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Research Article

Genetic diversity and population structure of Chinese ginseng accessions using SSR markers

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Abstract The need to preserve and use plant genetic resources is widely recognized, and the prospect of dwindling plant genetic diversity, coupled with increased demands on these resources, has made them a topic of global discussion. In the present study, the genetic diversity and population structure of 73 ginseng accessions collected from six regions in China were analyzed using eight simple sequence repeat (SSR) markers. Major allele frequencies ranged between 0.38 ~ 0.78, with a mean allele frequency value of 0.571. The number of alleles discovered ranged from 3 to 10 per accession, with a mean number of 7; 56 alleles were discovered in total. Gene diversity (GD) and polymorphic information content (PIC) values were similar to each other, and they ranged from 0.36 ~ 0.77 (mean 0.588) and 0.33 ~ 0.74 (mean 0.548), respectively. Accessions were divided into three clusters based on their phylogenetic relationships and genetic similarities, and although the populations were similar, they were not classified according to the region. Regional genetic diversity was also similar, with slight differences observed based on the number of accessions per region. It is expected that the findings of the present study can provide basic data for future studies on ginseng genetic diversity and for breeding ginseng cultivars.

Keywords *Panax ginseng*, genetic diversity, simple sequence repeats

Introduction

Ginseng (*Panax ginseng* Meyer) is a rare perennial plant belonging to the Araliaceae family, and it has been used in Korea and China for a long time as an herbal medicine. Approximately 12 species have been described in the *Panax* genus, but there are 3 species (*Panax ginseng*, *P. quinquefolius*, *P. notoginseng*) cultivated because of their economic value and used mostly as ingredients for herbal medicine and processed ginseng products (Wen and Zimmer, 1996). Among them, *P. ginseng* differs from the other 2 species in various proven efficacies, including activation of the immune system (Liu et al. 1995), anticancer effects (Shin et al. 2000; Yun et al. 2001), blood glucose regulation (Dey et al. 2003), anti-hyperlipidemic activity (Kim and Park, 2003), increased stamina (De Andrade et al. 2007), stress relief (Wang and Lee, 2000), and liver and kidney protection (Kang et al. 2007). Ginseng is a perennial crop with a simple morphology that makes it difficult to create or select cultivars, and thus about 20 cultivars have been developed in Korea (15 cultivars) and China (5 cultivars), relying on pure line isolation (Jo et al. 2016). However, because these cultivars were cultivated using this method and breeding with limited genetic resources, they are have low intervarietal genetic diversity (Lee et al. 2015; Kim et al. 2010; Kwon et al. 2003). Therefore, developing a system that would allow the acquisition of genetic resources with diverse traits from Korea and abroad and assessment of their diversity is important for increasing ginseng breeding efficiency (Jo et al. 2015).

Recent advances in molecular biology have led to the

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development of DNA fingerprinting and various DNA markers that allow the study of biodiversity at the DNA level. However, genetic analyses using DNA markers in ginseng were conducted later than those for other major crops such as rice. Wen and Zimmer (1996) and Ngan et al. (1999) analyzed differences in the 5.8S rDNA and internal transcribed spacer (ITS) region sequences in ginseng, while Fushimi et al. (1997) and Komatsu et al. (2001) used the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method to analyze differences in 18S rRNA gene sequences. The RAPD (random amplification of polymorphic DNA) method has been used to analyze gene diversity among *P. quinquefolius* (Bai et al. 1997; Lim et al. 2007), identify *P. quinquefolius* and *P. ginseng* (Boehm et al. 1999; Shim et al. 2003), and analyze the genetic relationships of native *P. ginseng* (Seo et al. 2003). Analyses using SSR markers, which offer the advantage of being codominant markers with excellent reproducibility, have been used in the identification of major *P. ginseng* cultivars and analysis of genetic diversity among various domestic and foreign ginseng cultivars (Bang et al. 2011a; Bang et al. 2011b; Bang et al. 2013; Hamada et al. 1982; Tautz and Renz, 1984) while sequence-related amplified polymorphism (SRAP) analysis has been used to study genetic diversity in Chinese ginseng cultivated in China (Xu et al. 2010). Analyses of genetic diversity and the relationships among crops increase the efficiency of breeding cultivars by expanding genetic mutations based on accurate genetic information and improvements in breeding (Tatineni et al. 1996).

