The organic extract contains mainly non-polar bio-organic compounds, such as ursolic acid (UA), oleanolic acid (OA), triterpenoids, flavonoids, betulinic, tannins, and rosmarinic acid (Lamaison, Petitjean-Freytet, and Carnat, 1991; Lee *et al*, 1998; Psotová *et al*, 2003; Ryu *et al*, 2000; Jia *et al*, 2009). UA is used as a control component in the *Pharmacopoeia of the People's Republic of China 2010*, in which most of the pharmacological activities are attributed. Polysaccharides are biopolymers possessing a wide array of biological effects and therapeutic potentials. Prunelline, a polysaccharide isolated from the aqueous extract of *P. vulgaris* exhibited anti-HIV (Tabba *et al*, 1989) and anti-type 1 and 2 herpes simplex virus activities (Xu *et al*, 1999). Furthermore, the crude aqueous extract from *P. vulgaris* was found to inhibit anaphylactic shock and

* Corresponding author: Guo QS Address: Institute of Chinese Medicinal Materials, Nanjing Agricultural University, Nanjing 210059, China
Tel/Fax: +86-25-8439 5980 E-mail: gqs@njau.edu.cn
Received: December 15, 2009; Revised: March 10, 2010; Accepted: September 16, 2010
Fund: National Natural Science Foundation of China (30772730)

immediate-type allergic reactions (Shin, Kim, and Kim, 2001). At the same time, the studies of seed physiology, cultivation, and genetic diversity of *P. vulgaris* have been carried out in our laboratory (Guo *et al*, 2009; Liao and Guo, 2009).

P. vulgaris is widely distributed in tropical and temperate regions including Europe, North Africa, Siberia, Western Asia, India, Pakistan, Nepal, Bhutan, Japan, North Korea, and America. In China, the plant grows wildly, such as Jiangsu, Shanxi, Gansu, Xinjiang, Henan, Hunan, Anhui, Jiangxi, Zhejiang, Fujian, Taiwan, Guangdong, Guangxi, Guizhou, Sichuang, Yunnan, and other provinces. Therefore, different populations had complex origins, which seriously impacted on the stability of pharmacodynamics.

The quality control of herbal medicine is an important concern for both the health authorities and the public health (Eisenberg *et al*, 1998). Different populations had rich genetic diversity in the levels of gene and chromosome, leading to a variety diversity of the forms of structure, growth and development, metabolism, and other physiological levels, which will directly or indirectly affect the medicinal quality, and resulting in the diversity of the quality among different populations (Chen, Wei, and Wang, 2003). And it is well known that medicinal plants collected at different times and from different localities may considerably differ in their types and quantities of chemical components (Liu *et al*, 2010), resulting in different therapeutic efficacy. It is generally believed that these differences are essentially caused by various environmental conditions and habitats in which the medicinal plants are grown and harvested.

Now Chinese genetic resources of *P. vulgaris* exhibit a lot in morphological and phytochemical variability which remains largely undocumented. Systematic morphological and chemical characterization of *P. vulgaris* germplasm is of great significance for future programmes on enhancement of quality and yield. This study is aimed at identification of elite populations and their spatial distribution in China linking the morphological variability with the chemical relation of populations within clusters.

Materials and methods

Plant materials

According to climate and soil type of China, the seeds and fruiting spikes of 28 *P. vulgaris* representative populations were collected during June to July, 2006 from different provinces including the main distribution areas such as Jiangsu, Anhui, and Zhejiang Provinces (Table 1). For chemical analysis, fruiting spikes of *P. vulgaris* collected from the 28 populations were dried and ground into powder before stored at 4 ℃ for use. For the morphological analysis, the seeds of 28 populations were planted in plots of 2.0 m × 5.0 m size at 20 cm × 20 cm spacing arranged in randomized block design with three replications in October, 2006 in Shangfeng Farm, Nanjing Agricultural University, China. Normal cultural practices were followed during the crop season. Data on morphological traits were recorded in 2008.

