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SRAP 분석에 의한 중국 재배삼의 유전적 다양성

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Genetic Diversity and Genetic Structures in Ginseng Landraces (Cultivars) by SRAP Analysis

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ABSTRACT : We investigated genetic diversity among and within the populations of cultivated ginseng *(Panax ginseng* C. A. Meyer) using SRAP profiles. A total of 24 ginseng plants were sampled from the three populations (two from China, one from Korea). Since all these populations are previously shown closely related to each other assister groups, we used *Panax quinquefolium* L. and wild ginseng as a reference species, which is not "within the sister group". All individuals from the three populations were screened with a total of 36 primer pairs with 26 primers generated from 328 SRAP bands of DNA gels. The mean gene diversity (H_E) was estimated to be 0.057 within populations (range 0.032-0.067), and 0.086 at the species level. The genetic differentiation (Gst = 0.31) indicates that genetic variation apportioned 30% among populations and 70% within populations. Generally, the result of this study indicates that ginseng contains high molecular variation in its populations.

Key Words **:** Ginseng, SRAP, Genetic Variance

INTRODUCTION

Ginseng (*Panax ginseng* C. A. Meyer) has been grown as a medicinal herb in several countries including China and Korea. Dried root of ginseng has been applied as a tonic, prophylactic and anti-aging agent in many Asian countries including China, Japan and Korea (Carlson, 1986; Cheung *et al*., 1994). Ginseng is becoming one of the best plant for medicinal herbs and is widely cultured in the Northeast of China. With the development of market demand, quality of ginseng needs to be improved and elite cultivars are urgently required for ginseng production. Because of many biological complexities such as natural heterozygote and cross-incompatibility, the analysis of the genetic relationships among inbred lines is of interest not only for germplasm conservation, but also for breeding purposes.

Ginseng is one of the most economically important medicinal plant in the world (Carlson, 1986; Cheung *et al*., 1994; Ngan *et al*., 1999), but there are fewer reports on genetic variations and relationship in ginsengs (Zhuravlev *et al*., 1998; Ma *et al*., 1999, Seo *et al*., 2003, In *et al*., 2005). Genetic structure among and within a population depends on the life history of a species (Fisher and Matthies, 1998; Fisher *et al*., 2000). Thus, the knowledge of the genetic structure allows us to infer the past history of a species. At the same time, genetic variations represent a starting point of further evolution and an important prerequisite for the prediction of evolutionary responses.

Sequence-related amplified polymorphism (SRAP) (Li and Quiros, 2001), which preferentially amplifies open reading frames (ORFs), was employed and the observed polymor-

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Table 1. Plant material used in this study.

No.	Name	local	Accession code	population
1	Jimei (cultivar)	Ji'an	JM2	
2	Ermaya	Ji'an	EM	
3	Biantiao	Ji'an	BT17	Jian
4	Yuanbangyuanlu	Ji'an	YB3	
5	Shizhu	Kuadian	SZ5	
6	Changbo	Ji'an	CB7	
7	Fuxing (cultivar)	Fusong	FX1	
8	Baoquan (cultivar)	Changbai	BQ3	
9	Damaya	Fusong	DM12	
10	Qingjing	Fusong	QJ2	
11	Zijing	Fusong	ZJ1	Fusong
12	Lvjing	Fusong	LJ2	
13	Sansui ginseng	Fusong	SS22	
14	Jinsui ginseng	Fusong	JS21	
15	Korean ginseng1	Korea	KG1	
16	Korean ginseng2	Korea	KG2	
17	Korean ginseng3	Korea	KG3	Korea
18	Korean ginseng4	Korea	KG4	
19	Korean ginseng5	Korea	KG5	
20	Korean ginseng6	Korea	KG6	
21	Korean ginseng7	Korea	KG7	
22	North Korean ginseng	North Korea	NG4	
23	Wild ginseng	Fusong	WG11	
24	American ginseng	Jingyu	AG5	

phism fundamentally originated in the variation of the length of these introns, promoters and spacers both among individuals and among species. SRAP had been applied extensively in genetic linkage map construction (Sun *et al*., 2007), genetic diversity analysis, and comparison. The aim of the present study is to analyze the variability of ginseng inbred lines to provide precision genetic information for the future breeding program.

MATERIALS AND METHODS

1. Plant Materials

We collected 24 ginseng accessions from two populations of cultivated ginseng grown in northeastern china and a population from Korea and used a North Korea ginseng, a wild ginseng and an American ginseng (*Panax quinquefolium* L.) as reference individuals (Table 1).