The objective of the present study was to use SSR markers to analyze the diversity and population structure of Chinese ginseng accessions to provide basic data for future preservation and breeding of ginseng genetic resources. If the genetic relationships of accessions are identified and a classification system is established, then utilization of ginseng breeding materials can be maximized.

Materials and Methods

Plant material and DNA extraction

Seventy-three ginseng (*P. ginseng*) accessions were collected from 6 regions (Fusong, Jian, Helong, Yanji, Antu, and Wangqing) in China and were (and still currently are) stored and grown in the Industrial Plant Science & Technology greenhouse at the Chungbuk National University, Cheongju, South Korea (Table 1). DNA was extracted from the freeze-dried leaves of a single 3-year-old plant of each accession using a Genomic DNA prep kit (Nanohelix, Korea) following the

Table 1 Chinese ginseng accessions and the region where they were collected

No	Region	No	Region	No	Region
CBG0061	Fusong	CBG0243	Jian	CBG0311	Yanji
CBG0062	Fusong	CBG0244	Jian	CBG0314	Yanji
CBG0063	Fusong	CBG0245	Jian	CBG0356	Wangqing
CBG0065	Jian	CBG0246	Jian	CBG0357	Wangqing
CBG0068	Helong	CBG0247	Jian	CBG0358	Wangqing
CBG0069	Helong	CBG0248	Jian	CBG0359	Wangqing
CBG0076	Fusong	CBG0252	Jian	CBG0360	Wangqing
CBG0078	Antu	CBG0259	Jian	CBG0361	Wangqing
CBG0079	Antu	CBG0262	Fusong	CBG0363	Wangqing
CBG0219	Yanji	CBG0269	Fusong	CBG0369	Wangqing
CBG0220	Yanji	CBG0272	Fusong	CBG0379	Antu
CBG0221	Yanji	CBG0274	Fusong	CBG0382	Wangqing
CBG0222	Yanji	CBG0285	Fusong	CBG0385	Helong
CBG0223	Yanji	CBG0290	Fusong	CBG0386	Helong
CBG0226	Yanji	CBG0294	Yanji	CBG0388	Antu
CBG0228	Yanji	CBG0295	Yanji	CBG0390	Antu
CBG0230	Yanji	CBG0297	Jian	CBG0391	Helong
CBG0232	Yanji	CBG0299	Jian	CBG0394	Antu
CBG0233	Yanji	CBG0300	Jian	CBG0398	Antu
CBG0234	Yanji	CBG0302	Fusong	CBG0401	Antu
CBG0235	Yanji	CBG0303	Fusong	CBG0403	Antu
CBG0238	Yanji	CBG0304	Fusong	CBG0436	Fusong
CBG0239	Yanji	CBG0308	Fusong	CBG0437	Fusong
CBG0241	Yanji	CBG0309	Yanji		
CBG0242	Yanji	CBG0310	Yanji		

