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A STUDY ON THE PHENOTYPIC DIVERSITY OF *SINOPODOPHYLLUM HEXANDRUM* (ROYLE) YING

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Abstract

Genetic diversity is a basic component of biological diversity, and phenotypic diversity is an important research direction of genetic diversity. The variation in phenotypic traits often has important implications for environmental adaptation and evolution, contributing to an understanding of the ways, mechanisms, and influencing factors of biological adaptation and biological evolution. In this study, the traits of leaves, flowers, fruits, and plant height of *Sinopodophyllum hexandrum* (Royle) Ying from different natural distribution regions were measured and analyzed to investigate its phenotypic diversity, which provided a theoretical basis for the analysis of genetic diversity, the protection of germplasm resources, the breeding of improved varieties, and the innovative development and utilization of *S. hexandrum*. By the phenotypic diversity investigation of 30 characters of 160 *S. hexandrum* from eight different regions, the results indicated that the phenotypic variation of *S. hexandrum* was extremely rich, and most traits had wide variation and significant differences among populations (among surveyed regions) and within populations (within surveyed regions). The coefficient of variation (*CV*) of 30 phenotypic traits in *S. hexandrum* ranged from 7.98% (L_{TW}) to 31.90% ($L_{TW/LL}$), with an average of 12.22%. The phenotypic differentiation coefficient (V_{ST}) ranged from 0.045% (L_{Se}/W_{Se}) to 61.305% (W_{Pe}), with an average of 17.776%. The degree of phenotypic differentiation was in this sequence as plant height (25.827%)> floral organs (19.376%)> fruiting organs (16.248%)> leaf organs (14.914%), indicating that the degree of differentiation of phenotypic traits in reproductive organs was higher than that in vegetative organs, and the phenotypic stability of reproductive organs was poor. The degree of variation among different morphological characteristics of *S. hexandrum* was quite different, but it showed a certain regularity, viz. the differentiation among individuals within surveyed regions was greater than that among surveyed regions, and the stability of the phenotypic traits of individuals within surveyed regions was worse than that among surveyed regions.

Key words: *Sinopodophyllum hexandrum*; Different regions; Phenotypic diversity; Variation coefficient; Differentiation degree.

Introduction

Sinopodophyllum hexandrum (Royle) Ying, also known as copper chopsticks, is a perennial rhizomatous herb plant of the *Sinopodophyllum* genus of the *Berberidaceae* family, which is mainly distributed in Shaanxi, Gansu, Sichuan, Yunnan, Tibet, Ningxia, Qinghai and other places in China, and has been listed in CITES Appendix II (Anon., 2011; Guo *et al.*, 2015; CITES, 2021; Lai *et al.*, 2022). Modern pharmacological studies have proved that lignans in *S. hexandrum* have high antitumor activity (Anon., 2015). The arylnaphthalene lignan has the strongest anticancer activity, and its content is more than three times that of the similar species, *Podophyllum peltatum* (Giri & Narasu, 2000). Podophyllum has a good effect on the treatment of skin cancer, condyloma acuminatum, cervical cancer and breast cancer, and is the precursor substance for the synthesis of anticancer drugs such as VP-16, VM-26, GP7, NK611 and other drugs (Issell, 1982; Giri & Narasu, 2000; Canel *et al.*, 2001; Moraes *et al.*, 2002; Rickard-Bon & Thompson, 2003; Yousefzadi *et al.*, 2010). At present, the domestic and foreign research on *S. hexandrum* mainly focuses on the chemical fingerprint and the comparison of the active ingredient content (Purohit *et al.*, 1999; Giri & Narasu, 2000; Farkya *et al.*, 2004; Lin *et al.*, 2008; Zhou *et al.*, 2008; Qin *et al.*, 2009; Xiong *et al.*, 2010; Kong *et al.*, 2010; Sun *et al.*, 2011; Huang *et al.*, 2012), biological activity and pharmacological activity (Canel *et al.*, 2000; Reddy *et al.*, 2010), phylogenetic evolution (Li *et al.*, 2011), and genetic diversity (Xiao *et al.*, 2006a, 2006b; Xiao, 2006; Alam *et al.*, 2008, 2009; Naik *et al.*, 2010; Xiao *et al.*, 2015; Liu *et al.*, 2015).

Phenotypic diversity is one of the important contents of genetic diversity research, and the variation in phenotypic traits often has adaptive and evolutionary significance (Zeng & Bai, 2007). Therefore, the study of phenotypic traits can provide insight into the ways, mechanisms and influencing factors of biological adaptation and evolution (Ge & Hong, 1994). Using phenotypic traits to study the genetic diversity of populations can be directly observed and analyzed in the field, which has the advantages of simplicity, speed and economy, especially when it is necessary to understand genetic variation in a short period of time or when other methods can't be carried out, morphological means are a valuable choice (Luo *et al.*, 2003; Li & Gu, 2005). Zhang *et al.*, (2018) analyzed the phenotypic diversity of three different populations of *Cistanches Herba*, a medicinal plant

from Xinjiang, and the results indicated that *Cistanches Herba* showed different phenotypic characteristics in different distribution areas. Ye *et al*., (2020) analyzed nine phenotypic traits of Xihuangcao and found that Xihuangcao were significantly different among populations. Dan *et al*., (2017) analyzed the phenotypic diversity of *S. hexandrum* from different populations in Nyingchi, Tibet, and found that the phenotypic diversity of *S. hexandrum* from different populations was high and the variation of phenotypic traits was discontinuous. However, few reports are available for the phenotypic diversity of *S. hexandrum*. Therefore, in this experiment, the phenotypic traits of leaves, flowers, fruits and plant height of wild *S. hexandrum* in the representative distribution area of China (involving eight production areas in seven provinces) were used as the inspection indicators to clarify the degree of phenotypic variation and the regularity of phenotypic variation of *S. hexandrum*, and to provide a new theoretical basis for its genetic diversity research, the selection and breeding of improved varieties, and the innovative development and utilization.

Materials and Methods

Plant material: The sampling points were set in Jingyuan, Ningxia (S1), Mei County, Shaanxi (S2), Huzhu, Qinghai (S3), Yongdeng, Gansu (S4), Kangding, Sichuan (S5), Shangri-la, Yunnan (S6), Nyingchi, Tibet (S7), Diebu, Gansu (S8) (Guo *et al.*, 2015; Lai *et al.*, 2022). The environmental conditions of eight sampling sites were shown in Table 1 and the photos of *S. hexandrum* from eight sample points were shown in Fig. 1. Four natural populations were selected at each sampling point, and the distance between populations was at least 30 km. Five individuals were randomly collected within each population, and the distance between individuals was at least 10 m (Xiao *et al.*, 2006a, 2006b; Sertse *et al.*, 2011). The sample information of the eight sample sites were shown in Table 2.

Table 1. Environmental conditions of eight sampling sites.