Morphological variation analysis

Twelve morphometric traits were recorded. Fifteen

Table 1 *P. vulgaris* populations from different locations in China used for phenotypic traits and chemical composition analysis

No.	Locations	Population code	Latitude	Longitude
1	Nanjing, Jiangsu	NJ	32°02′57N	117°14′17E
2	Jiangning, Jiangsu	JN	32°04′57N	117°14′17E
3	Gaochun, Jiangsu	GC	31°15′22N	118°57′38E
4	Xuyu, Jiangsu	XY	32°58′36N	118°46′46E
5	Yixing, Jiangsu	YX	31°21′25N	119°48′46E
6	Zhenjiang, Jiangsu	ZJ	32°11′12N	119°27′20E
7	Yizheng, Jiangsu	YZ	32°26′41N	118°59′05E
8	Guangde, Anhui	GD	31°01′42N	119°31′28E
9	Lujiang, Anhui	LJ	31°14′45N	117°59′42E
10	Jingde, Anhui	JD	30°15′39N	118°32′58E
11	Anqing, Anhui	AQ	30°29′52N	117°03′06E
12	Hexian, Anhui	HX	31°42′55N	118°21′28E
13	Chuzhou, Anhui	CZ	32°18′00N	118°18′47E
14	Guichi, Anhui	GC	30°31′09N	117°29′59E
15	Hefei, Anhui	HF	31°30′41N	117°14′17E
16	Jianou, Fujian	JO	27°01′46N	118°11′39E
17	Guilin, Guangxi	GL	25°16′52N	110.16′57E
18	Huzhou, Zhejiang	HZ	30°36′05N	119°47′35E
19	Luding, Sichuan	LD	31°42′03N	99°58′03E
20	Mianyang, Sichuan	MY	32°29′37N	104°13′51E
21	Jinshan, Sichuan	JS	31°56′02N	101°43′00E
22	Yaan, Sichuan	YA	29°35′36N	102°35′33E
23	Guiyang, Guizhou	GY	26°34′23N	106°45′25E
24	Jian, Jiangxi	JA	27°33′18N	115°08′50E
25	Lean, Jiangxi	LA	27°16′02N	115°46′17E
26	Chengxian, Gansu	CX	36°05′31N	103°41′51E
27	Kunming, Yunnan	KM	25°03′53N	102°43′07E
28	Fengdu, Chongqing	FD	29°34′52N	106°33′02E

Plants per plot randomly selected were used for the observations. Plant height (PH), leaf length (LL), leaf width (LW), internode length (IL), and stolon diameter (SD) were noted in April 2008. Corolla length (COL), corolla throat width (CTW), calyx length (CL), and calyx width (CW) were recorded at full-bloom stage in June 2008. Fruiting spikes length (FSL) and fruiting spikes width (FSW) were counted in August after harvest. Maturity period (MD) was observed during the spike growth.

The second leaves from top were measured for LL and LW. SD was obtained at bottom of primary branch. IL was estimated as the distance of the second internode from the root of primary branch. FSL, FSW, and the floral characters of COL, CTW, CL, and CW were measured by vernier caliper. MD was counted by coding from "1" to "5". "1" was represented late ripening when *P. vulgaris* was matured in late June. In contrast, "5" meaned the maturity period in early May which belonged to early ripening.

Analysis of ursolic acid and oleanolic acid

Calibration curve UA (742–8701) and OA (0709–9803) reference substances were purchased from Chinese National Institute for the Control of Pharmaceutical and Biological Products. A series of OA and UA standards were prepared by diluting a stock solution of OA or UA (10 mg/mL ethanol) with ethanol to obtain 0.5, 1, 2, 3, 4, 6, and 10 mg/mL. The stability of these standards was investigated at room temperature.

Preparation of sample solutions Powdered sample (1 g) was macerated with ether overnight in a Soxhlet apparatus at room temperature and extracted for 6 h at 40 ℃, then filtered. After evaporation of the ether to dryness under vacuum, the residue was defatted twice with 10 mL of petroleum ether for 2 min and redissolved in 10 mL ethanol, then filtrated with a 0.45 μm filter. Sample solution (20 μL) was injected into the HPLC system.

Determination of sample solutions Separation was achieved with a reversed-phase column [Spherisorb® ODS (250 mm × 4.6 mm, 5 μm)] and ethanol-ammonium acetate-triethylamine (80∶20∶0.02) was employed as the mobile system (Fig. 1). The column temperature was kept constant at 25 ℃, and the flow rate was kept at 1.0 mL/min. Detection wavelength was 210 nm.

