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Genetic diversity of *Rhododendron simsii* Planch. natural populations at different altitudes in Wujiashan Mountain (central China)

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Abstract. Altitude could greatly influence species distribution and even their genetic diversity. However, it is unclear how altitude has affected the genetic diversity and population structure of *Rhododendron simsii* Planch., an dominant forestry species in north temperate forest. In this research, 22 polymorphic EST-SSR markers were utilized to assess the genetic diversity of *R. simsii* population distributed at different altitudes of Wujiashan Mountain, a major peak of Dabie Mountains (central China). Totally, 203 alleles were obtained, and each locus gave out 5 to 19 alleles. High genetic diversity existed, as Nei's gene diversity (*h*) and Shannon's Information index (*I*) ranged from 0.728 to 0.920 and 1.430 to 2.690, with the mean value of 0.821 and 1.916, respectively. In particular, 11.1% of genetic differentiation was maintained between populations, while 88.9% occurred within populations. Moreover, moderate gene flow (2.001) among populations was observed, which could effectively resist genetic drift. The genetic diversity of all these five *R. simsii* populations varied significantly with elevation, basically showing high-low-high pattern with elevation increase. Without human intervention, genetic diversity of *R. simsii* populations might increase with the altitude. At the significance level ($p < 0.05$), negative correlation was found between genetic diversity and attenuation rate of light intensity (r=-0.873). Soil of Wujiashan Mountain was acid (the pH value ranged from 4.33 to 4.70), which was rich in organic matter, available phosphorus, available potassium, and alkali hydrolysable nitrogen, as these soil factors interacted with each other to affect the growth of *R. simsii* population. This research would contribute a lot to the knowledge of evolutionary history of *R. simsii* species and benefit subsequent management and conservation actions.

Key words. *Rhododendron simsii* Planch.; EST-SSR; genetic diversity; altitude; germplasm protection.

INTRODUCTION

Genetic studies are important for understanding the genetic structure of populations and their ability to respond to natural selection (Lee 2002; Allendorf and Lundquist 2003). Genetic diversity reflects the ability of plant species to adapt environment changes during evolution. Moreover, understanding of genetic diversity in plants, including origin, maintenance and distribution, could give great insight into the modes of speciation, adaptation, as well as population dynamics (Bussell 1999). Genetic composition of a certain species is often influenced by various factors, including the history of introduction, founder effects, life-history characteristics, reproductive method, and even the effect of gene drift (Liu et al. 1998; Ye et al. 2003; Dewalt and Hamrick 2004; Liang et al. 2008). In particular, life-history characteristics, including reproductive method, could affect genetic diversity within- and among- populations (Dewalt and Hamrick 2004). Founder effects and genetic drift could reduce the heterozygosity and increase inter-population differentiation (Liang et al. 2008). Moreover, spatial distribution of genetic structure, reflecting adaptation evolution, environmental changes and natural selection effect, is often closely related to breeding mechanisms of the species (Ishihama et al. 2005).

Genetic and geographical structure in natural populations along elevational gradients are often influenced by life history, ecological traits, and biogeographic history (Quiroga and Premoli 2007; Truong et al. 2007). Elevation, or altitudinal gradient, is an assemblage of environmental variables, which could markedly influence the distribution of population genetic variation (Hahn et al. 2012). Therefore, understanding of current distribution pattern of population genetic diversity and differentiation along altitudinal gradients is vital for conservation and reasonable utilization (Mcmahon et al. 2007).

The *Rhododendron* genus, belonging to Ericaceae family, is widely distributed around the northern hemisphere and presents as different ecological types (Popescu and Kopp 2013). Besides high horticultural and medicinal properties, *Rhododendron* plants play important roles in the stability of ecological system. In particular, *R. simsii* is the dominant species in the community of "Dabie Mountains woods" (central China), (Wang et al. 2017). However, *Rhododendron*-based tourism, habitat fragmentation caused by human activities, as well as changes in ecological environment, all have exerted great influence towards natural *Rhododendron* population (Wang et al. 2017). Therefore, research on genetic diversity and ecological conservation of wild *R. simsii* is essential. However, analysis of genetic diversity and population structure of wild *R. simsii* population is limited, especially the populations located on Dabie Mountains.

Microsatellite, or simple sequence repeats (SSR), is abundant, co-dominant, widely distributed in genomes, highly polymorphic, and easily detectable, which has been widely used in genotype mapping, population structure and genetic diversity analysis (Ambreen et al. 2018; Ukoskit et al. 2018). In particular, SSR marker developed from expressed sequence tags (EST), the EST-SSR, showed more convenience in genetic studies, which has a high transferability to related species (Xu et al. 2018; Zhang et al. 2018). In this research, EST-SSR markers were used to investigate the genetic diversity of *R. simsii* populations at different altitudes in Wujiashan Mountain.