No.	Locations	Annual precipitation (mm)	Average annual temperature (℃)	Annual sunshine hours (h)	Soil type
S_1	Jingyuan, Ningxia	641.5	6.9	2370	Grey-cinnamon soils
S_2	Mei County, Shaanxi	609.5	12.9	2015.2	Dark brown soil
S_3	Huzhu, Qinghai	477.4	5.8	2581.7	Alpine meadow soil
S_4	Yongdeng, Gansu	290	5.9	2659	Alpine meadow soil
S_5	Kangding, Sichuan	830	7.5	1738	Humus loam
S_6	Shangri-la, Yunnan	649.4	5.5	2141.0	Subalpine shrub soil
S_7	Nyingchi, Tibet	650	8.7	2022.2	Alpine shrub soil
S_8	Diebu, Gansu	634.6	6.7	2242.2	Alpine meadow soil

Fig. 1. The photos of *S. hexandrum* from eight sampling sites.

Table 2. Sample information from the eight sampling sites.

No.	Locations	Population	Code	Coordinates	Sample size	Altitude (m)
S_1	Jingyuan, Ningxia	Baiyunshan	BYS	E106°15′N35°37′	20	2232
		Yehegu	YHG	E106°13′N35°31′	20	2370
		Zhiwuyuan	ZWY	E106°18′N35°22′	20	2080
		Qiaozigou	QZG	E106°22′N35°15′	20	2564
S_2	Mei County, Shaanxi	Pingansi	PAS	E107°43′N34°1′	20	2815
		Mingxingsi	MXS	E107°44′N34°0′	20	2637
		Yuhuangmiao	YHM	E107°22′N34°5′	20	1780
		Liulingou	LLG	E108°10′N33°52′	20	1013
S_3	Huzhu, Qinghai	Zhalongkou	ZLK	E102°34′N36°53′	20	2264
		Zhalonggou	ZLG	E102°37′N36°47′	20	2698
		Yuanlongou	YLG	E102°27′N36°54′	20	3069
		Xiahe	XH	E102°42′N36°44′	20	3169
S_4	Yongdeng, Gansu	Suoergou	SEG	E102°43′N36°40′	20	2389
		Lalagou	LL	E102°43′N36°35′	20	2733
		Dachang	DC	E102°44′N36°44′	20	2449
		Datanzigou	DTZ	E102°46′N36°33′	20	2530
S_5	Kangding, Sichuan	Yajiageng	YJG	E101°57′N30°0′	20	2946
		Laoyulin	LYL	E101°59′N29°55′	20	3788
		Shengkangcun	SKC	E102°1′N30°4′	20	3207
		Zhonggucun	ZGC	E101°54′N30°16′	20	3554
S_6	Shangri-la, Yunnan	Rime	RM	E99°37′N27°51′	20	3528
		Naipi	NP	E99°36′N28°2′	20	3432
		Xiaozhongdian	XZD	E99°56′N27°28′	20	3590
		Mugaocun	MGC	E99°34′N27°30′	20	2250
S_7	Nyingchi, Tibet	Zhangmaicun	ZMC	E94°20′N29°40′	20	3097
		Selong	SL	E94°11′N29°44′	20	3173
		Pula	PL	E94°22′N29°27′	20	3256
		Duosongba	DSB	E94°13′N29°37′	20	3855
S_8	Diebu, Gansu	Zemo	ZM	E103°21′N33°45′	20	2728
		Dalong	DL	E103°14′N35°2′	20	2620
		Dalagou	DLG	E103°22′N33°52′	20	2677
		Nagai	NG	E103°14′N33°51′	20	2963

Phenotypic trait selection and determination: Thirty main phenotypic traits of leaves, flowers, fruits and plant height were selected to measure (Table 3) (Abdessalem *et al.*, 2014; Dan *et al.*, 2017; Xu *et al.*, 2021; Li *et al.*, 2021). Nine indexes of leaf were measured by tape measure, vernier caliper (799 series, Starrett Company, USA) and leaf area meter. The 14 indexes of the flower were measured by straightedge and vernier caliper. The number of seeds in the fruit was determined by manual counting method, and the other 5 indexes were determined by vernier caliper. Twenty seeds were randomly selected from each plant for the determination of seed traits. The plant height index (H_P) was measured by tape measure. And each indicator was measured in triplicate.

Data processing: Statistical analysis of the data was performed with SPSS 25.0 software (IBM, USA), including calculation of the coefficient of variation (*CV*), phenotypic differentiation coefficient (V_{ST}), population repeatability and individual repeatability, and nested analysis of variance.

The formula for calculating the coefficient of variation (*CV*):

$$CV = \left(\frac{\text{SD}}{\text{Mean}}\right) \times 100\% \quad (1)$$

where:
SD = the standard deviation of the data.
Mean = the mean of the data.

The formula for calculating the phenotypic differentiation coefficient (V_{ST}):

$$V_{ST} = \frac{\sigma_{t/s}^2}{\sigma_{t/s}^2 + \sigma_s^2} \quad (2)$$

where:
$\sigma_{t/s}^2$ = the average variance (variance component) among populations.
σ_s^2 = the average variance (variance component) within populations.

Table 3. Quantitative morphological characteristics of *S. hexandrum*

Organ	Code	Morphological indicators	Abbreviates
Leaf	1	Leaf length	L_L
	2	Leaf width	W_L
	3	Square of leaf area	S_{LA}
	4	Leaf length/ Leaf width	L_L/L_W
	5	Leaf tip to widest length	L_{TW}
	6	Leaf tip to widest length / Leaf length	L_{TW}/L_L
	7	Petiole length	L_{Pe}
	8	Petiole width	W_{Pe}
	9	Leaf lateral veins No.	N_L
Flower	10	Petal length	L_P
	11	Petal width	W_P
	12	petal length / petal width	L_P/W_P
	13	Anther length	L_A
	14	Filament length	L_{Fi}
	15	Stamen length	L_{Sta}
	16	Filament length/ Stamen length	L_{Fi}/L_{Sta}
	17	Anther length/stamen length	L_A/L_{Sta}
	18	Gynoecium length	L_G
	19	Androecium-Gynoecium length	$L_{A\text{-}G}$
	20	Stigma length	L_{Stig}
	21	Sepals length	L_{Se}
	22	Sepals width	W_{Se}
	23	Sepals length/ Sepals width	L_{Se}/W_{Se}
Fruit	24	Fruit length	L_F
	25	Fruit width	W_F
	26	Number of seeds	N_S
	27	Seed length	L_S
	28	Seed width	W_S
	29	Seed length/ Seed width	L_S/W_S
Plant	30	Plant height	H_P

The formula for calculating population repeatability and individual repeatability:

Population repeatability (R_P):

$$R_P = \frac{MS_1 - MS_2}{MS_1 + (P-1)MS_2} \quad (3)$$

Individual repeatability (R_I):

$$R_I = \frac{MS_2 - MS_3}{MS_2 + (F-1)MS_3} \quad (4)$$

where:
MS_1 = the mean square among populations for each trait.
MS_2 = the mean square within populations for each trait.
MS_3= the mean square error of each trait.
P = the number of populations.
F = the number of individuals.