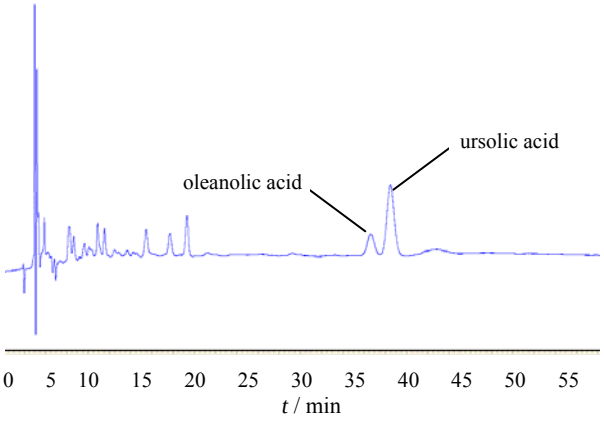

Fig. 1 HPLC chromatogram of UA and OA in *P. vulgaris*

Extraction and measurement of flavonoids

The optimum extraction condition was using 35% ethanol at 30 times of sample volume, refluxing at 87 ℃ for 3.5 h. The content of flavonoids was measured at 510 nm corresponding to maximum absorbance by UV spectrophotometer (Yu and Yu, 2007). According to content and the relationship between absorbance seek reference substance rutin standard curve regression equation, which is *y* equivalent of 0.056 kg plus 0.960*x*, and correlation coefficients is 0.9994. The results content and absorbance have a good linear relationship.

Extraction and measurement of polysaccharides

The extraction process was based on methods described by Psotová *et al* (2003). Powdered sample (20 g) was pretreated with 95% ethanol twice to remove some colored materials and some small molecules, then was extracted in a Soxhlet apparatus with petroleum ether (60−90 ℃). The organic solvent was volatilized and pretreated dry powder was obtained. The pretreated dry powder (20.0 g) was extracted with 1 L for each time of distilled water three times at 90 ℃ for 4 h. All the aqueous extracts were pooled and centrifuged to remove any water-insoluble components. The collected supernatant was concentrated by rotary evaporator and precipitated by 80% ethanol at 4 ℃ overnight. The resulting precipitates were dissolved and dialyzed against distilled water. The retentate was clarified by centrifugation and freeze-dried to obtain a dark brown powder called PPV (15.0 g).

The content of the polysaccharides was measured according to Hou *et al* (2008).

Statistical methods

For comparison of morphological and chemical traits, data analysis was calculated by mean value. The

quantitative data of cluster analysis were done by SPSS 16.0 software.

Results

Morphological diversity

The 28 populations of *P. vulgaris* exhibited perceptible variations in a spectrum of morphological traits (Table 2). The variability ranges observed were PL, IL, SD, LL, LW, COL, corolla width (COW), CTW, CL, CW, fruit length, and fruit width, which were 7.93－33.40 cm, 2.38－9.35 cm, 1.68－2.58 cm, 2.23－4.40 cm, 0.98－1.74 cm, 7.91－14.36 mm, 1.88－4.72 mm, 4.57－9.24 mm, 2.92－4.80 mm, 28.25－61.06 mm, and 9.58－12.10 mm, respectively. On basis of divergence, 28 populations of *P. vulgaris* could be grouped into six clusters (Fig. 2). The mean values of six clusters for 28 quantitative traits were listed in Table 3. Cluster A was the largest having 12 populations followed by cluster E, which had the highest mean PH, IL, LL, COL, CTW, CL, CW, and FSW, in spite of having moderate maturity period. Cluster B consisted of the populations XY, GC, LA, and FD showing the latest maturity period and moderate mean values for most of the traits. Populations JO and JD falling in cluster C exhibited the higher mean value FSL, PH, and LSD. Cluster D contained only HX populations with the lowest PH, IL, and moderate mean values for most of the other traits. The plants of cluster E mostly originating from western area of China, were small to medium sized showing the lowest mean values for most of the characters. Populations RD, GL, and YA falling cluster F had the earliest maturity period and average the other traits.

Chemical diversity

The results showed abundant diversity of chemical constituents as measured by the quantity of the components

