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

Hyejin An · Jong-Hyun Park · Chi Eun Hong · Sebastin Raveendar · Yi Lee · Ick-Hyun Jo · Jong-Wook Chung

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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 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
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: GGAGGTGATTGATGTTGGAATCC | (AGA)³    | FAM                | 124             | Um et al. 2016 EF140899  |
|               | R: GCTCTCTCATATCCTATTTCCC |           |                    |                 |                          |
| WCGSSR2       | F: AATCAGAAACAAAGAAGCTAAAAC | (ATG)⁴   | VIC                | 113             | Um et al. 2016 EF140892  |
|               | R: CTCTCTCATATCCTATTTCCC | (CTGATG)⁵ |                    |                 |                          |
| WCGSSR3       | F: CTACACGCTTTCTCTCTCTTACA | (CTCCTTT)³| NED                | 171             | Um et al. 2016 EF140900  |
|               | R: TGCTGATCAAAGATTCGAGGC   |           |                    |                 |                          |
| WCGSSR4       | F: CTTGGAGTGGAGATTGAATCTAT| (GAAA)⁶  | PET                | 554             | Um et al. 2016 EF531990.1 |
|               | R: GTTGGATGCTTCAGCAT       |           |                    |                 |                          |
| WCGSSR5       | F: TCTCTCTCTAAGTTAAGTTTCAAA| (ATAG)¹¹ | FAM                | 158             | EF140893.1                |
|               | R: ATTATACACTCTCTCTCTCTCTAC| (AG)³    | VIC                | 295             | GU565702.1                |
| WCGSSR6       | F: TGGATGATTCAGATTTCTCTG   | (GGAACC)³| NED                | 488             | BZ957342.1                |
|               | R: TCAAATCCCCCTACCTAAAACC |           |                    |                 |                          |
| WCGSSR7       | F: GGCTTAAGGGCTAAGTTAAGTAAG| (GAA)¹⁸  | PET                | 590             | GU565701.1                |
|               | R: CGTAAATCCCGTTGGAGGC     |           |                    |                 |                          |

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 (MAF), number of alleles (Na), observed heterozygosity (Ho), 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: ∆K = M (|L(K + 1) − 2 L(K) + L(K − 1)|/s[L(K)], where 2[L(K)] represents the kth 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 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 $\Delta K$ 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      | $M_{AF}$ | $N_A$ | $H_O$ | GD | PIC |
|-------------|----------|-------|-------|----|-----|
| 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 |

$^aM_{AF}$: major allele frequency

$^bN_A$: number of alleles

$^cH_O$: observed heterozygosity

$^dGD$: genetic diversity

$^ePIC$: 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* | MAF | NA | HO | GD | PIC |
|---------|------------|-----|----|----|----|-----|
| POPI    |            | 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|
| 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|

*MAF: major allele frequency, NA: number of alleles, HO: observed heterozygosity, GD: genetic diversity, PIC: polymorphic information content