The linear model for nested analysis of variance:

$$Y_{ijk} = \mu + S_i + T_{(i)j} + \varepsilon_{(ij)k} \quad (5)$$

where:
Y_{ijk} = the k^{th} observation value of the j^{th} individual plant in the i^{th} population.
μ = the overall mean.
S_i = the effect value of the i^{th} population.
$T_{(i)j}$ = The effect value of the j^{th} individual plant in the i^{th} population.
$\varepsilon_{(ij)k}$ = the random error.

Results and Analysis

Analysis of variation in mean values of phenotypic traits in *S. hexandrum*: The results of the variation in mean values of phenotypic traits in *S. hexandrum* were shown in Table 4. There were significant differences among the different traits of *S. hexandrum* (p<0.05). In the eight growth regions of S_1~S_8, for 30 traits of the selected 160 individuals, the range of variation in mean values was 0.112 ± 0.015 (S_5, W_S) ~ 41 ± 3.733 (S_5, N_S), the coefficient of variation (*CV*) values ranged from 4.42% (S_4, $L_{A\text{-}G}$) to 29.20% (S_5, L_{TW}/L_L), and the RR values ranged from 7.73% (S_5, L_F) to 70.29% (S_5, W_{Se}). The largest *CV* value was the ratio of the leaf tip to widest length to leaf length (L_{TW}/L_L) for *S. hexandrum* from the S_5 region, with a value of 29.20%, the RR value of 23.10%, and a mean value of 0.5239 ± 0.153 cm. It was followed by the sepal width (W_{Se}) of *S. hexandrum*, which was also from the S_5 region, with a value of 25.89%, an RR value of 70.29%, and an average of 1.205 ± 0.312 cm. However, the *CV* value of the androecium-gynoecium length ($L_{A\text{-}G}$) trait was the smallest (S4), with a value of 4.42%, the RR value of 44.04% and a mean value of 1.628 ± 0.072 cm. These data showed that the L_{TW}/L_L and W_{Se} trait had greater phenotypic variation and the differentiation were more severe, while the $L_{A\text{-}G}$ trait phenotypic variation was smaller and the trait was more stable.

Table 4. The morphological characteristics variations of *S. hexandrum* among eight regions.