Table 2 Summary of the eight polymorphic SSR markers

SSR Marker ID	Primer sequence (5'→ 3')	SSR motif	Fluorescence label	Allele Size (bp)	Reference and GenBank No.
WCGSSR1	F: GGAGGTGATTGATGTAGTGGAATCC R: GGCTCTCCTATACTCACTATTTCCC	$(AGA)^{13}$	- FAM	124	Um et al. 2016 EF140899
WCGSSR2	F: AATCAGAAACAAAGAAAGCTAAAAC R: CTCTCTCATCTCTCTCTCTTCC	$(ATG)^{7}$ $(CTGATG)^{2}$	- VIC	113	Um et al. 2016 EF140892
WCGSSR3	F: CTACACGCTTTTTCATAGCTTACA R: TGTCTGCATAAAAGAGTTCGAGGC	$(CTCCTTT)^{4}$	NED -	171	Um et al. 2016 EF140900
WCGSSR4	F: CCTGCTGGAGATTGAAGTCAT R: GTTGGAATGCTTCAGCAGAT	$(GAAA)^{6}$	PET -	554	Um et al. 2016 EF531909.1
WCGSSR5	F: TCTCTTCTCAAGTTAATTTTTCCAA R: ATTTACAACTCTCTTCTTCCTCTAC	$(ATAG)^{11}$	- FAM	158	- EF140893.1
WCGSSR6	F: TGGATGATTTCGACATTTCTG R: TCAAATCCCCTAACCCTAACC	$(AG)^{13}$	VIC -	295	- GU565702.1
WCGSSR7	F: GGGCTAAGGGCAAAATTAGA R: CTGAATTACCGAACCGAACC	$(GGAACC)^{3}$	NED -	488	- BZ957342.1
WCGSSR8	F: TGGAAAATTGTTTGAGAGATCA R: AGGAGACCATGAAGGATTCG	$(GAA)^{18}$	PET -	590	- GU565701.1

manufacturer's protocol. The final DNA concentration was adjusted to 20 ng/μL using an Epoch Microplate Spectrophotometer (BioTek, Winooski, VT, USA).

PCR conditions

To assess diversity and analyze the population clusters and population structure of the collected ginseng accessions, 8 markers was used for the present study (Table 2). PCR reaction solutions had a total volume of 20 μL consisting of 0.1 μL of dNTPs (10 μM), 0.5 μL each of the forward and reverse primers (10 pmol/μL), and 0.2 μL of Taq (5 units). Solutions were subjected to 25 cycles of pre-denaturation at 94°C for 3 min followed by denaturation (94°C, 30 sec), annealing (55 ~ 64°C, 45 sec), and extension (72°C, 1 min), which were followed by a final extension for 15 min at 72°C. The size of the amplified fragments was measured using a Fragment Analyzer (Advanced Analytical Technologies Inc, Arkeny, IA, USA).

Genetic diversity

To analyze major allele frequencies (M_{AF}), number of alleles (N_A), observed heterozygosity (H_O), gene diversity (GD), and polymorphic information content (PIC) for each marker, PowerMarker V3.25 (Liu and Muse, 2005) was used.

Genetic relationships and population structure

For cluster and population structure analysis, distance-based clustering and a model-based clustering algorithm (STRUCTURE ver. 2.22) were used. For the cluster analysis, two main steps were undertaken. 1) The CS Chord 1967 method included in PowerMarker was used to calculate the genetic distance between accessions, while the UPGMA (unweighted pair group method with arithmetic mean) and neighbor-joining clustering methods were used to construct an unrooted phylogenetic tree of the Chinese ginseng accessions (Tamura et al. 2007), and the MEGA4 software package was used to draw the tree graphs. 2) GenAlex 6.5 (Peakall et al. 2012) was used to analyze principal component coordination. For the population structure analysis, ten runs were performed for each value (1 ~ 10) of the possible number of population clusters (K) in a sample of individuals. After applying 100,000 interactions and 100,000 burn-ins to each K, the log-likelihood value for each K was analyzed to determine the population with the highest log-likelihood value. Mean log-likelihood value of the data [LnP(D)] (± standard error, and ± 0.95 confidence interval) as a function of the value of K (K = 1-10) over 10 runs was obtained with STRUCTURE ver. 2.22 to detect the most accurate value of K. Then, the △K analysis proposed by Evanno and Goudet (2005) was used to choose the best K: $\triangle K = M(|L(K+1) - 2L(K) + L(K-1)|)/s[L(K)]$, where $2L(K)$ represents the k^{th} LnP(D), M is the mean of 10 runs, and S their standard deviation. STRUCTURE ver. 2.22 assumes a model with K populations, each population characterized by a set of allele frequencies at each locus. The likelihoods of a series of K values are calculated, and a best K value, based on likelihood tests, is selected after a series of runs with different K values.