MATERIALS AND METHODS

Description of Wujiashan Mountain and Materials

Wujiashan Mountain (115°46'31.37"-115°50'39.20"E, 31°04'43.20"- 31°07'31.60"N, 3.02×10^4 hm^2), is one of the beautiful spot in Dabie Mountains. According to our field investigation, the constructive species making up the brush and forest were mainly species belonging to the families of Lauraceae, Cornaceae, Leguminosae, Anacardiaceae, Fagaceae, and Caprifoliaceae. Fresh leaves of *R. simsii* were collected at different altitudes on Wujiashan Mountain in August 2017 (Table 1). Particularly, the minimum interval between individuals was set as 100m.

Development of EST-SSR markers

Transcriptome data (SRP099282) of *R. simsii* flower tissue was used for the development of EST-SSR markers

Table 1 The location of *R. simsii* populations studied sampled from Wujiashan Mountain.

Population code	Sampling altitudes	Number of individuals	Longitude (E)	Latitude (N)	Percentage of polymorphic loci
1	972m	15	115°47'18"	31°06'53"	100%
2	1,071m	15	115°47'06"	31°06'04"	100%
3	1,167m	15	115°47'01"	31°06'07"	100%
4	1,270m	15	115°46'49"	31°06'08"	100%
5	1,370m	15	115°46'40"	31°06'08"	100%

with MicroSAtellite (MISA, http://pgrc.ipk- gatersleben.de/misa). These SSR-containing unigenes (di-nucleotide units) with sufficient flanking regions (more than 100bp) were chosen for prime pair design with online software Primer 3 (Wang et al. 2010).

DNA Extraction and Genetic diversity analysis based on EST-SSR markers

The modified CTAB (cetyltrimethyl ammonium bromide) method was adopted to extract genomic DNA, which was further diluted to 50ng/μL (Wang et al. 2017). The 10μL PCR amplification system was set, including 5μL 2×*Taq* Plus PCR MasterMix (TianGen, Beijing, China), 0.2 μM for each primer, as well as 50 ng genomic DNA. The PCR amplification conditions included initial denaturation at 95°C for 10 min, followed by 35 amplification cycles (94°C for 30 s, annealing at optimal temperature for 40 s, and 72°C for 50 s), as well as a 7 min elongation step at 72°C. Then, the PCR amplification products were separated on 6% (w/v) denaturing polyacrylamide gels, which were further visualized by silver staining.

Analysis of soil nutrients and attenuation rate of light intensity in sample plots

Five randomized soil cores (3cm in diameter) were taken up from each sampling spot (0-15cm depth), which were dried off in air and sieved through the 1mm sieve according to WiśniowskaKielian and Klima (2010). Available phosphorus and potassium forms were extracted from the soil with lactate reagent according to the Egner-Riehm's method (Sienkiewicz et al. 2011). In particular, the content of available phosphorus (mg/kg of the soil dry matter) were determined through spectrophotometric method using Beckman DU 640 apparatus, while content of available potassium were obtained with atomic absorption spectrometry (AAS) using PU 9100X Philips. Furthermore, contents of alkali hydrolysable nitrogen were determined with alkaline persulfate digestion (Ding et al. 2013). Contents of the soil organic matter (SOM) were calculated with potassium dichromate oxidation method. Soil pH values were measured in 0.01mol/L $CaCl_2$ slurry (1:2.5 soil/solution) using a reference glass electrode (Ding et al. 2013).

The light intensity upon the upper leaf surface and below the bottom leaf of each *R. simsii* plant was measured with luminometer (AS 810), and 50 plant were randomly selected in each sampling pot. The attenuation rate of light intensity was equal to the upper light intensity divided by lower light intensity. STATISTICA (version 6.1, StatSoft) was employed to determine the statistical parameters and correlation coefficients.

Data Analysis

DNA bands were scored for each sample. Population genetic parameters were calculated with POPGENE version 1.31 software, including number of alleles (N_A) per locus, effective number of alleles (N_E) per locus, expected heterozygosity (H_E), observed heterozygosity (H_O), tests for linkage disequilibrium (LD), Nei's (1973) gene diversity (*h*), Shannon's information index (*I*), total-population inbreeding coefficient (F_{IT}), intra-population inbreeding coefficient (F_{IS}), inter-population genetic differentiation coefficient (F_{ST}), gene flow, genetic identify (*GI*), and genetic distance (*D*) between populations (Wu et al. 2011). Moreover, genetic distance matrix among pairs of populations resulting from POPGENE analysis was utilized to create a dendrogram by MEGA software version 4.0. In addition, the correlations between genetic diversity and altitudinal distances, as well as soil factors were tested using DMRT with the software SPSS 17.0. The statistical significance between populations was estimated by two-tailed Student's t test ($P < 0.05$).