Variables	S_1				S_2				S_3			
	Mean	**SD**	***CV***	**RR**	**Mean**	**SD**	***CV***	**RR**	**Mean**	**SD**	***CV***	**RR**
L_L	16.325	1.562	9.57%	19.80%	17.455	1.617	9.26%	18.89%	18.435	2.228	12.09%	21.14%
W_L	8.331	0.673	8.08%	13.37%	9.452	0.728	7.70%	12.47%	8.661	1.139	13.15%	20.54%
S_{LA}	11.662	1.237	10.61%	20.22%	12.845	1.292	10.06%	18.86%	12.636	1.703	13.48%	23.92%
L_L/L_W	1.960	0.242	12.35%	17.25%	1.847	0.297	16.08%	21.82%	2.129	0.288	13.53%	23.63%
L_{TW}	9.657	0.673	6.97%	17.28%	9.777	0.728	7.45%	17.74%	9.877	1.339	13.56%	23.63%
L_{TW}/L_L	0.592	0.076	12.85%	23.16%	0.560	0.131	23.39%	18.21%	0.536	0.042	7.84%	5.97%
L_{Pe}	15.668	2.123	13.55%	23.37%	16.463	2.178	13.23%	22.64%	15.779	2.189	13.87%	27.42%
W_{Pe}	0.952	0.067	7.04%	23.84%	1.022	0.122	11.94%	28.57%	1.152	0.133	11.55%	16.67%
N_L	7.000	0.856 a	12.23%	42.86%	9.000	0.911 b	10.12%	34.06%	9.000	1.322 b	14.69%	29.61%
L_P	3.785	0.457 a	12.07%	17.54%	3.926	0.512 b	13.04%	18.57%	3.984	0.423 b	10.62%	13.28%
W_P	1.566	0.125 a	7.98%	14.75%	1.654	0.180 a	10.88%	17.90%	1.768	0.241 b	13.63%	28.05%
L_P/W_P	2.417	0.246 a	10.18%	27.80%	2.374	0.301 a	12.68%	31.05%	2.253	0.312 b	13.85%	32.71%
L_A	2.332	0.231 a	9.91%	22.90%	2.431	0.286 b	11.76%	24.64%	2.541	0.297 c	11.69%	7.83%
L_{Fi}	1.436	0.127 a	8.84%	23.54%	1.535	0.182 b	11.86%	13.22%	1.634	0.213 c	13.04%	3.12%
L_{Sta}	3.768	0.434 a	11.52%	18.23%	3.966	0.489 b	12.33%	18.96%	4.175	0.564 c	13.51%	8.43%
L_A/L_{Sta}	0.619	0.064 a	10.34%	5.82%	0.613	0.072 a	11.75%	16.48%	0.609	0.046 b	7.56%	10.02%
L_{Fi}/L_{Sta}	0.381	0.026 a	6.82%	17.06%	0.387	0.051 a	13.18%	33.59%	0.391	0.042 a	10.73%	7.92%
L_G	3.235	0.436 a	13.48%	31.65%	3.546	0.491 b	13.85%	2.51%	3.425	0.402 c	11.74%	20.12%
$L_{A\text{-}G}$	1.321	0.067 a	5.07%	50.79%	1.625	0.122 b	7.51%	45.29%	1.532	0.203 c	13.25%	21.93%
L_{Stig}	1.132	0.065 a	5.74%	37.37%	1.333	0.120 b	9.00%	36.61%	1.226	0.111 c	9.05%	7.18%
L_{Se}	3.521	0.554 a	15.73%	17.92%	3.724	0.609 b	16.35%	18.69%	3.637	0.622 c	17.10%	8.14%
W_{Se}	1.324	0.235 a	17.75%	57.63%	1.635	0.290 b	17.74%	50.64%	1.522	0.101 c	6.64%	28.12%
L_{Se}/W_{Se}	2.659	0.268 a	10.08%	18.95%	2.278	0.243 b	10.67%	24.98%	2.390	0.271 c	11.34%	15.44%
L_F	5.236	0.635 a	12.13%	23.57%	5.335	0.690 a	12.93%	24.35%	5.446	0.701 a	12.87%	16.51%
W_F	2.657	0.353 a	13.29%	21.38%	2.462	0.308 b	12.51%	25.71%	2.768	0.379 c	13.69%	8.42%
N_S	36.000	3.656 a	10.16%	16.67%	25.000	3.211 b	12.84%	24.26%	28.000	4.022 c	14.36%	23.80%
L_S	0.352	0.031 a	8.81%	14.77%	0.272	0.036 b	13.24%	2.57%	0.250	0.017 c	6.80%	6.80%
W_S	0.152	0.021 a	13.82%	44.54%	0.143	0.017 b	11.89%	17.48%	0.143	0.008 b	5.59%	18.88%
L_S/W_S	2.316	0.262 a	11.31%	28.20%	1.902	0.217 b	11.41%	37.75%	1.748	0.113 c	6.46%	18.19%
H_P	25.112	3.442 a	13.77%	12.86%	28.556	3.497 b	12.49%	11.71%	19.254	2.108 c	11.09%	20.42%
Variables	S_4				S_5				S_6			
	Mean	**SD**	***CV***	**RR**	**Mean**	**SD**	***CV***	**RR**	**Mean**	**SD**	***CV***	**RR**
L_L	20.665	1.567 d	7.58%	15.87%	14.215	1.639 e	11.53%	23.33%	22.132	1.749 f	7.90%	16.89%
W_L	11.241	0.678 c	6.03%	10.32%	9.341	0.750 b	8.03%	12.83%	13.224	0.760 d	5.75%	12.24%
S_{LA}	15.241	1.242 c	8.15%	15.77%	11.523	1.314 a	11.40%	21.19%	17.108	1.324 d	7.74%	16.74%
L_L/L_W	1.838	0.247 b	13.44%	20.89%	1.522	0.205 c	13.47%	27.73%	1.674	0.204 c	12.19%	27.66%
L_{TW}	12.477	0.678 b	5.43%	13.75%	7.447	0.750 c	10.07%	23.54%	15.437	0.760 d	4.92%	14.08%
L_{TW}/L_L	0.604	0.081 b	13.42%	30.31%	0.524	0.153 a	29.20%	23.10%	0.697	0.093 b	13.33%	21.22%
L_{Pe}	17.334	2.128 c	12.28%	21.39%	17.336	2.200 c	12.69%	21.61%	20.216	2.210 d	10.93%	21.11%
W_{Pe}	1.256	0.072 c	5.73%	21.74%	1.149	0.144 b	12.53%	18.36%	1.254	0.154 c	12.28%	19.38%
N_L	11.000	0.861 c	7.83%	27.69%	9.000	0.933 b	10.37%	23.16%	9.000	0.943 b	10.48%	33.33%
L_P	4.015	0.462 b	11.51%	17.68%	2.658	0.324 c	12.19%	28.14%	4.125	0.544 d	13.19%	24.18%
W_P	1.776	0.130 b	7.32%	15.60%	1.124	0.152 c	13.52%	10.23%	1.934	0.212 d	10.96%	29.18%
L_P/W_P	2.261	0.251 b	11.10%	31.76%	2.365	0.323 a	13.66%	31.97%	2.133	0.306 c	14.35%	40.00%
L_A	2.732	0.236 d	8.64%	21.23%	1.971	0.238 e	12.08%	31.35%	2.674	0.318 d	11.89%	32.43%
L_{Fi}	1.524	0.132 b	8.66%	25.20%	1.823	0.204 d	11.19%	23.15%	1.939	0.214 e	11.04%	34.62%
L_{Sta}	4.256	0.439 c	10.31%	17.22%	3.794	0.511 a	13.47%	20.32%	4.613	0.521 d	11.29%	22.12%
L_A/L_{Sta}	0.642	0.069 c	10.75%	12.77%	0.520	0.041 d	7.89%	23.10%	0.580	0.071 e	12.25%	7.07%
L_{Fi}/L_{Sta}	0.358	0.031 b	8.66%	31.00%	0.480	0.053 c	11.03%	31.01%	0.420	0.053 d	12.61%	16.65%
L_G	4.445	0.441 d	9.92%	24.07%	2.252	0.313 e	13.90%	35.88%	3.532	0.423 b	11.98%	43.29%
$L_{A\text{-}G}$	1.628	0.072 b	4.42%	44.04%	0.934	0.114 d	12.21%	27.30%	1.501	0.154 c	10.26%	37.71%
L_{Stig}	1.435	0.070 d	4.88%	32.68%	0.827	0.102 e	12.33%	9.79%	1.334	0.152 b	11.39%	48.35%
L_{Se}	2.912	0.559 d	19.20%	23.25%	3.313	0.631 e	19.05%	21.58%	3.712	0.641 b	17.27%	25.97%
W_{Se}	1.504	0.240 c	15.96%	53.79%	1.205	0.312 d	25.89%	70.29%	1.425	0.322 d	22.60%	69.12%
L_{Se}/W_{Se}	1.936	0.273 d	14.10%	28.41%	2.749	0.245 e	8.91%	21.39%	2.605	0.274 a	10.52%	27.87%
L_F	5.516	0.640 a	11.60%	23.21%	4.115	0.512 b	12.44%	7.73%	4.821	0.622 c	12.90%	37.11%
W_F	2.756	0.358 c	12.99%	22.28%	1.766	0.230 d	13.02%	19.93%	2.315	0.241 e	10.41%	34.13%
N_S	30.000	3.661 d	12.20%	20.15%	41.000	3.733 e	9.10%	14.84%	29.000	3.743 c	12.91%	27.59%
L_S	0.251	0.026 c	10.36%	39.04%	0.257	0.028 c	10.89%	14.01%	0.279	0.038 b	13.62%	21.86%
W_S	0.131	0.017 c	12.98%	28.24%	0.112	0.015 d	13.39%	15.18%	0.144	0.009 b	6.25%	50.63%
L_S/W_S	1.916	0.267 b	13.94%	36.48%	2.295	0.319 a	13.90%	19.04%	1.938	0.219 d	11.30%	39.95%
H_P	30.346	3.447 d	11.49%	10.87%	30.557	3.519 d	11.73%	10.99%	37.638	3.529 e	9.54%	10.21%

Table 4. (Cont'd.).