Results and Discussion

SSR polymorphism

Diversity analyses using 8 markers for the 73 Chinese ginseng accessions collected contained a total of 56 alleles. The N_A discovered ranged from 3 (WCGSSR2) to 10 (WCGSSR5) with a mean of 7. M_{AF}, which indicates the distribution of major alleles, ranged from 0.38 (WCGSSR8) to 0.78 (WCGSSR7) with a mean of 0.571. With respect to GD and PIC, which represent genetic diversity, both were lowest in WCGSSR7 (0.36 and 0.33, respectively) and highest in WCGSSR8 (0.77 and 0.74, respectively) while their means were 0.588 and 0.548, respectively (Table 3). Bang et al. (2011a), found that the mean N_A found in *P. ginseng* cultivars using SSR markers is 2.6 and the mean PIC value is 0.480. Moreover, it has been reported that domestic and foreign ginseng accessions and collected species have a mean N_A of 4.3 and mean genetic diversity of 0.553 (Bang et al. 2011b). Thus, the SSR markers used in the present study found in higher genetic diversity than in previous studies. Whether this difference was due to the number of tested samples or the actual genetic diversity of the cultivars needs to be examined with a larger number of cultivars. However, Maluf et al. (2005) reported that SSR markers are more effective in detecting genetic diversity than other markers, indicating that the findings in the present study may be used effectively in the future for analyzing the diversity of ginseng genetic resources.

Distance-based phylogeny and population genetic structure

The present study created a phylogenetic tree based on genetic distances using the CS Chord 1967 method, and, as a result, the ginseng accessions were divided into three clusters. Four accessions belonged to cluster I: 2 from the Fusong region, 1 from Yanji, and 1 from Wangqing. Eleven accessions belonged to cluster II: 5 from the Antu region, 3 from Helong, 2 from Fusong, and 1 from Wangqing. Lastly, 58 accessions belonged to cluster III: 21 from the Yanji region, 12 from Fusong, 12 from Jian, 7 from Wangqing, 4 from Antu, and 2 from Helong. The principal component coordination analysis results were consistent with the results from the cluster analysis based on the genetic relationships calculated by CS Chord 1967 (Figs. 1A and B).

The population structure analysis showed that as the potential number of populations (K) increased, the log-likelihood value also increased (Fig. 2A). The △K analysis determined that the highest K-value was 3 (Fig. 2B). Among the 73 accessions, 69 had a 70% chance of belonging to 1 of the 3 subpopulations, while 4 were classified as admixture types (Fig. 2C). Four accessions belonged to population I (POPI): 2 from the Fusong region, 1 from Yanji, and 1 from Wangqing. Thirteen accessions belonged to population II (POPII): 7 from the Antu region, 3 from Helong, 2 from Fusong, and 1 from Wangqing. Lastly, 52 accessions belonged to population III (POPIII): 21 from the Yanji region, 11 from Fusong, 10 from Jian, 6 from Wangqing, 2 from Helong, and 2 from Antu.

The cluster and population structure analyses results exhibited the same trend as those from previous studies,

Table 3 Summary of allelic and genetic diversity in 73 Chinese ginseng accessions based on analysis with eight simple sequence repeat (SSR) markers

Marker	M_{AF}[a]	N_A[b]	H_O[c]	GD[d]	PIC[e]
WCGSSR1	0.48	7	0.25	0.66	0.61
WCGSSR2	0.64	3	0.26	0.51	0.45
WCGSSR3	0.47	8	0.47	0.71	0.68
WCGSSR4	0.76	8	0.22	0.41	0.39
WCGSSR5	0.57	10	0.23	0.64	0.61
WCGSSR6	0.50	7	0.89	0.63	0.56
WCGSSR7	0.78	4	0.00	0.36	0.33
WCGSSR8	0.38	9	0.27	0.77	0.74
Mean	0.571	7	0.324	0.588	0.548