RESULTS

Genetic diversity of Rhododendron populations

Among 57 EST-SSR markers, 22 were polymorphic (Table 2), which gave out 203 bands. *R. simsii* had high genetic diversity at species level, and the polymorphic percentage in five populations were all 100%. N_A and N_E ranged from 5 to 19 and 3.674 to 12.437, with the mean value of 9.227 and 6.083, respectively (Table 3). The length of amplified bands ranged from 161 to 268 bp. The average Shannon's information index (*I*) and Nei's gene diversity (*h*) were 1.916 and 0.821, respectively (Table 3). In particular, the highest *I* and *h* was observed at EST-SSR117 locus, while the lowest existed at SSR019 locus (Table 3). Moreover, H_O and H_E ranged from 0.208 to 1.000 and 0.744 to 0.926, with the mean value of 0.862 and 0.828, respectively (Table 3).

The genetic diversity of population was lower than that of the species. At population level, the average N_A and N_E were 5.56 and 4.23, and the mean *I* and *H* were 1.498 and 0.734, respectively (Table 4). The level of genetic variation of these five populations from the highest to lowest revealed by *I* was pop 5> pop 1> pop 3> pop 2> pop 4. In particular, pop 5 gave out

Table 2 Characteristics of SSR primers used in this research. Shown for each primer pair are forward and reverse primer sequences, repeat motif, annealing temperature (Ta), and the size range of alleles fragment (bp).

Locus	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Repeat motif	*Ta* (°C)	Size range (bp)
SSR019	ATCCCATCCCATCTCTCTC	CACAGATGAGAGAAGAGAGC	(CT)25	55	202-212
SSR025	TCGTGTTGGGTTTCTATTGT	TCCATCAAACTACCAACACC	(CT)25	55	236-256
SSR031	GCAATCTTTCCTCCCATCTT	CTTCTGAATGGGTGCTACTT	(AG)26	56	233-245
SSR032	GAAACGTGTCTGTTTTCTCC	CTACCCCAATTTCCACTACC	(CT)28	56	207-231
SSR070	TCTTCCGATTCCATCATTCC	TGGGCGTGATTTGGTTATAA	(CT)22	54	179-203
SSR078	TTCCAGTTCCAATTCATCGG	CCCAACAACAATTCCATCAC	(CT)22	56	161-179
SSR081	GCCCTATCCCTCAACTTTAC	GAGGAGCGTGGTTAGTAATT	(TC)21	55	230-252
SSR082	GTATGGGACCTGTGATTTCC	CTCCAACTAGCTACTCCAAC	(AG)24	57	229-243
SSR090	TTGAAGAACACTCAAGTTGC	ACGTAGAACATTGCTTTCCT	(GA)21	56	187-201
SSR093	GGTATCCGGTTTTCATCACT	ATACCCACTAGCAACAGAGA	(GA)23	55	234-248
SSR097	AGAAAACTGGGAGATGTGTC	AGGTGATCATCTTTGCATGT	(CT)21	55	247-267
SSR105	CCCCTCTTTCTCTCTAGGAT	GAGAGAGAAGCCGATTACAG	(TC)22	56	186-200
SSR110	TAACCTGCCAGTGGAATTAC	TCTACGTACGCCATTGAAAT	(CT)22	55	224-234
SSR111	CTGCAGACATGACATGAAAC	TTTGCTTACCACTCCCATTT	(AG)21	55	244-260
SSR113	TATTGTACAGCTCCCCTTTG	CCTCAATGTTCTATCGACGT	(CT)23	56	186-200
SSR114	TATTGTACAGCTCCCCTTTG	GAACATGTTAAAGCGCTTGA	(TC)21	54	171-183
SSR116	ATTGCTTCTGATACCATCCG	TATCAGCTTTCGAGTTGTCC	(TC)21	55	211-223
SSR117	GCTATTCACTCGTCAAATGC	ATTGTGGGAATGAAGGTCTC	(GA)22	55	229-268
SSR123	CCCTTCCTCTTCTCAAATCC	CGTCATTTTCACACACAGAG	(CT)23	54	174-189
SSR125	CTCTCCCAAAATTAGCCGAT	GAATTGGCTGTTGGATGATG	(CT)21	55	234-246
SSR129	TGAAGCTGTTTTAGACTCCC	CATGATGGGAAAGCAAAGTG	(TC)22	55	161-175
SSR130	CCATGACGAACCCTATTGAT	TCCTGATATTCCTTTGCACA	(AG)21	56	235-245

the most alleles (128), while pop 4 produced the least alleles (119), which were all polymorphic (Table 4). The *I* ranged from 1.423 (pop 4) to 1.565 (pop 5), while *h* ranged from 0.705 (pop 4) to 0.762 (pop 1). The mean H_O and H_E ranged from 0.836 (pop 2) to 0.885 (pop 5) and 0.730 (pop 4) to 0.807 (pop 1), respectively. Basically, the genetic diversity of five populations showed a high-low-high variation pattern, as genetic diversity of *R. simsii* populations sampled at high and low altitude was higher than populations collected at middle altitude. Using an unpaired two-tailed Student t-test, the difference between populations was not statistically significant ($p<0.05$)