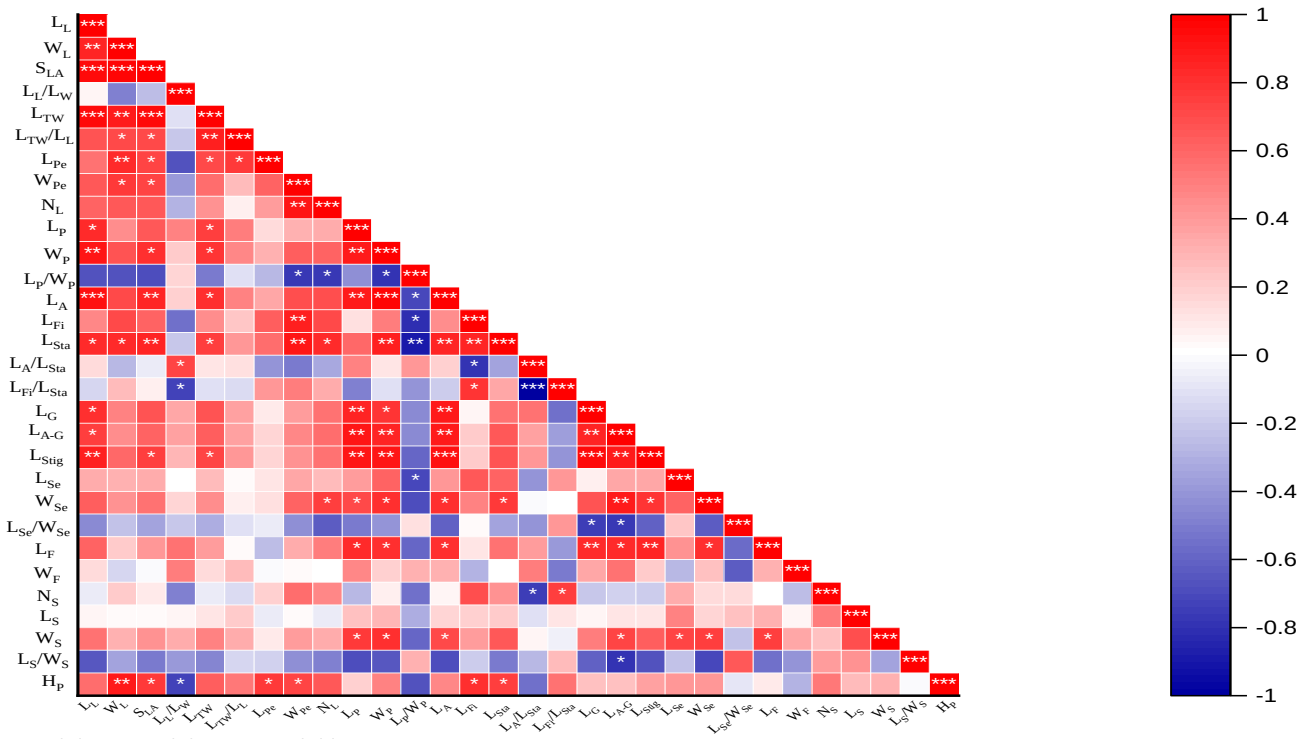
Variables	S_7				S_8				Average			
	Mean	**SD**	***CV***	**RR**	**Mean**	**SD**	***CV***	**RR**	**Mean**	**SD**	***CV***	**RR**
L_L	16.545	1.621 b	9.80%	22.90%	14.665	1.553 e	10.59%	25.83%	17.555	1.692	9.79%	20.58%
W_L	8.446	0.732 a	8.67%	19.76%	7.776	0.664 e	8.54%	21.46%	9.559	0.766	8.24%	15.37%
S_{LA}	11.821	1.296 a	10.96%	24.64%	10.679	1.228 e	11.50%	27.28%	12.939	1.330	10.49%	21.08%
L_L/L_W	1.959	0.201 a	10.26%	34.25%	1.886	0.233 b	12.35%	35.58%	1.852	0.240	12.96%	26.10%
L_{TW}	9.256	0.732 a	7.91%	24.03%	8.857	0.664 e	7.50%	25.11%	10.348	0.791	7.98%	19.89%
L_{TW}/L_L	0.559	0.075 a	13.41%	28.42%	0.604	0.067 b	11.09%	26.33%	0.585	0.177	31.90%	22.09%
L_{Pe}	15.227	2.182 a	14.33%	27.69%	17.225	2.114 c	12.27%	24.48%	16.906	2.166	12.89%	23.72%
W_{Pe}	0.763	0.106 d	13.89%	32.63%	0.856	0.058 e	6.78%	29.09%	1.051	0.107	10.22%	23.79%
N_L	6.000	0.715 d	11.92%	33.33%	6.000	0.847 d	14.12%	50.00%	8.250	0.924	11.47%	34.25%
L_P	3.482	0.416 e	11.95%	28.63%	3.335	0.448 f	13.43%	29.90%	3.664	0.448	12.25%	22.24%
W_P	1.323	0.184 e	13.91%	42.63%	1.063	0.116 c	10.91%	34.24%	1.526	0.168	11.14%	24.07%
L_P/W_P	2.632	0.305 d	11.59%	34.39%	3.137	0.237 e	7.55%	32.03%	2.446	0.285	11.87%	32.71%
L_A	2.122	0.290 f	13.67%	40.86%	2.006	0.222 f	11.07%	43.22%	2.351	0.265	11.34%	28.06%
L_{Fi}	1.135	0.146 f	12.86%	59.12%	1.127	0.118 f	10.47%	32.92%	1.519	0.167	10.99%	26.86%
L_{Sta}	3.257	0.453 e	13.91%	31.32%	3.133	0.425 e	13.57%	32.56%	3.870	0.480	12.49%	21.15%
L_A/L_{Sta}	0.652	0.083 f	12.74%	10.59%	0.640	0.055 c	8.59%	10.78%	0.609	0.063	10.23%	12.08%
L_{Fi}/L_{Sta}	0.348	0.045 e	12.91%	28.12%	0.360	0.017 b	4.73%	27.24%	0.391	0.040	10.08%	24.07%
L_G	3.213	0.435 a	13.54%	49.14%	2.736	0.427 f	15.61%	46.75%	3.298	0.421	13.00%	31.68%
$L_{A\text{-}G}$	1.117	0.126 e	11.28%	44.14%	1.223	0.058 f	4.74%	48.49%	1.360	0.115	8.59%	39.96%
L_{Stig}	1.143	0.124 a	10.85%	38.93%	0.911	0.056 f	6.15%	37.87%	1.168	0.100	8.67%	31.10%
L_{Se}	3.123	0.573 f	18.35%	27.31%	2.692	0.345 g	12.82%	31.69%	3.329	0.567	16.98%	21.82%
W_{Se}	1.128	0.294 e	26.06%	87.32%	1.114	0.212 e	19.03%	61.49%	1.357	0.251	18.96%	59.80%
L_{Se}/W_{Se}	2.769	0.277 e	10.00%	26.22%	2.417	0.259 c	10.72%	30.04%	2.475	0.264	10.79%	24.16%
L_F	4.732	0.634 c	13.40%	37.81%	4.136	0.566 b	13.68%	43.25%	4.917	0.625	12.75%	26.69%
W_F	1.851	0.262 d	14.15%	42.68%	3.125	0.344 f	11.01%	25.28%	2.463	0.309	12.63%	24.98%
N_S	18.000	2.515 f	13.97%	44.44%	20.000	2.647 f	13.24%	40.00%	28.375	3.399	12.35%	26.47%
L_S	0.242	0.029 d	11.98%	35.12%	0.237	0.022 e	9.28%	35.86%	0.268	0.028	10.62%	21.26%
W_S	0.113	0.016 d	14.16%	62.57%	0.114	0.012 d	10.53%	36.58%	0.132	0.014	11.08%	34.26%
L_S/W_S	2.142	0.301 d	14.05%	46.04%	2.079	0.253 d	12.17%	28.19%	2.042	0.244	11.82%	31.73%
H_P	19.218	2.421 c	12.74%	19.84%	18.335	2.433 c	13.52%	20.94%	26.127	3.050	12.05%	14.73%