Table 2 Morphological traits of 28 *P. vulgaris* populations

Populations	PH / cm	IL / cm	SD / mm	LL / cm	LW / cm	COL / mm	CTW / mm	CL / mm	CW / mm	FSL / mm	FSW / mm	MD
NJ	29.60±3.89	7.14±0.92	2.42±0.38	3.75±0.55	1.38±0.17	14.46±1.59	4.20±0.49	8.66±0.83	4.47±0.97	55.64±11.42	11.33±1.29	5.0
JN	27.60±3.75	7.34±1.41	1.77±0.22	3.36±0.38	1.42±0.23	13.01±0.99	3.82±0.41	7.86±1.35	3.83±0.46	43.69±6.29	12.10±0.96	4.0
GC	19.38±4.96	5.62±0.53	2.08±0.37	3.91±0.67	1.14±0.17	13.72±1.07	3.96±0.40	7.33±0.86	2.92±0.65	49.56±9.04	11.66±1.41	1.0
XY	18.64±3.07	5.61±1.06	2.19±0.25	3.17±0.23	1.17±0.12	13.35±1.02	4.41±0.87	8.17±0.55	3.59±0.34	39.63±7.44	10.74±0.75	3.5
YX	23.30±3.30	7.11±1.36	2.05±0.34	3.52±0.60	1.23±0.19	13.24±0.91	4.40±0.32	8.27±0.82	4.05±0.40	50.63±9.16	11.46±0.49	4.0
ZJ	31.90±3.70	9.35±1.55	2.63±0.32	4.35±0.49	1.51±0.27	13.49±0.86	4.35±0.36	7.50±0.68	3.97±0.56	61.06±11.46	10.68±1.29	4.0
YZ	28.60±3.72	6.12±0.92	1.99±0.33	3.75±0.32	1.40±0.13	13.29±0.90	4.72±0.39	9.24±0.95	4.36±0.39	51.29±5.25	11.06±1.06	5.0
GD	28.80±1.92	6.46±0.95	1.68±0.24	3.35±0.30	1.23±0.18	13.12±1.03	4.50±0.43	8.04±0.69	3.91±0.35	50.45±7.27	11.21±1.43	3.0
LJ	31.63±3.78	7.00±1.52	2.50±0.34	4.23±0.64	1.66±0.34	13.91±0.86	4.27±0.68	6.99±0.97	3.71±0.37	53.72±6.95	9.89±0.77	5.0
JD	27.83±4.26	5.75±0.75	2.00±0.28	2.40±0.41	1.10±0.11	12.76±0.59	3.80±0.19	6.12±0.61	3.77±0.43	63.75±8.24	11.95±1.01	3.0
AQ	25.40±3.17	8.00±1.64	2.65±0.52	4.40±0.40	1.57±0.14	13.85±0.76	4.16±0.43	7.63±0.72	4.13±0.48	61.97±9.96	11.11±0.89	3.0
HX	15.33±2.31	3.40±0.36	2.20±0.21	3.19±0.62	1.82±0.34	12.46±0.96	4.38±0.40	7.05±0.73	3.68±0.39	53.77±8.88	10.60±1.42	2.0
CZ	28.90±1.14	6.71±1.65	1.95±0.30	3.94±0.71	1.31±0.20	13.03±1.01	4.31±0.51	7.75±0.46	4.09±0.37	55.61±9.08	11.08±0.72	4.0
GC	25.80±6.18	6.70±1.99	2.05±0.36	3.92±0.68	1.74±0.33	12.14±0.95	3.79±0.24	8.53±0.97	3.90±0.38	53.71±9.39	10.92±0.70	4.0
HF	33.40±3.61	7.19±1.42	1.98±0.25	3.94±0.25	1.44±0.20	14.39±1.07	4.10±0.41	8.46±0.94	4.80±0.47	58.14±6.72	12.02±0.75	4.0
JO	21.63±4.53	6.19±0.92	1.80±0.20	3.32±0.59	1.32±0.16	11.01±0.48	2.96±0.27	4.81±0.45	4.53±0.40	49.03±8.79	10.81±0.74	5.0
GL	24.71±5.09	8.33±1.61	1.93±0.19	3.25±0.83	1.22±0.41	8.45±0.93	2.40±0.41	5.78±0.74	3.42±0.28	42.05±9.13	9.66±1.38	3.0
HZ	26.00±2.98	6.80±0.56	2.58±0.22	3.73±0.49	1.46±0.22	13.46±0.61	4.27±0.48	6.99±0.91	3.64±0.33	49.44±5.20	10.45±0.61	5.0
LD	23.45±1.42	7.85±1.50	1.91±0.25	2.43±0.23	1.13±0.16	8.68±1.42	1.85±0.26	5.13±0.47	2.98±0.19	35.29±3.77	9.63±0.89	3.0
MY	19.55±2.45	5.71±1.67	2.29±0.42	3.78±0.67	1.47±0.14	9.41±1.12	2.07±0.29	5.84±0.73	3.01±0.22	33.43±4.34	9.62±0.52	3.0
JS	7.93±1.83	2.38±0.52	1.90±0.27	3.28±0.61	1.69±0.26	7.91±1.20	2.01±0.20	5.67±0.60	3.23±0.12	30.88±7.23	9.75±0.86	4.0
YA	15.00±3.37	4.14±1.06	1.72±0.18	2.23±0.36	0.98±0.16	8.72±0.96	2.43±0.32	5.89±0.42	3.62±0.34	30.23±4.55	9.58±0.62	1.0
GY	20.17±2.48	6.29±1.81	2.16±0.35	3.12±0.71	1.36±0.23	8.81±0.84	1.88±0.32	4.57±0.55	2.92±0.28	35.61±3.77	9.86±0.76	5.0
JA	16.43±1.71	5.77±2.95	1.98±0.22	3.53±0.54	1.50±0.18	9.51±0.68	2.29±0.36	6.56±0.36	3.31±0.41	32.48±4.59	10.93±1.34	1.0
LA	19.75±3.80	6.13±1.62	2.11±0.44	3.41±0.87	1.13±0.38	10.09±0.74	2.81±0.26	5.84±0.44	3.37±0.25	51.38±8.17	10.98±0.47	3.5
CX	15.25±2.55	5.05±0.79	2.00±0.41	3.85±0.43	1.64±0.23	10.82±0.48	2.45±0.20	6.73±0.84	3.59±0.24	34.27±8.98	9.49±0.78	1.0
KM	15.71±2.36	6.69±0.99	2.08±0.10	3.11±0.58	1.38±0.27	8.01±1.10	1.96±0.16	5.74±0.65	3.93±0.39	28.25±4.75	10.23±1.02	1.5
FD	24.55±2.38	6.78±1.41	2.41±0.63	3.37±0.62	1.35±0.25	12.27±0.52	3.54±0.26	5.96±0.69	3.86±0.37	44.92±7.81	9.91±0.58	3.5
$\bar{x} \pm s$	23.08±6.23	6.34±1.43	2.11±0.27	3.49±0.54	1.38±0.21	11.76±2.12	3.43±1.00	6.90±1.26	3.74±0.49	46.42±10.53	10.67±0.80	3.35
RSD	0.27	0.23	0.13	0.15	0.15	0.18	0.29	0.28	0.13	0.23	0.75	0.34