Genetic differentiation among populations at different altitudes

Significant genetic differentiation presented among these five *R. simsii* populaitons ($P<0.001$). An AMOVA of the distance matrix for all individuals partitioned overall variation into two levels, including 'among species' and 'among populations'. The F_{IS} and F_{IT} values ranged from -0.508 to 0.447 and -0.215 to 0.715, with the mean value of -0.178 and -0.047, respectively (Table 3). The F_{IS} value was negative for all five populations, ranging from -0.223 (pop 4) to -0.136 (pop 3), inferring that relatively high level of outcross occurred within populations (Table 4). F_{ST} value was calculated to be 0.111, suggesting that only 11.1 percent of overall genetic variation occurred between populations, while 88.9 percent took place within populations (Table 3). Furthermore, genetic variation mainly occurred at the SSR019 locus, followed by SSR105, SSR117, and SSR123 loci. In particular, gene flow was 2.001, which occurred frequently at SSR114, SSR097, SSR129, SSR082, and SSR090 loci (Table 3). However, the gene flow was a low-frequency event at SSR019 locus with the *Nm* value of 0.265, inferring that this locus might undergo genetic drift during population evolution (Whitlock and McCauley 1999).

Cluster analysis of different R. simsii populations

Genetic distance between pop 1 and pop 3 was the biggest (D = 0.8169), while their genetic identify was the lowest (GI = 0.4418). However, genetic distance between pop 3 and pop 4 was the smallest (D = 0.3979), while their genetic identify was the highest (GI = 0.6717) (Table 5). Based on the matrix of genetic distance, UPG-

Table 3 Genetic diversity of *R. simsii* populations based on SSR markers, including Number of alleles (N_A), effective number of alleles (N_E), Shannon's information index (I), Nei's gene diversity (h), observed heterozygosity (H_O), expected heterozygosity (H_E), intra-population inbreeding coefficient (F_{IS}), total-population inbreeding coefficient (F_{IT}), inter-population genetic differentiation coefficient (F_{ST}), and gene flow (Nm).

Locus	N_A	N_E	I	h	H_O	H_E	F_{IS}	F_{IT}	Fst	Nm
SSR019	5	3.674	1.430	0.728	0.208	0.744	0.447	0.715	0.486	0.265
SSR025	11	6.751	2.090	0.852	0.964	0.860	-0.286	-0.154	0.103	2.184
SSR031	7	4.018	1.598	0.751	0.900	0.757	-0.278	-0.182	0.075	3.105
SSR032	13	7.904	2.245	0.874	1.000	0.880	-0.243	-0.146	0.078	2.937
SSR070	13	9.047	2.361	0.890	1.000	0.896	-0.225	-0.130	0.078	2.960
SSR078	10	6.468	2.036	0.845	1.000	0.852	-0.338	-0.169	0.126	1.738
SSR081	12	5.787	2.005	0.827	1.000	0.833	-0.356	-0.189	0.123	1.785
SSR082	9	5.842	1.923	0.829	1.000	0.835	-0.269	-0.204	0.052	4.606
SSR090	8	6.630	1.961	0.849	1.000	0.857	-0.247	-0.175	0.058	4.048
SSR093	8	6.512	1.944	0.846	1.000	0.853	-0.276	-0.172	0.082	2.817
SSR097	11	6.054	2.015	0.835	1.000	0.841	-0.254	-0.193	0.049	4.874
SSR105	8	5.678	1.870	0.824	1.000	0.831	-0.508	-0.215	0.195	1.036
SSR110	6	3.947	1.527	0.747	0.561	0.752	0.201	0.251	0.062	3.754
SSR111	11	5.161	1.915	0.806	1.000	0.813	-0.363	-0.205	0.116	1.904
SSR113	8	6.383	1.949	0.843	0.754	0.850	0.019	-0.078	0.060	3.889
SSR114	7	5.070	1.758	0.803	0.732	0.810	0.053	0.097	0.047	5.116
SSR116	7	4.398	1.660	0.773	0.797	0.778	-0.117	-0.045	0.063	3.716
SSR117	19	12.437	2.690	0.920	0.843	0.926	-0.114	0.097	0.189	1.071
SSR123	9	7.861	2.126	0.873	0.968	0.880	-0.332	-0.109	0.168	1.241
SSR125	7	4.350	1.624	0.770	0.712	0.776	-0.016	0.059	0.074	3.133
SSR129	8	5.630	1.886	0.822	0.908	0.829	-0.161	-0.102	0.05	4.708
SSR130	6	4.221	1.538	0.763	0.623	0.769	0.080	0.208	0.139	1.547
Mean	9.227	6.083	1.916	0.821	0.862	0.828	-0.178	-0.047	0.111	2.001
St. Dev	3.146	1.990	0.295	0.050	0.201	0.050				

Table 4 Genetic diversity of *R. simsii* populations at different altitudes.