Note: Morphological variable abbreviates were showed in Table 3. The units of 'mean' and 'SD' were cm. Small letters in the same line indicated variation significance ($p<0.05$)

Correlation analysis on phenotypic trait: From Fig. 2, it could be seen that all traits of *S. hexandrum* were correlated with each other. Filament length/stamen length (L_{Fi}/L_{Sta}) and anther length/stamen length (L_A/L_{Sta}) were extremely significantly negatively correlated ($p<0.001$) with a correlation coefficient of 1. Stamen length (L_{Sta}) and petal length/petal width (L_P/W_P) showed a highly significant negative correlation ($p<0.01$) with a correlation coefficient of 0.90. Leaf length (L_L) and anther length (L_A), leaf length (L_L) and square of leaf area (S_{LA}), leaf length (L_L) and leaf tip to widest length (L_{TW}), square of leaf area (S_{LA}) and leaf tip to widest length (L_{TW}), filament length/ stamen length (L_{Fi}/L_{Sta}) and stigma length (L_{Stig}), square of leaf area (S_{LA}) and leaf width (W_L), anther length (L_A) and petal width (W_P) all showed extremely significant positive correlations ($p<0.001$) with correlation coefficients of 0.94, 0.95, 0.95, 0.95, 0.95, 0.97, and 0.97, respectively.

Analysis of differences in phenotypic traits among (within) surveyed regions: The results of the nested analysis of variance were shown in Table 5. The differences of 30 phenotypic traits within surveyed areas were all significant ($p<0.05$), whereas the variation of partial phenotypic traits among the surveyed regions were significant ($p<0.05$). A total of 11 traits, including L_L, W_L, N_L, L_P, W_P, L_{Sta}, W_{Se}, L_F, W_F, L_S/W_S and H_P, reached significant levels among surveyed regions, accounting for 36.67% of all traits, while the remaining 19 traits among surveyed regions were not significant. From the above data, it could be seen that the morphological variation of *S. hexandrum* was more manifested among individuals within the surveyed area.

Analysis of phenotypic differentiation of *S. hexandrum* among (within) surveyed regions: The degree of phenotypic differentiation of *S. hexandrum* in different survey areas was counted, and the statistics results of variance component, percentage of variance component and phenotypic differentiation coefficient (V_{ST}) among surveyed regions (among populations) and within surveyed regions (within populations) were shown in Table 6. From Table 6, it could be seen that the mean value of the variance component of the 30 morphological characters among populations was 8.580%, the mean value of the variance component within populations was 25.081%, and the error was 7.190%, it indicated that the variance component within populations were significantly larger than that among populations.



*p<=0.05;**p<=0.01; ***p<=0.001

Fig. 2. The figure of the correlation between phenotypic trait.
Note: "*" indicates significant correlation ($p \leq 0.05$); "**" indicates highly significant correlation ($p \leq 0.01$); "***" indicates extremely significant correlation ($p \leq 0.001$).

Table 5. Variance analysis of morphological characteristics of *S. hexandrum*.

Variables	Among surveyed regions	Within surveyed regions	Errors
L_L	1123.563*	1576.359*	65.321
W_L	356.243*	314.657*	12.356
S_{LA}	51.495	35.464*	5.364
L_L/W_L	5.647	1.003*	0.067
L_{TW}	87.642	61.347*	5.364
L_{TW}/L_L	2.103*	1.112*	0.568
L_{Pe}	234.681	186.493*	15.687
W_{Pe}	125.667	90.225*	10.234
N_L	65.875*	55.676*	11.354
L_P	356.682*	310.473*	34.623
W_P	534.516*	456.172*	54.6215
L_P/W_P	3.658	1.357*	0.067
L_A	678.368	587.685*	65.324
L_{Fi}	3353.473	3102.473*	123.654
L_{Sta}	661.355*	541.023*	56.324
L_A/L_{Sta}	46.379	35.147*	5.328
L_{Fi}/L_{Sta}	38.397	21.106*	5.3654
L_G	123.654	90.335*	11.349
L_{A-G}	225.687	170.556*	20.55
L_{Stig}	33.460	21.337*	3.3247
L_{Se}	1102.358	990.654*	165.667
W_{Se}	556.754*	430.125*	18.657
L_{Se}/W_{Se}	12.359	3.456*	0.536
L_F	687.945*	541.236*	15.665
W_F	1376.357*	1021.481*	150.524
N_S	2578.316	2013.472*	110.235
L_S	458.387	352.443*	28.647
W_S	69.558	56.338*	5.657
L_S/W_S	24.271*	15.673*	3.214
H_P	1564.356*	1128.309*	94.652

Note: *Indicated significance ($p<0.05$)

Table 6. Variance component and differentiation coefficient among/within surveyed regions in *S. hexandrum*.

Variables	variance component			Percentage of variance component (%)			Phenotypic differentiation coefficient (%)
	Among surveyed regions ($\sigma_{t/s}^2$)	Within surveyed regions (σ_s^2)	Errors	Among surveyed regions	Within surveyed regions	Errors	V_{ST}
L_L	1.123	25.102	0.042	25.726	41.25	0.563	4.282
W_L	5.024	123.356	1.321	7.992	23.542	0.004	3.913
S_{LA}	0.49	7.658	25.314	6.576	22.12	12.313	6.014
L_L/W_L	0.647	10.249	0.027	0.126	0.684	0.643	5.938
L_{TW}	0.64	32.546	63.364	11.706	57.256	11.313	1.929
L_{TW}/L_L	2.103	6.336	0.526	7.804	29.354	0.021	24.920
L_{Pe}	4.682	37.851	1.687	26.804	62.354	0.007	11.008
W_{Pe}	125.66	79.314	10.234	0.667	0.234	6.063	61.305
N_L	15.873	40.546	1.354	9.808	25.358	0.146	28.134
L_P	0.682	11.587	0.623	12.802	34.352	0.003	5.559
W_P	2.051	13.1658	16.021	13.104	40.654	54.245	13.479
L_P/W_P	5.008	18.221	0.007	31.804	67.354	0.005	21.559
L_A	0.102	7.215	0.324	0.804	16.354	3.654	1.394
L_{Fi}	3.473	8.635	45.654	0.258	0.368	33.516	28.684
L_{Sta}	1.358	9.608	0.324	21.785	57.335	0.238	12.384
L_A/L_{Sta}	10.003	23.554	0.001	0.137	15.687	0.612	29.809
L_{Fi}/L_{Sta}	15.007	24.556	30.346	5.196	10.354	16.309	37.932
L_G	2.784	27.335	0.367	0.315	0.235	0.321	9.243
L_{A-G}	5.687	26.658	0.556	0.581	0.387	0.303	17.582
L_{Stig}	3.001	41.259	0.327	0.976	14.574	0.016	6.780
L_{Se}	12.376	15.687	1.623	3.807	19.357	0.353	44.101
W_{Se}	6.452	12.547	29.678	15.777	41.327	15.515	33.960
L_{Se}/W_{Se}	0.015	33.654	0.006	2.1048	17.6548	1.676	0.045
L_F	7.564	48.657	15.665	0.352	0.365	8.356	13.454
W_F	25.667	47.258	1.527	26.815	67.365	0.545	35.196
N_S	41.3123	56.472	7.249	0.135	1.657	0.116	42.248
L_S	0.834	23.163	0.074	0.1813	5.3687	1.612	3.475
W_S	0.531	17.885	75.157	9.8184	25.3684	40.667	2.883
L_S/W_S	0.045	19.663	0.014	13.246	38.796	5.223	0.228
H_P	10.257	29.457	2.256	0.181	15.369	1.343	25.827
Average	10.348	29.306	11.056	8.580	25.081	7.190	17.776