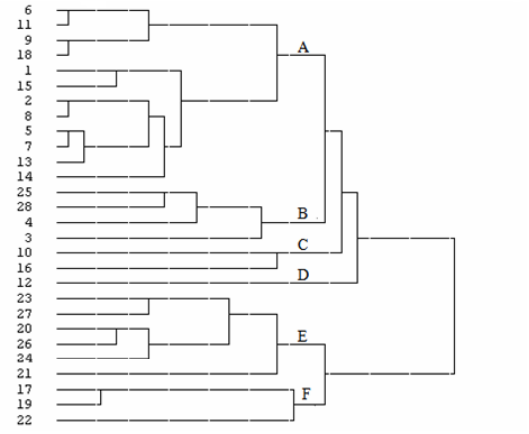

Fig. 2 UPGMA dendrogram based on similarity matrix constructed from the 12 morphological traits of 28 *P. vulgaris* populations

in *P. vulgaris* fruits from different populations (Table 4). UA was employed as the standard for quality control of *P. vulgaris* in *the Pharmacopoeia of the People's Republic of China 2010*. The contents of UA in different populations varied from 0.12 to 0.39 mg/g. Based on three chemical constitutes, 28 *P. vulgaris* populations could be identified as four groups. The results of the hierarchical cluster analysis showed that the samples from different localities could be divided into four distinct groups (Fig. 3). Cluster I was exhibited slightly higher amounts of UA, flavonoids, and polysaccharides with average 2.0 mg/g, 7.96%, and 7.50%, respectively. This group included the majority of accession from Jiangsu, Anhui and some from Gansu, Guangxi, and Guizhou. Cluster II was identified as a flavonoids-poor chemotype, in which flavonoid content ranged from 2.16% to 7.05%, with average of 4.96%. This group included populations mainly from Jiangxi, Fujian, Yunnan, and some from Anhui, Jiangsu, and Sichuan. Cluster III characterized by polysaccharides-rich and flavonoids-poor. Only a single population from Zhejiang belonged to this group, which located in the most eastern part of China. Cluster IV was identified as UA-rich chemotype and exhibited highest amount of flavonoids, ranging from 8.96% to 9.53% and on average 9.29%. This group included populations from Sichuan, which locates in the most western part of China.