Population codes	N_A	Mean N_A	*Mean* N_E	I	h	H_O	H_E	F_{IS}
Pop 1	120	5.46±1.57	4.52±1.30	1.551±0.271	0.762±0.065	0.884±0.222	0.807±0.069	-0.153±0.262
Pop 2	121	5.50±1.50	3.97±1.27	1.457±0.321	0.717±0.110	0.836±0.239	0.750±0.114	-0.151±0.293
Pop 3	124	5.64±1.68	4.27±1.35	1.495±0.396	0.727±0.148	0.849±0.249	0.757±0.154	-0.136±0.358
Pop 4	119	5.41±1.71	3.92±1.21	1.423±0.387	0.705±0.154	0.866±0.257	0.730±0.159	-0.223±0.294
Pop 5	128	5.82±1.62	4.50±1.30	1.565±0.291	0.757±0.077	0.885±0.200	0.784±0.079	-0.167±0.260
Mean	122.4	5.56±0.17	4.23±0.28	1.498±0.060	0.734±0.025	-	-	-

MA cluster analysis assigned these five populations into two groups (Figure 1). Group I possessed pop 3, pop 4, and pop 5, while pop 1 and pop 2 were clustered into group II. Group I could be further divided into two subgroups, Ia and Ib. Particularly, Ib consisted of pop 3 and pop 4. Population 3 appeared to be more closer with population 4 than other populations. The dendrogram indicated that *R. simsii* population clustering had obvious region specificity, as the first group included populations sampled at higher altitudes, while the second possessed populations collected at the lower altitudes of Wujiashan Mountain (Figure 1).

Soil nutrients and attenuation rate of light intensity in five sample plots

Contents of available phosphorus and available potassium ranged from 13.696 mg/kg (pop 2) to 20.850

Table 5 Nei's genetic identity (above diagonal) and genetic distance (below diagonal) between different populations.

Pop ID	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5
Pop 1	-	0.5720	0.4418	0.4718	0.4865
Pop 2	0.5587	-	0.6309	0.5660	0.5332
Pop 3	0.8169	0.4605	-	0.6717	0.6194
Pop 4	0.7512	0.5692	0.3979	-	0.6712
Pop 5	0.7206	0.6288	0.4790	0.3987	-

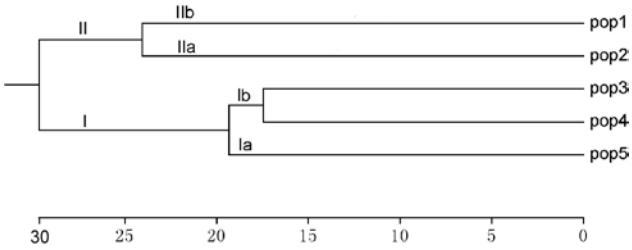

Fig. 1. Dendrogram of five *R. simsii* populations generated with MEAG4 cluster analysis.

mg/kg (pop 5) and 144.378 mg/kg (pop 1) to 306.197 mg/kg (pop 5), with the mean values of 17.329 mg/kg and 204.198 mg/kg, respectively (Figure 2A, 2B and Supplementary file 1). The content of available phosphorus differed slightly between various populations, with a decreasing order of pop 5, pop 3, pop 4, pop 1, and pop 2 (Figure 2A). Moreover, content of soil organic matter in Wujiashan Mountain was 720.953 mg/kg (Figure 2C and Supplementary file 1). Basically, the content of soil organic matter increased with altitude rising, which was highest in pop 5 (838.565mg/kg). Along the elevation, contents of alkali hydrolysable nitrogen were also increased: the lowest value existed in pop 1, while the highest value was observed in pop 5 (Figure 2D). Overall, soil of Wujiashan Mountain was estimated to be rich in nutrients necessary for the growth of *R. simsii*.

The pH value of soil ranged from 4.33 (pop 4) to 4.70 (pop 3), and the mean value was calculated to be 4.494 (Figure 2E). Moreover, the attenuation rate of light intensity in *R. simsii* populations differed significantly, varying from 0.438 to 0.594 (Figure 2F). In particular, pop 5 had the lowest attenuation rate of light intensity (0.438), followed by pop 1 (0.455). However, the highest attenuation rate of light intensity was observed in pop 4 (0.594), followed by pop 3 (0.529).

Correlation between population genetic differentiation and environmental factors

The correlation analysis showed that genetic diversity between populations was not significantly related to altitude ($r_{(I,\ altitude)}$=-0.014, p>0.05; $r_{(h,\ altitude)}$ = -0.136, p>0.05). Moreover, N_A, H_O, H_E, and F_{IS} also showed no relationship with altitude: $r_{(NA,\ altitude)}$=0.599, p>0.05; $r_{(HO,\ altitude)}$=0.599, p>0.05; $r_{(HE,\ altitude)}$=-0.343, p>0.05; $r_{(Fis,\ altitude)}$=-0.474, p>0.05. At the significance level ($p < 0.05$), negative correlation was observed between genetic diversity and attenuation rate of light intensity with r value of -0.873 (Figure 3A). However, no significant correlation were observed between genetic diversity and available phosphorus, available potassium, alkali hydrolysable nitrogen, soil organic matter, as well as pH value of soil at the significance level ($p < 0.05$) by Mantel's test, with the r value ranging from 0.236 to 0.526. In particular, contents of available phosphorus were positively correlated with content of alkali hydrolysable nitrogen (r=0.953, p=0.012) and the content of soil organic matter (r=0.879, p=0.05) (Figure 3B and C). Furthermore, similar correlation also existed between alkali hydrolysable nitrogen content and soil organic matter content (r = 0.935, p = 0.020) (Figure 3D).