[a]M_{AF}: major allele frequency
[b]N_A: number of alleles
[c]H_O: observed heterozygosity
[d]GD: genetic diversity
[e]PIC: polymorphic information content

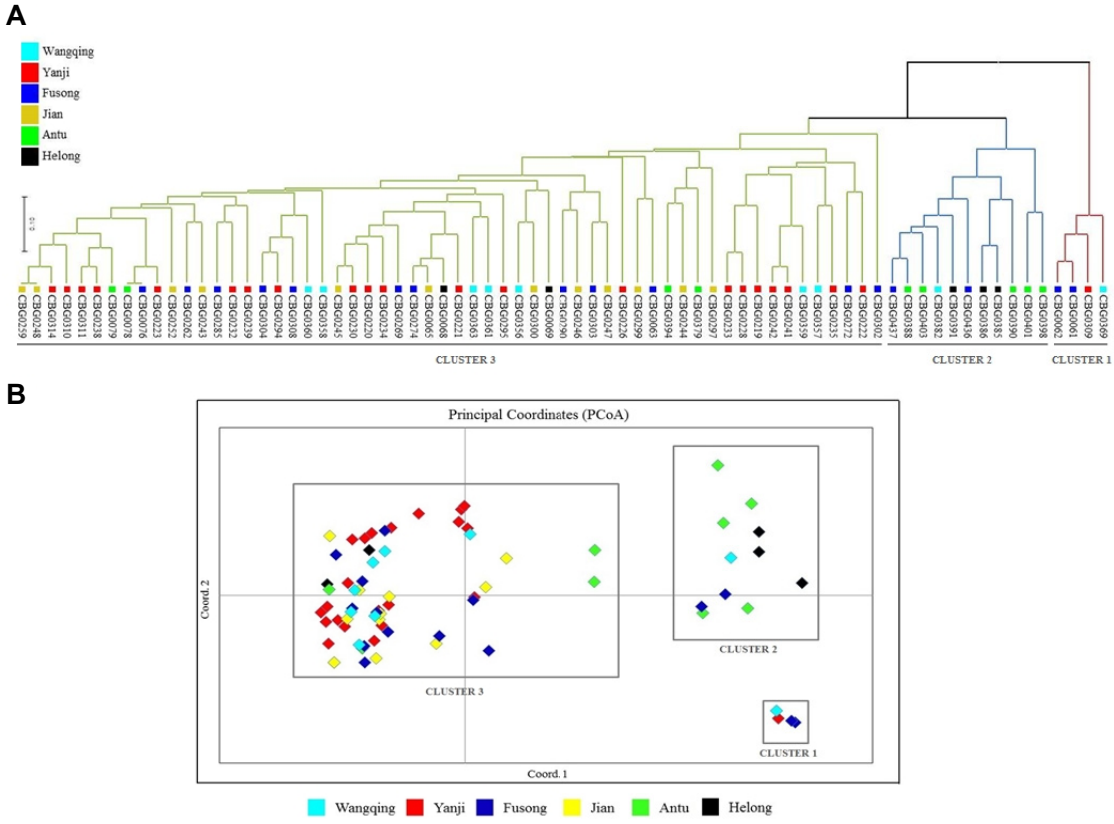

Fig. 1 (A) Model-based clustering tree (UPGMA) based on a CS Chord 1967 matrix and a (B) principal component coordinates analysis of 73 Chinese ginseng accessions collected from six regions in China