Discussion

The present study has highlighted the presence of considerable morphological variation in the 28 populations collected from different parts of China. Two

Table 3 Cluster mean for 28 *P. vulgaris* populations in morphological traits

Cluster	No. of populations	PH / cm	IL / cm	SD / mm	LL / cm	LW / cm	COL / mm	CTW / mm	CL / mm	CW / mm	FSL / mm	FSW / mm	MD
A	12	28.41	7.16	2.19	3.85	1.45	13.45	4.24	7.99	4.11	53.78	11.11	3.79
B	4	20.58	6.03	2.20	3.46	1.20	12.36	3.68	6.82	3.44	46.37	10.82	1.88
C	2	24.73	5.97	1.90	2.86	1.21	11.89	3.38	5.46	4.15	56.39	11.38	4.50
D	1	15.33	3.40	2.20	3.19	1.82	12.46	4.38	7.05	3.68	53.77	10.6	3.00
E	6	15.84	5.31	2.07	3.44	1.51	9.08	2.11	5.85	3.33	32.49	9.98	2.75
F	3	21.05	6.77	1.85	2.64	1.11	8.62	2.23	5.60	3.34	35.86	9.62	4.67

Table 4 Three chemical compositions of 28 *P. vulgaris* populations

Populations	UA / (mg·g^{-1})	Flavonoids / %	Polysaccharides / %	Populations	UA / (mg·g^{-1})	Flavonoids / %	Polysaccharides / %
NJ	2.32 ± 0.18	8.87 ± 0.57	7.24 ± 0.49	HF	2.20 ± 0.13	7.15 ± 0.45	7.75 ± 0.46
JN	2.14 ± 0.15	5.41 ± 0.31	5.84 ± 0.35	JO	1.26 ± 0.08	2.85 ± 0.17	5.94 ± 0.34
GC	2.18 ± 0.16	10.29 ± 0.60	7.34 ± 0.49	GL	1.77 ± 0.12	7.11 ± 0.43	6.78 ± 0.66
XY	2.63 ± 0.15	7.45 ± 0.44	7.90 ± 0.53	HZ	1.93 ± 0.14	3.48 ± 0.20	8.67 ± 0.50
YX	1.75 ± 0.11	8.10 ± 0.51	7.45 ± 0.48	LD	3.95 ± 0.23	9.38 ± 0.54	9.00 ± 0.53
ZJ	2.01 ± 0.11	8.68 ± 0.54	7.80 ± 0.47	MY	3.53 ± 0.92	9.53 ± 0.57	7.56 ± 0.44
YZ	2.08 ± 0.13	8.92 ± 0.56	8.09 ± 0.47	JS	2.95 ± 0.28	8.96 ± 0.52	7.13 ± 0.50
GD	2.27 ± 0.14	5.76 ± 0.33	5.28 ± 0.10	YA	2.92 ± 0.20	6.37 ± 0.37	6.12 ± 0.36
LJ	1.48 ± 0.15	9.25 ± 0.53	5.83 ± 0.36	GY	1.50 ± 0.12	7.26 ± 0.49	6.89 ± 0.40
JD	1.33 ± 0.08	7.04 ± 0.40	8.40 ± 0.48	JA	2.21 ± 0.12	4.07 ± 0.32	6.48 ± 0.38
AQ	2.19 ± 0.16	5.07 ± 0.30	7.08 ± 0.45	LA	2.36 ± 0.16	2.16 ± 0.13	5.34 ± 0.31
HX	1.16 ± 0.07	5.15 ± 0.42	6.51 ± 0.38	CX	2.06 ± 0.14	8.80 ± 0.52	7.20 ± 0.42
CZ	2.20 ± 0.13	6.83 ± 0.40	8.48 ± 0.49	KM	2.17 ± 0.13	7.53 ± 0.47	5.94 ± 0.36
GC	2.40 ± 0.14	5.66 ± 0.33	7.81 ± 0.64	FD	1.50 ± 0.09	5.24 ± 0.34	7.59 ± 0.48

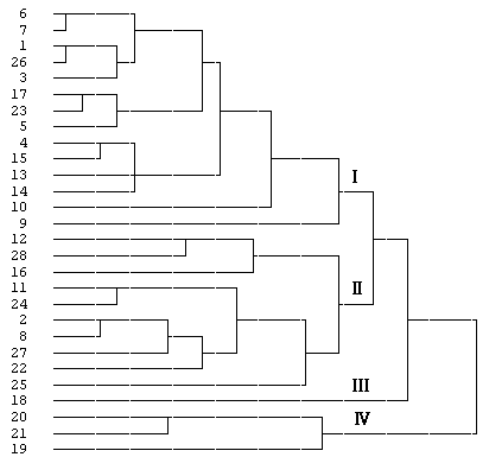

Fig. 3 UPGMA dendrogram based on similarity matrix constructed from the three chemical compositions of 28 *P. vulgaris* populations