Table 6 Contents of available phosphorus, available potassium, alkali hydrolysable nitrogen, soil organic matter, and the pH values of five sampling spots.

Populations	Available phosphorus (mg/kg)	Available potassium (mg/kg)	Soil organic matter (g/kg)	Alkali hydrolysable nitrogen (mg/kg)	pH value	Attenuation rate of light intensity (%)
pop 1	14.578±6.429	144.378±21.666	670.808±39.513	192.356±38.317	4.67	0.455±0.165
pop 2	13.696±3.975	186.313±43.959	658.812±31.972	221.104±43.587	4.43	0.489±0.246
pop 3	20.302±8.97	167.584±10.924	734.673±40.214	327.148±38.510	4.70	0.529±0.229
pop 4	17.220±5.475	216.518±62.862	701.905±37.975	258.014±37.964	4.33	0.594±0.242
pop 5	20.850±2.125	306.197±32.939	838.565±35.178	375.484±42.802	4.34	0.438±0.294
Mean	17.329	204.198	720.953	274.821	4.494	0.501
Standard deviation	3.241	62.838	72.041	75.564	0.179	0.063

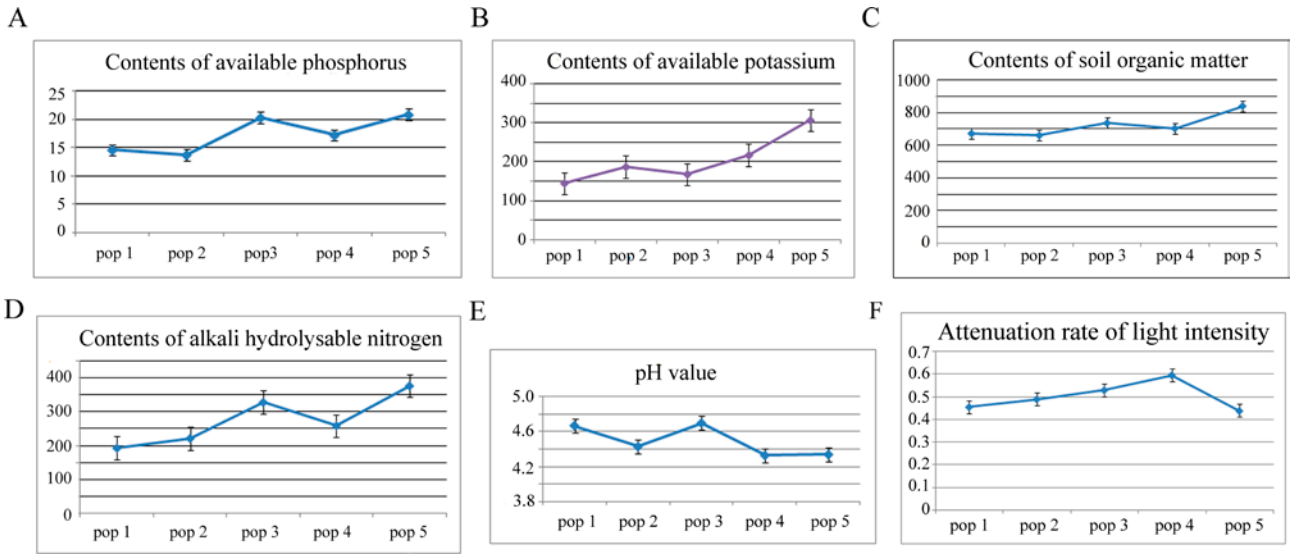

Fig. 2. Contents of aAvailable phosphorus content (A), available potassium content (B), soil organic matter content (C), alkali hydrolysable nitrogen content (D), soil acidity (E), and attenuation rate of light intensity (F) of five *R. simsii* populations. Values were represented as mean value ± standard deviation.