The range of V_{ST} for the 30 traits within the surveyed regions was 0.045% (ratio of sepals length to sepals width, L_{Se}/W_{Se}) ~ 61.305% (petiole width, W_{Pe}), and the mean value of V_{ST} was 17.776%. The maximum value of V_{ST} (61.305%) was obtained for petiole width (W_{Pe}), followed by the traits of sepals length (L_{Se}) and number of seeds (N_S) with V_{ST} values of 44.101% and 42.248%, respectively. Among the three major organs of leaf, flower and fruit, the mean value of V_{ST} for the floral organ traits was the largest at 19.376%, followed by the fruiting and leaf organs with the mean value of V_{ST} of 16.248% and 14.914%, respectively. The mean value of V_{ST} for the floral organs (19.376%) was significantly greater than the mean V_{ST} value for the 30 traits (17.776%), indicating a greater degree of variation in the floral organs than in the fruiting and leaf organs. In addition, the value of V_{ST} for plant height (H_P) (25.827%) was greater than the mean value of V_{ST} for floral organs, which may be due to the fact that plant growth is easily influenced by external environmental factors, such as temperature, rainfall, soil nutrients, topography, and altitude.

Analysis of phenotypic characteristic stability: The population repeatability (R_P) and individual repeatability (R_I) of the *S. hexandrum* survey regions were determined, and the measurement results were shown in Table 7. As can be seen from Table 7, the values of R_P were all greater than the values of R_I, that is, the population repeatability among the surveyed regions was all greater than the individual repeatability within the surveyed regions, and the phenotypic traits of *S. hexandrum* were more stable among surveyed regions than that within surveyed regions at the individual level. The mean values of R_P and R_I for the 30 phenotypic traits were 0.369 and 0.114, respectively. The eight traits of leaf organs had the highest mean R_P value of 0.463, followed by fruiting organs (6 traits) and floral organs (15 traits) with values of 0.409 and 0.317, respectively, while plant height (H_P) had the smallest mean value of R_P (0.136). Similarly, the means value of R_I had the same order on these different organs, that is, $R_{I\ \text{leaf}}$ (0.140) > $R_{I\ \text{fruit}}$ (0.114) > $R_{I\ \text{flower}}$ (0.104) > $R_{I\ \text{plant height}}$ (0.045). From these data, it could be seen that leaf organ traits were more stable than fruiting and floral organs, and the stability of the floral organs was poor at both the population (among survey regions) and individual (within survey regions) levels in *S. hexandrum*, but the differences in the stability of these organ traits were less pronounced. The stability of the plant height was the worst, and the stability of the organ traits of the leaves, flowers and fruits was quite different. This result was consistent with its phenotypic differentiation coefficient results.

Table 7. Repeatability among/within surveyed regions in *S. hexandrum*.

Variables	Repeatability	
	Among surveyed regions (R_P)	Within surveyed regions (R_I)
L_L	0.456	0.145
W_L	0.246	0.025
S_{LA}	0.358	0.047
L_L/W_L	0.878	0.367
L_{TW}	0.413	0.122
L_{TW}/L_L	0.657	0.246
L_{Pe}	0.548	0.137
W_{Pe}	0.145	0.034
N_L	0.386	0.025
L_P	0.35	0.053
W_P	0.143	0.064
L_P/W_P	0.146	0.035
L_A	0.064	0.043
L_{Fi}	0.088	0.056
L_{Sta}	0.261	0.06
L_A/L_{Sta}	0.373	0.142
L_{Fi}/L_{Sta}	0.253	0.032
L_G	0.424	0.039
L_{A-G}	0.341	0.23
L_{Stig}	0.415	0.215
L_{Se}	0.512	0.201
W_{Se}	0.551	0.312
L_{Se}/W_{Se}	0.455	0.056
L_F	0.654	0.243
W_F	0.354	0.046
N_S	0.453	0.142
L_S	0.426	0.115
W_S	0.354	0.103
L_S/W_S	0.215	0.034
H_P	0.136	0.045
Average	0.510	0.164

Discussion

The primitive angiosperms often exhibit great variation in morphological structure (Endress, 1987). As one of the most primitive angiosperms, *S. hexandrum* also has a polymorphism and complexity of variation in morphological characteristics. The coefficient of variation (*CV*) can be used for comparative analysis of differences among different phenotypic traits in populations or individuals (Luo & Chun, 2004). In this study, the phenotypic diversity of 30 characters of 160 *S. hexandrum* from eight different regions were analyzed, and the value of *CV* ranged from 4.42% (S_4, L_{A-G}) to 29.20% (S_5, L_{TW}/L_L) (Table 4), which indicated that phenotypic traits were obviously different from different growing regions, and there were abundant variations among populations and within populations of *S. hexandrum*. Correlation analysis can determine the degree of correlation between variables (Yin *et al.*, 2021). All traits in this study were correlated with each other, with 59 pairs of traits showing significant correlation (p<0.05), 31 pairs showing highly significant correlation (p<0.01), and 8 pairs showing extremely significant correlation (p<0.001). Most of the traits were positively correlated with each other, and only 23 pairs of traits were negatively correlated with each other (Fig. 2). Nested analysis of variance is the analysis of variance of multivariate hierarchical classification experiment design (also known as nested design), which is suitable for the comparative analysis of variables in a multivariate complex system (Yuan & Zhou, 2000). The results of the nested analysis of variance revealed that the variance in 30 phenotypic traits within populations were all significant, whereas the variation of partial phenotypic traits among populations was significant (P<0.5) (Table 5), which may mean that the morphological diversity of *S. hexandrum* is mainly caused by the different genetic bases of individuals within surveyed regions. Phenotypic differentiation analysis showed the value of V_{ST} ranged from 0.045% (L_{Se}/W_{Se}) to 61.305% (W_{Pe}), with an average of 17.776%, implying that the degree of phenotypic differentiation had high level. The average of the variance components of the 30 morphological characteristics among populations was 8.580%, and the average of the variance components within populations was 25.081%, indicating that the variance contribution within populations was greater than that among populations (Table 6). Moreover, repeatability can reflect the stability of species phenotypic characteristics, the value of population repeatability was much greater than that of individual repeatability (Table 7). Phenotypic traits among the population were more stable than that within the population, which also clarified the results of phenotypic differentiation analysis. In terms of organ types, the degree of phenotypic differentiation of floral organs (V_{ST} =19.376%) > the degree of phenotypic differentiation of fruiting organs (V_{ST} =16.248%) > the degree of phenotypic differentiation of leaf organs (V_{ST} =14.914%); $R_{I\ leaf}$ (0.140) >$R_{I\ fruit}$ (0.114) >$R_{I\ flower}$ (0.104). Among the three major organs of leaf, flower and fruit, the phenotypic differentiation coefficient of floral organs was the largest, indicating that its traits were the most unstable, followed by the fruiting organs. According to the actual field investigation and the related literatures, it is found that the size, color and number of seeds of the berries of *S. hexandrum* are obviously different. In the process of growth and development, the pollination and fertilization process of *S. hexandrum* is influenced by a variety of complex factors, such as pollination medium, temperature, precipitation, illumination and plant nutrients. These factors lead to differences in the degree of pollination and fertilization, resulting in large differences in the setting of fruit. This may explain the reason why the degree of phenotypic differentiation of the reproductive organs of *S. hexandrum* was higher than that of the vegetative organs, and the stability was worse than that of the vegetative organs.