morpho-types could be clearly distinguished. The populations of clusters A, B, C, and D had distinctly high PH and large flower. However, the shape of populations was smaller in clusters E and F. In order to analyze morphological variation, 28 populations from different regions of the country grown at a single location were subjected to the same environmental conditions. Therefore, observed variations could be largely genetic. But it observed that some clusters included populations from different geographical locations while populations from similar locations fell in different clusters (Mathur, Sharma, and Kumar, 2003). For example, the populations originating from Jiangsu Province got grouped in more than one cluster (Fig. 2). It indicated that the factor other than geographical distribution may be responsible for genetic divergence in agreement with other studies (Singh, Shukla, and Singh, 1998; Rameshkumar and Singh, 2005). Thus there was no relationship between genetic divergence and geographical origin as populations from one state entered into more than one cluster and vice versa as reported by earlier researchers (Stebbin, 1960; Rao, Ramachandran, and Sharma, 1980). Tendency to form such type of clustering irrespective of geographical boundaries showed that the regional isolation was not the only factor contributing to diversity in natural populations.

The analysis of variance revealed abundant diversity among 28 populations of *P. vulgaris* in chemical traits. The differences in chemical components will affect the quality of pharmaceutical products and the standardization of this herbal medicine. Furthermore, the safety and quality of raw materials from a medicinal plant depend significantly on its intrinsic and extrinsic factors. As WHO has defined, the intrinsic factors include genetic influences, and extrinsic factors include environmental conditions, collection methods, cultivation, harvest, and post-harvest (WHO, 2003). The fruit spikes of *P. vulgaris* for chemical analysis were collected from the authentic locations. So it is a common understanding that the variation of chemical characteristics are influenced by the intrinsic (genetic) and extrinsic (environmental) factors, such as soils, temperatures, and the plant available concentration of nutrients. This may indicate that the differences in *P. vulgaris* in chemical components from different regions are attributable not only to environmental conditions in which the plants were collected or grown, but also to some extent to the genetic background that has shown an independent profile of the populations.

To effectively control and evaluate the quality of medicinal plants, it is necessary to take both chemical composition and genetic variation of the target populations of the plants from different geographical origins into consideration. For example, if a specific type of medicine plants from a region is recognized as an effective component and its genetic background also shows a consistently specific profile, it can be developed to fine varieties for the production and utilization of human (Guo, 1998).

Combining of chemical and genetic diversity in *P. vulgaris* populations, it will provide a useful guide for their efficient utilization, conservation, breeding, and management of good agricultural and collection practices. When selecting populations that may contain high active components, the populations such as LD and MY with high genetic diversity should be included for conservation and sustainable utilization as sources for establishing a cultivation population. In addition, information on morphological and chemical characteristics of these populations can be further used in breeding programmes for combining desirable traits in commercial cultivars. For example, populations of cluster A in morphological dendrogram and cluster IV in chemical dendrogram are suggested to be used in varietal improvement program since they showed the

big shape of plant and high amount of active components.

There were no obvious correlations between morphological trait cluster and chemical component cluster. One reason may be due to the difference of sources in samples of chemical and morphological analyses. The samples of chemical analysis derived from the authentic locations, while the materials of morphological analysis were cultivated at Shangfeng Farm in Jiangsu, whose seeds were brought from the origin. Another reason could be morphological markers which were easily affected by conditions, growth period, and other factors, so genetic expression was not very stable. Therefore, to study genetic variation, there were considerable uncertainty and limitations by morphological markers. If the relationship between the chemical composition and the genetic background should be identified, we need to further study from the gene level. Despite of that, the results demonstrate that the general trend that populations of Sichuan Province had higher contents of active ingredient with small plants, which were in the same group in both dendrograms (from chemical components and morphological character). In the same, many populations were in the cluster A and I of both dendrograms, such as ZJ, LJ, NJ, HF, YX, YZ, CZ, and GC, which held the traits of the long spike and excellent comprehensive traits.

References

Chang HM, But PPH, 1987. *Pharmacology and Applications of Chinese Medicinal Materials*. Singapore: World Scientific, 964.

Chen XH, Wei SL, Wang WQ, 2003. Germplasm resources and the quality of Chinese traditional medicines. *Res Inf Tradit Chin Med* 5: 11-14.

Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, Kessler RC, 1998. Trends in alternative medicine use in the United States, 1990－1997: Results of a follow-up national survey. *JAMA* 280: 1569-1575.

Guo QS, Zhang XX, Shen XL, Chen YH, 2009. Effects of seed priming on drought tolerence in *Prunella vulgaris*. *China J Chin Mater Med* 34: 1195-1198.