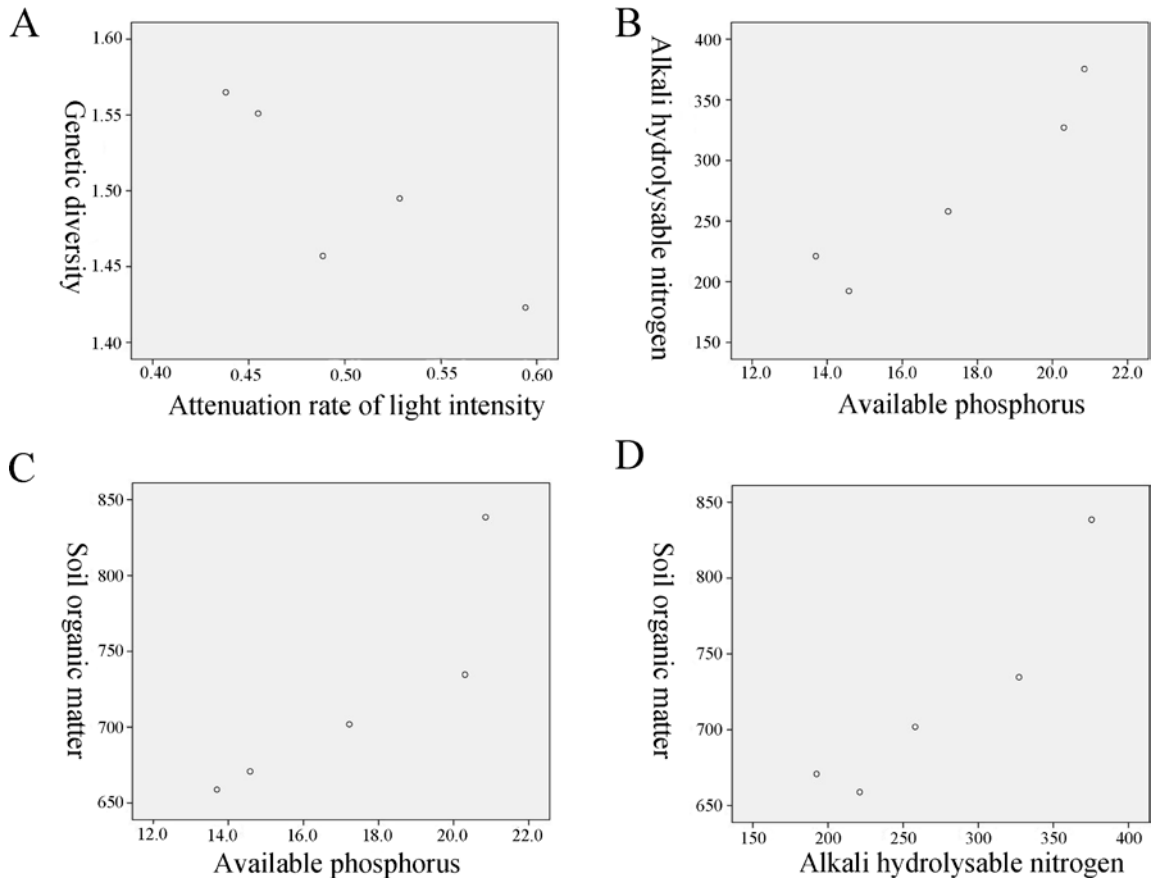

Fig. 3. Correlation between genetic diversity and attenuation rate of light intensity (A), content of alkali hydrolysable nitrogen and available phosphorus (B), soil organic matter and available phosphorus content (C), as well as soil organic matter and alkali hydrolysable nitrogen content (D).

DISCUSSION

Genetic diversity is the result of long-term evolution of a species, which represents the evolutionary potential (Cheng et al. 2017). Moreover, population evolution and the ability to adapt to environment may largely depend on genetic diversity. According to Bussell (1999), deep research on origin, maintenance, as well as distribution of genetic diversity in a species could enhance the understanding of modes of speciation, adaptation, and even population dynamics in the future. *R. simsii*, one of the most valuable woody plants, is dominant shrub with narrow distribution in Dabie Mountains (Li et al. 2015). Global environmental change and travel increase have threatened native biodiversity of wild *R. simsii*, especially the populations located on Dabie Mountain, whose current status need for significant attention.

In this study, high level of genetic diversity was observed in *R. simsii* populations, with *I* and H_E ranging from 1.423 to 1.565 and 0.730 to 0.807, respectively, as H_E ranging from 0.3 to 0.8 means that the tested population possessed high genetic diversity (Frankham et al. 2002; Edwards et al. 2014). The genetic diversity was significantly higher than *Corylus heterophylla* populations in Xingtangsi forest park (*I*=0.4790), *Acer ginnala* sampled at different altitudes in Qiliyu (*I*=0.5070), *Firmiana danxiaensis* located in Danxia landform of China (H_E: 0.364±0.019), *R. decorum* in southwest China (H_E: 0.758±0.048), *Erigeron arisolius* (H_E: 0.748±0.069*),* and *R. jinggangshanicum* population (H_E: 0.642±0.200) sampled from Mount Jinggangshan of China (Yan et al. 2010; Di et al. 2014; Chen et al. 2014; Wang et al. 2013a; Edwards et al. 2014; Li et al. 2015). In our opinion, the ancestor of *R. simsii* located on Wujiashan Mountaian might have a rich genetic basis, which is well preserved during evolution. *R. simsii*, as perennial shrub with overlapping generations, is both wind-pollinated and insect-pollinated plant. Sexual reproduction could increase genetic variation within population, which correspondingly allow natural selection to proceed effectively (Ayres and Ryan 1999; Burt 2000). Therefore, the high genetic diversity existed in *R. simsii* natural populations might be related to the biological characteristics and living conditions. Furthermore, sexual reproduction might be another critical reason for high genetic diversity.