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

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References

Bai D, Brandle J, Reeleder R (1997) Genetic diversity in North American ginseng (Panax quinquefolius L.) grown in Ontario detected by RAPD analysis. Genome 40:111-5
Bang KH, Chung JW, Kim YC, Lee JW, Jo IH, Seo AY, Kim OT, Hyun DY, Kim DH, Cha SW (2011a) Development of SSR markers for identification of Korean ginseng (Panax ginseng C.A. Meyer) cultivars. Korean J. Medicinal Crop Sci 19: 185-90
Bang KH, Jo IH, Chung JW, Kim YC, Lee JW, Seo AY, Park JH, Kim OT, Hyun DY, Kim DH, Cha SW (2011b) Analysis of genetic polymorphism of Korean ginseng cultivars and foreign accession using SSR markers. Korean J. Medicinal Crop Sci 19:347-53
Bang KH, Chung JW, Kim YC, Jo IH, Kim JU, Shin MR, Hyun DY, Kim DH, Cha SW, Kim KH, Moon JY, Noh BS, Kim HS (2013) Analysis of genetic polymorphism of Korean ginseng cultivars and breeding lines using RAPD markers. Korean J Int Agric 25:184-93
Boehm CL, Harrison HC, Jung G, Nienhuis J (1999) Organization of American and Asian ginseng germplasm using randomly amplified polymorphic DNA (RAPD) markers. J Amer Soc Hort Sci 124:252-6
De Andrade E, de Mesquita AA, de Claro JA, de Andrade PM, Ortiz V, Paranhos M, Srougi M (2007) Study of the efficacy of Korean Red Ginseng in the treatment of erectile dysfunction. Asian Journal of Andrology 9:241-4
Dey L, Xie JT, Wang A, Wu J, Maleekar SA, Yuan CS (2003) Anti-hyperglycemic effects of ginseng: comparison between root and berry. Phytomedicine 10:600-5
Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611-20
Fushimi H, Komatsu K, Isobe M, Namba T (1997) Application of PCR-RFLP and MASA analyses on 18S ribosomal RNA gene sequence for the identification of three Ginseng drugs. Biol Pharm Bull 20:765-9
Hamada SR, Petrino MG, Kakunaga T (1982) A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. Proc Natl Acad Sci USA 79:6465-9
Ibiza VP, Blanca J, Cañizares J, Nuez F (2012) Taxonomy and genetic diversity of domesticated Capsicum species in the Andean region. Genet Resour Crop Evol 59:1077-88
Jo IH, Lee SH, Kim YC, Kim DH, Kim HS, Kim KH, Chung JW, Bang KH (2015) De novo transcriptome assembly and the identification of gene-associated single-nucleotide polymorphism markers in Asian and American ginseng roots. Mol Genet Genomics 290:1055-65
Jo IH, Bang KH, Hong CE, Kim JU, Lee JW, Kim DH, Hyun DY, Ryu H, Kim YC (2016) Analysis of the chloroplast genome and SNP detection in a salt tolerant breeding line in Korean ginseng. J Plant Biotechnol 43:417-21
Kang KS, Kim HY, Yamabe N, Park JH, Yokozawa T (2007) Preventive effect of 20(S)-ginsenoside Rg3 against lipopolysaccharide-induced hepatic and renal injury in rats. Free Radic Res 41:1181-8
Kim SH, Park KS (2003) Effects of Panax ginseng extract on lipid metabolism in humans. Pharmacological Research 48:511-3
Kim YJ, Kim MK, Shim JS, Pulla RK, Yang DC (2010) Somatic embryogenesis of two new Panax ginseng cultivars, Yunpoong and Chunpoong. Russ J Plant Physiol 57:283-9
Komatsu K, Zhu S, Fushimi H, Qui TK, Cai S, Kadota S (2001) Phylogenetic analysis based on 18S rRNA gene and matK gene sequence of Panax vietnamensis and five related species. Planta Med 67:461-5
Kwon WS, Lee JH, Park CS, Yang DC (2003) Breeding process and characteristics of Gopoong, a new variety of Panax ginseng C.A. Meyer. J Ginseng Res 27:86-91
Lee JH, Lee JS, Kwon WS, Kang JY, Lee DY, In JG, Kim YS, Seo JH, Baeg IH, Chang IM, Grainger K (2015) Characteristics of Korean ginseng varieties of Gumpoong, Sunun, Sunpoong, Sunone, Cheongsun, and Sunhyang. J Ginseng Res 39:94-104
Lim W, Mudge KW, Weston LA (2007) Utilization of RAPD markers to assess genetic diversity of wild populations of North American ginseng (Panax quinquefolius). Planta Med 73:71-6
Liu J, Wang S, Liu H, Yang L, Nan G (1995) Stimulatory effect of saponin from Panax ginseng on immune function of lymphocytes in the elderly. Mechanisms of Ageing and Development 83:43-53
Liu K, Muse SV (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinform 21:2128-9
Maluf MP, Silvestrini M, Ruggiero LM, Filho OG, Colombo CA (2005) Genetic diversity of cultivated Coffea arabica inbred lines assessed by RAPD, AFLP and SSR marker systems. Sci Agricola 62:366-73
Ngan F, Shaw P, But P, Wang J (1999) Molecular authentication of Panax species. Phytochemistry 50:787-91
Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in excel. Population genetic software for teaching and research-an update. Bioinform 28:2537-9
Seo SD, Yik JA, Cha SK, Kim HH, Seong BJ, Kim SI, Choi JE (2003) Analysis of diversity of Panax ginseng collected in Korea by RAPD technique. Korean J Med Crop Sci 11:377-84
Shin HR, Kim YJ, Yun TK, Morgan G, Vainio H (2001) The cancer-preventive potential of Panax ginseng: a review of human and experimental evidence. Cancer Causes Control 11:565-76
Shim YH, Choi JH, Park CD, Lim CJ, Cho JH, Kim HJ (2003) Molecular differentiation of Panax species by RAPD analysis. Arch Pharm Res 26:601-5
Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol 24:1596-9
Tatineni V, Cantrell RG, Davis DD (1996) Genetic diversity in elite cotton germplasm determined by morphological characteristics and RAPDs. Crop Sci 36:186-92
Tautz D, Renz M (1984) Simple sequence are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res 12:4127-38
Um YR, Jin ML, Kim OT, Kim YC, Kim SC, Cha SW, Chung KW, Kim SR, Chung CM, Lee Y (2016) Identification of Korean ginseng (Panax ginseng) cultivars using simple sequence repeat markers. Plant Breed Biotech 4:71-8
Wang LC, Lee TF (2000) Effect of ginseng saponins on cold tolerance in young and elderly rats. Planta Med 66:144-7
Wen J, Zimmer EA (1996) Phylogeny and biogeography of Panax L. (the ginseng genus, Araliaceae): inferences from ITS sequences of nuclear ribosomal DNA. Molecular Phylogenetics and Evolution 6:167-77
Xu YH, Jin H, Kim YC, Bang KH, Cha SW, Zhang LX (2010) Genetic diversity and genetic structures in ginseng landraces (cultivars) by SRAP analysis. Korean J Med Crop Sci 183:180-5
Yun TK, Lee YS, Lee YH, Kim SI, Yun HY (2001) Anticarcinogenic effect of Panax ginseng C. A. Meyer and identification of active compounds. Korean Medical Science 16:56-18