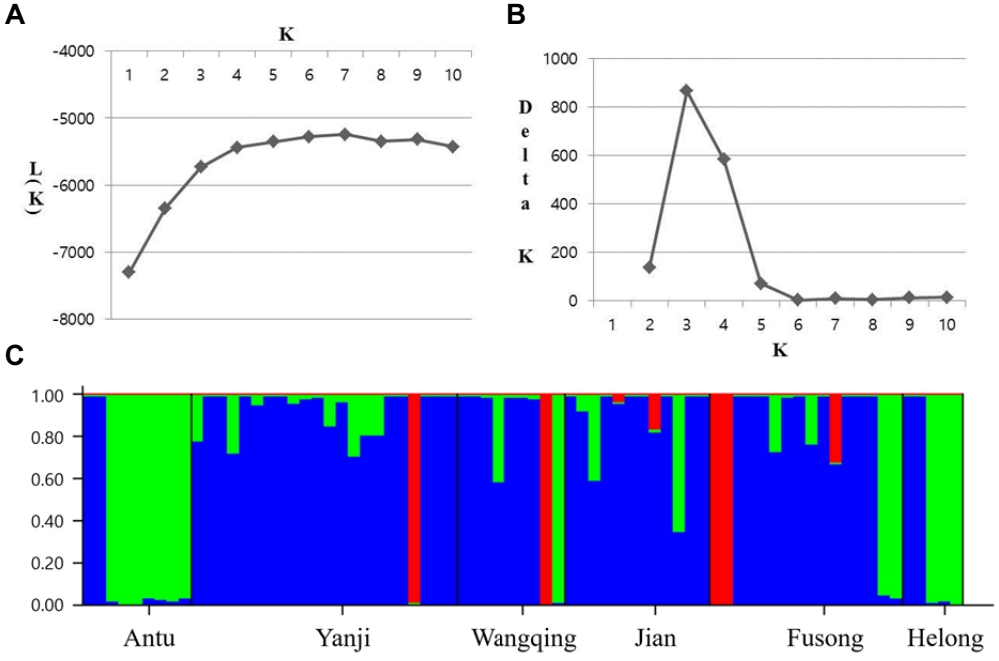

Fig. 2 Estimated (A) Mean log-likelihood value of the data LnP (D) and (B) ΔK based on the number of populations (K-value) and (C) the estimated population structure determined with use of the software program STRUCTURE for 73 Chinese ginseng accessions. Each accession is represented by three colors, designated by the K-value