Heterozygote excess was found in this wide *R. simsii* populations (F_{IS} = -0.178), inferring that outcross might occurred, especially in pop 4 (F_{IS} = -0.223) (Nagylaki 1998). Relatively low levels of inbreeding coefficient and outcross also existed in *R. jinggangshanicum* (F_{IS} = 0.023), *R. championiae* (F_{IS} = 0.012), and *R. moulmainense* populations (F_{IS} = 0.045) (Ng and Corlett 2000; Li et al. 2015).Furthermore, 88.9 percent of genetic variation occurred within populations, while only 11.1 percent was maintained between populations (F_{ST} = 0.111, P < 0.001). In particular, genetic variation of *R. simsii* populationswas slightly lower than *R. jinggangshanicum* distributed on Jinggangshan Mountain (93.13%, P < 0.001), but higher than *R. decorum* sampled from Southwest China (85.11%, P < 0.001) and *R. concinnum* collected in Qinling Mountains (85.3%, P < 0.001) (Zhao et al. 2012; Wang et al. 2013b; Li et al. 2015). Gene flow was2.001, higher than *R. arboreum* population (*Nm* = 1.13). Therefore, these *R. simsii* populations might effectively counteract the effect of genetic drift and resist the population differentiation (Kuttapetty et al. 2014). Dendrogram showed typical region specificity, so gene flow might easily occur between neighboring populations.

Genetic diversity of these five *R. simsii* populations varied significantly with elevation (pop 5> pop1>pop 3>pop 2>pop 4), and basically showed high-low-high pattern. In relation to pop 5 with the highest genetic diversity, the contents of available phosphorus, potassium, soil organic matter, and the alkali hydrolysable nitrogen were all the most, while the attenuation rate of light intensity was lowest. During field observation, we found that *R. simsii* population increased basically with altitudes, which reached the maximum at 1,280 meters. According to Leimu et al. (2006), genetic diversity and population size was positively correlated, as well as fitness and population size. Therefore, high genetic diversity at high altitude might be due to the large population size, as effective population size is sufficient to prevent the genetic drift caused by loss of genetic diversity during long-term evolution. Moreover, populations located at 1,280 meters might also possess high level of ecological adapt-ability. Furthermore, the community structure in Wujiashan Mountain had almost no artificial destruction, especially at the high altitude. Local famers plant *R. simsii* as ornamental plant, therefore different genotypes might have been brought to the population at low altitudes. Gene mutation and recombination further enhance the genetic diversity of *R. simsii* populations at low altitudes (Liang et al. 2008).

Soil of Wujiashan Mountain was acid with the pH value ranging from 4.33 to 4.70, and was rich in organic matter, available phosphorus, available potassium, and alkali hydrolysable nitrogen. The typical acid soil is very suitable for the growth of *R. simsii*. Substrate availability could influence microbial metabolic pathways to regulate carbon and even nutrient demand (Mondini et al. 2006). The soil conditions might also exert influence towards the metabolic pathways of microbes associated with *R. simsii*, which further affect the growth of *R.*

simsii population. However, no obvious correlation was observed between these soil factors with genetic diversity of *R. simsii populations*, except the attenuation rate of light intensity.

Relatively high genetic diversity maintained within *R. simsii* populations located on Wujiashan Mountain was observed. No obvious correlation was observed between genetic diversity and altitude. However, genetic diversity was in negative correlation with attenuation rate of light intensity. In particular, the available phosphorus, potassium, soil organic matter, and the alkali hydrolysable nitrogen in soil might interact with each other to affect the growth of *R. simsii* population. This research will be beneficial for the understanding of evolutionary history and population dynamics of *R. simsii* population located on Wujiashan Mountain. In addition, the study is also important for preserving *R. simsii* genetic resources, as well as broadening genetic basis of *Rhododendron* cultivars.

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Supplementary file 1 The correlation coefficient (below) and significant level (above) among genetic diversity, contents of available phosphorus, available potassium, alkali hydrolysable nitrogen, soil organic matter, soil pH value, and the attenuation rate of light intensity.

Item	Genetic diversity	Available phosphorus	Available potassium	Alkali hydrolysable nitrogen	Soil organic matter	pH value	Attenuation rate of light intensity
Genetic diversity	——	0.619	0.703	0.611	0.363	0.614	0.050
Available phosphorus	0.304	——	0.298	0.012	0.05	0.91	0.965
Available potassium	0.236	0.587	——	0.154	0.069	0.127	0.735
Alkali hydrolysable nitrogen	0.311	0.953	0.739	——	0.02	0.696	0.867
Soil organic matter	0.526	0.879	0.849	0.935	——	0.588	0.604
pH value	0.308	-0.071	-0.77	-0.0241	-0.33	——	0.796
Attenuation rate of light intensity	-0.873	0.027	-0.021	-0.105	-0.317	-0.161	——