Guo QS, 1998. Study on genetic diversity of *Pinellia ternata* (Thunb.) Breit. Nanjing Agricultural University: 59.

Hou XJ, Chen W, 2008. Optimization of extraction process of crude polysaccharides from wild edible *Bachu* mushroom by response surface methodology. *Carbohyd Polym* 72: 67-74.

Jia XB, Feng L, Chen Y, Gao CL, Shao ZZ, Song SH, Liu GM, 2009. Ideas and methods on material basis of chemoprevention of *Prunella valgaris* on lung canaer. *Chin Tradit Herb Drugs* 40(2): 316-318.

Lamaison JL, Petitjean-Freytet C, Carnat A, 1991. Medicinal Laminaceae with antioxidant properties, a potential source of rosmarinic acid. *Pharm Acta Helv* 66: 185-188.

Lee KH, Lin Y, Wu TS, Zhang DC, Yamagishi T, Hayashi T, Hall IH, Chang JJ, Wu RY, Yang TH, 1998. The cytotoxic principles of *Prunella vulgaris*, *Psychotria serpens* and *Hyptis capitata*: Ursolic acid and related derivatives. *Planta Med* 54: 308-311.

Liao L, Guo QS, 2009. Establishment of ISSR marker technology and optimization of its system in *Prunella vulgaris*. *Chin Tradit Herb Drugs* 40: 1131-1135.

Liu GM, Jia XB, Chen Y, Zhu FX, Feng L, Jiang J, Shi F, 2010. Diversity of chemical constituents in *Prunella* L. at different sources by HPLC. *Chin Tradit Herb Drugs* 41(8): 1384-1386.

Liu Y, Song SJ, Xu SX, 2003. Advances in the study on the chemical constituents and biological activities of *Prunella vulgaris*. *J Shenyang Pharm Univ* 20: 55-59.

Psotova J, Kolar M, Sousek J, Svagera Z, Vicar J, Ulrichova J, 2003. Biological activities of *Prunella vulgaris* extract. *Phytother Res* 17: 1082-1087.

Rameshkumar R, Singh SP, 2005. Genetic divergence in *Cuphea lanceolata* －a medicinal plant. *J Med Aromat Plant Sci* 27: 238-242.

Rao VR, Ramachandran M, Sharma JL, 1980. Multivariate analysis of genetic divergence in sunflower. *Indian J Gene* 40: 74-85.

Ryu SY, Oak MH, Yoon SK, Cho DI, Yoo GS, Kim TS, Kim KM, 2000. Anti-allergic and anti-inflammatory triterpenes from the herb of *Prunella vulgaris*. *Planta Med* 66: 358-360.

Mathur S, Sharma S, Kumar S, 2003. Description of variation in the Indian accessions of the medicinal plant *Centella asiatica* (L.) Urban. *Plant Gene Res Newsl* 135: 47-52.

Shin TY, Kim YK, Kim HM, 2001. Inhibition of immediatetype allergic reactions by *Prunella vulgaris* in a murine model. *Immunopharmacol Immunotoxicol* 23: 423-435.

Singh SP, Shukla S, Singh M, 1998. Genetic divergence in relation to breeding for fatty acids in opium poppy (*Papaver somniferum* L). *J Genet Breed* 52: 301-306.

Stebbins GL, 1960. *Variation and Evolution in Plants*. New York: Columbia University Press.

Sun WG, Liao HL, Ye ZM, He GX, 2003. Advances in the study on the chemical constituents and pharmacological action of *Prunella vulgaris*. *Chin J Inf Tradit Chin Med* 10: 86-88.

Tabba HD, Chang RS, Smith KM, 1989. Isolation, purification and partial characterization of prunellin, an anti-HIV component from aqueous extracts of *Prunella vulgaris*. *Antivir Res* 11: 263-273.

WHO, 2003. *Guidelines on Good Agricultural and Collection Practices* (*GACP*) *for Medicinal Plants*. Geneva, Switzerland: World Health Organization.

Xu HX, Lee SH, Lee SF, White RL, Blay J, 1999. Isolation and characterization of an anti-HSV polysaccharide from *Prunella vulgaris*. *Antivir Res* 44: 43-54.

Yu JS, Yu JP, 2007. Study on extraction techniques and variety trends of the flavonoids in *Sarcandrae glabra*. *China J Chin Mater Med* 32: 307-309.

Zhang KJ, Zhang MZ, Wang QD, 2005. Inductive effect of *Prunella vulgaris* Injection on K562 cells apoptosis. Chin Tradit Herb Drugs 36(7): 1031-1035.