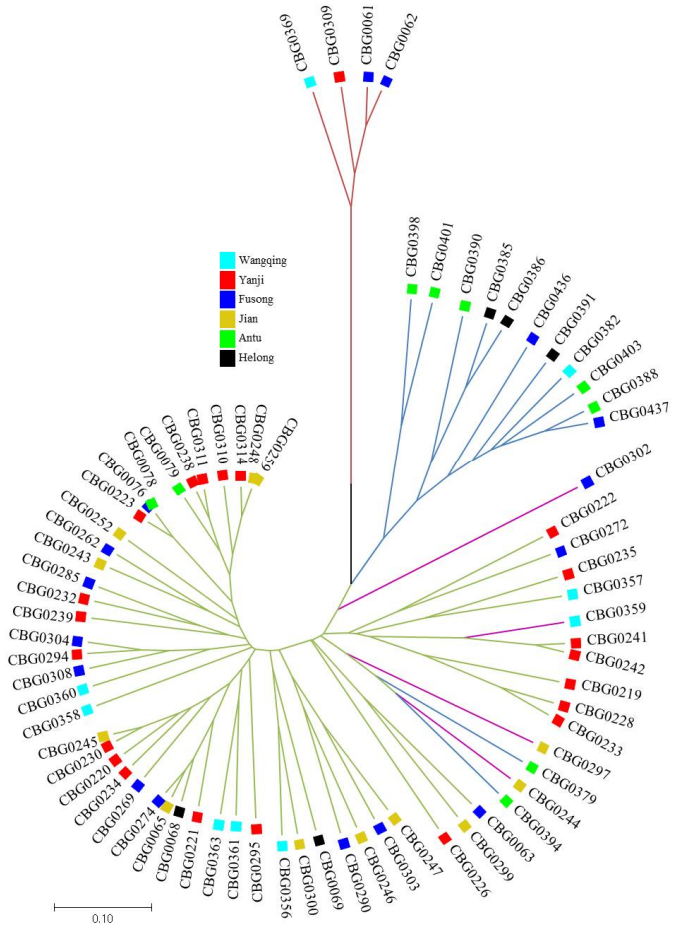

Fig. 3 Unrooted neighbor-joining tree based on CS Chord 1967 for 73 Chinese ginseng accessions. Each color represents a population identified by the software program STRUCTURE with over 70% similarity. Brown, cluster I; blue, cluster II; green, cluster III; violet, admixture

in that collection region did not affect cluster formation (Ibiza et al. 2012). To compare the results from the 2 analyses, excluding the 4 accessions that were of the admixture type, population structure analysis results were substituted into an unrooted tree; the resulting distribution patterns of all accessions, except CBG379 and CBG394, were the same (Fig. 3).

Genetic diversity of region and model-based populations

Table 4 shows a summary of diversity values based on collection region. The N_A was highest in Yanji at 5.5, and lowest in Helong at 2.88. Mean M_{AF} in the Jian and Yanji regions were 0.68 and 0.64, respectively, while it was similar among the other regions, ranging between 0.54 and 0.57. GD and PIC values were highest in the Fusong region at 0.6 and 0.57, respectively, and lowest in the Jian region at 0.45 and 0.41, respectively. There were almost no differences in genetic diversity between regions, and genetic diversity was lower when the number of accessions present was lower.

With respect to genetic diversity in the three populations determined by the population structure analysis, the N_A in POPs I, II, and III were 1.88, 3.88, and 5, respectively, while that of admixed Chinese ginseng accessions was 2.75. Mean M_{AF} was highest in POP I at 0.81 followed in decreasing order by those of POP III (0.64), POP II (0.63), and admixed accessions (0.61). In contrast, GD values were lowest in POP I at 0.23, followed in increasing order by POP III (0.47), POP II (0.5), and admixed accessions (0.51). The PIC value was lowest in POP I at 0.21, which was same as GD for this population, but the other PIC values were all 0.44, which differed from the GD values (Table 4).

The Chinese ginseng accessions used in the present study exhibited higher diversity than existing ginseng accessions in Korea. However, it is necessary to conduct analyses using a greater number of ginseng accessions for a more accurate comparison of diversity against ginseng genetic resources from China, Korea, and abroad. The findings

Table 4 Summary of allelic and genetic diversity as determined by the software STRUCTURE version 2.3.1 and by the region of collection of 73 Chinese ginseng accessions

Cluster		Diversity[a]				
		M_{AF}	N_A	H_O	GD	PIC
By Structure	POPI[b]	0.81	1.88	0.28	0.23	0.21
	POPII	0.63	3.88	0.47	0.5	0.44
	POPIII	0.64	5	0.29	0.47	0.44
	admix	0.61	2.75	0.31	0.51	0.44
	Mean	0.673	3.378	0.338	0.428	0.383
By Region	Antu	0.54	3.25	0.41	0.56	0.49
	Fusong	0.55	5	0.26	0.6	0.57
	Helong	0.55	2.88	0.4	0.55	0.47
	Jian	0.68	3.38	0.3	0.45	0.41
	Wangqing	0.57	4.63	0.36	0.58	0.54
	Yanji	0.64	5.5	0.32	0.51	0.47
	Mean	0.588	4.107	0.342	0.542	0.492

[a]M_{AF}: major allele frequency, N_A: number of alleles, H_O: observed heterozygosity, GD: genetic diversity, PIC: polymorphic information content
[b]POPI: population I, POPI: population II, POPIII, population III

in the present study are expected to serve as basic data for future diversity-based ginseng cultivar breeding.

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