The evolution of the various organ traits of *S. hexandrum* has a certain degree of asynchronism, and there are abundant phenotypic differences (diversity) among populations. These differences are associated with the ecological environment, and also relate to genetic factors. The existence of the differences is beneficial for the selection of excellent germplasm resources. Large-scale phenotypic variations in plants can usually produce new genotypes in populations. Simultaneously, the results of existing studies have shown that there is also

significant genetic differentiation both within and among the populations, enabling the *S. hexandrum* populations to adapt to various complex environmental conditions (Liu *et al.*, 2014). *S. hexandrum* species has wide geographical distributions with alpine mountains and frigid habitats in China and other countries at 1500～4500m elevations (Anon., 2011). *S. hexandrum* is mainly distributed in the forest stand transition zone, in which the terrain factors, climate factors, and other ecological factors are present. Through long-term geographical isolation and natural selection, abundant intraspecific variation and subpopulation variation has developed. Therefore, phenotypic variation results from the interaction between genetic and environmental factors is an important clue to genetic variation.

Genetic diversity is affected by multiple factors, such as geographical distribution, mating system, life form, pollen and seed dispersal (Hamrick *et al.*, 1992; Ohsawa *et al.*, 2008). Phenotypic variation is an important aspect of genetic diversity. *S. hexandrum* is a perennial herb whose rhizomes easily reproduce, which can slow down the genetic diversity. Pollen dispersal is generally restricted to a small region due to the large pollen size, which limits gene flow to increase or maintain genetic diversity (Liu *et al.*, 2014). In this study, low phenotypic variation among populations could be attributed to the predominant clonal reproduction and short seed dispersal in *S. hexandrum* in the present study areas. The extremely low phenotypic diversity among populations can have resulted from the severe bottleneck effect during their evolutionary process (Tang *et al.*, 2014). The inbreeding may further decrease their phenotypic diversity in the shrinking populations. By contrary, the high phenotypic variation across individuals may be caused by genetic drift (Hamrick & Godt, 1989). Wright (1965) noted that genetic drift would lead a new small population to emerge with a distinct genetic differentiation when the Nm (gene flow) value is lower than 1.0. The Nm of *S. hexandrum* populations (0.3587) was lower than 1.0, which suggested that some genetic drift may have emerged among the populations of this species (Liu *et al.*, 2016). According to the field investigation, the distribution of *S. hexandrum* populations obviously tends to fragment, which is consistent with the possibility of genetic drift.

A number of factors such as fragmented geographical distribution, lack of pollinators or seed dispersers can be a barrier to gene flow among populations (Slatkin, 1985). The limited gene flow among populations of *S. hexandrum* may be related to inbreeding of the species and limited seed propagation distance. Some studies have found that seed dispersal is the primary factor influencing variation of gene flow (Kalisz *et al.*, 1999). Heavy mature berries of *S. hexandrum* usually drop to the ground because of rain or wind, settling some seeds in the soil, whereas others are spread by cattle, birds, or humans. Therefore, the short distance of seed dispersal of *S. hexandrum* may result in limited gene flow among populations. Mountain ranges and rivers are possible barriers to either dispersal of pollen or rhizomes of *S. hexandrum*, reproductively isolating the populations. *S. hexandrum* is an herbaceous plant, and weak competitiveness compared to broad-leaved trees may also accelerate individual phenotypic variation. Historical events are also responsible for the variation in phenotypic diversity (Karron, 1991). Therefore, much lower phenotypic variation among populations than that within populations was found in this study.

There were abundant variations among populations and within populations of *S. hexandrum*, and the phenotypic variation among populations was much lower than that within populations, which indicated that the phenotypic variation of *S. hexandrum* were greatly affected by environmental factors, and the resources of *S. hexandrum* have phenotypic diversity. When considering the specific variation of inbred populations, the parental plants must be carefully selected for the creation of high-quality germplasm resources and the directional selection of elite varieties. Therefore, the selection and collection of *S. hexandrum* germplasm resources and the protection of their habitats should be prioritized in strategies for development and utilization.

Conclusions

The phenotypic diversity of 30 characters of 160 *S. hexandrum* from eight different regions were studied, it was found that the phenotypic variation of *S. hexandrum* was extremely rich, and most traits had wide variation and significant differences among populations (among surveyed regions) and within populations (within surveyed regions). The coefficient of variation (*CV*) of 30 phenotypic traits in *S. hexandrum* ranged from 7.98% (L_{TW}) to 31.90% (L_{TW}/L_L), with an average of 12.22%. The phenotypic differentiation coefficient (V_{ST}) ranged from 0.045% (L_{Se}/W_{Se}) to 61.305% (W_{Pe}), with an average of 17.776%. The degree of phenotypic differentiation was in this sequence as plant height (25.827%)> floral organs (19.376%)> fruiting organs (16.248%)> leaf organs (14.914%), it indicated that the degree of differentiation of phenotypic traits in reproductive organs was higher than that in vegetative organs, and the phenotypic stability of reproductive organs was poor. The degree of variation among different morphological characteristics of *S. hexandrum* was quite different, but it showed a certain regularity, that is, the differentiation among individuals within surveyed regions was greater than that among surveyed regions, and the stability of the phenotypic traits of individuals within surveyed regions was worse than that among surveyed regions.

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