2. DNA Extraction and SRAP Analysis

The total genomic DNA was extracted from young leaves or dried ginseng roots by the CTAB method as described by Doyle and Doyle (1987). 10 ng/ul DNA templates were made using TE (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0). All SRAP primer combinations were initially screened using a group of ten samples (Table 2). The sixteen primer pairs that produced scorable polymorphic bands were used to amplify the rest of the accessions. Each of 15 mL reaction consisted of 1.33 mM of primers, 200 mM of each dNTP, 1.5 ㎖ of 10 × PCR Buffer, 2 mM of MgCl2, 0.8 ㎎/㎖ Bovine serum albumin, 5.8 ㎖ ddH_2O, 1 unit of Taq polymerase and 20 ng of template. DNA Thermal Cycler (ABI2720, USA) was used and cycling parameters included 2 min of denaturing at 94℃, five cycles of three steps: 1 min of denaturing at 94℃, 1 min of annealing at 35℃ and 1 min of elongation at 72℃ In the following 35 cycles the annealing temperature was increased to 50℃, and for extension, one cycle 5 min at 72℃. PCR amplification products were separated by electrophoresis using 6% denaturing polyacrylamide gels and were visualized following silver staining method as described by Bassam *et al*., (1991).

Table 2. SRAP primer used in this experiment.

Forward primer (5′-3′)	Reverse primer (5′-3′)
me1 : TGAGTCCAAACCGGATA	em1 : GACTGCGTACGAATTAAT
me2 : TGAGTCCAAACCGGAGC	em2 : GACTGCGTACGAATTTGC
me3 : TGAGTCCAAACCGGAAT	em3 : GACTGCGTACGAATTGAC
me4 : TGAGTCCAAACCGGACC	em4 : GACTGCGTACGAATTTGA
me5 : TGAGTCCAAACCGGAAG	em5 : GACTGCGTACGAATTAAC
me6 : TGAGTCCAAACCGGTAG	em6 : GACTGCGTACGAATTGCAC

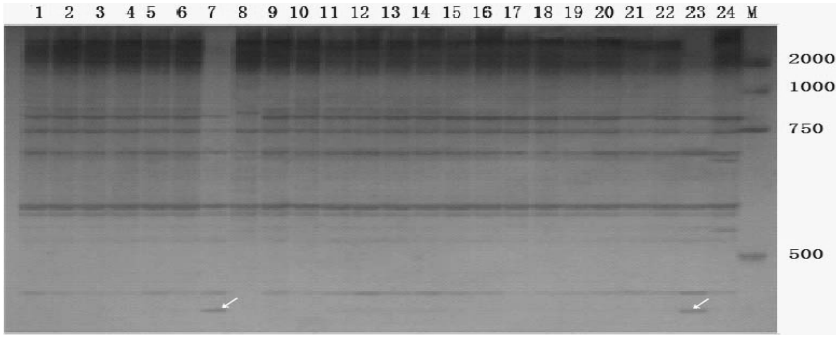

Fig. 1. SRAP Banding pattern of primer M5E3. 1-24, 24 ginseng accessions. Polyacrylamide gel was used for gel images having the DNA size marker 'M' (DL2000 marker).

3. Data Analysis

Each band was scored as present (1) or absent (0) and data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) software package (Rohlf, 1998). A similarity matrix was constructed based on Dice's coefficient which considers only one to one matches between two taxa for similarity. The similarity matrix was used to construct a dendrogram using the unweighted pair group method arithmetic average (UPGMA) to determine genetic relationships among the germplasm studied.

Genetic diversity analyzed using POPGENE 1.32 software (Francis and Yang, 2000). The following genetic diversity parameters were determined: 1) the number of polymorphic loci (*A*) and the percentage of polymorphic loci (*P*); 2) the average number of alleles per loci (*Na*); 3) the effective number of alleles per loci (*Ne*); 4) gene diversity (*H*) and Shannon's information index (*I*); 5) Nei's genetic distances (*D*) and genetic identity (*IN*); and 6) the coefficient of gene differentiation amongst populations within species was calculated using Nei's gene diversity method. The formula was $GST = DST/HT$, $HT = Hs + DST$, where HT is the total gene diversity, Hs is the gene diversity within population and DST is the gene diversity between populations. Gene flow was determined as $Nm = 0.5(1 - GST) / GST$. Analysis of Molecular Variance was carried out on the SRAP data using the WinAMOVA 1.55 program (Excoffier, 1992).

RESULTS

1. Polymorphism of SRAP amplified bands by different primer combinations

On the basis of preliminary testing, 16 sets of primers, which steadily produced well-defined and scorable amplification products, showed polymorphisms in all 24 genotypes. Fig. 1 shows the amplification profile of primer combination Me5/Em3, which showed a special band differed Fuxing and wild ginsneg from the another material used in the wild ginseng from another material used in the experiment. A total of 348 bands were observed among which 201were polymorphic (57.75%), ranging between 11 (Me5/Em2 and Me/Em1) and 29 (Me1/Em6) per primer combination, with an average of 21.75 bands per primer set.

2. Phylogenetic analysis

A dendrogram based on the Jaccard similarity coefficients of the 24 accessions was constructed (Fig. 2). The cluster from SRAP analysis grouped the 24 samples into 4 major groups at a similarity index value of 0.92. The first group consisted of 19 accessions mainly from China and Korea. The landraces Changbo, Yuanbangyuanlu and Shizhu formed

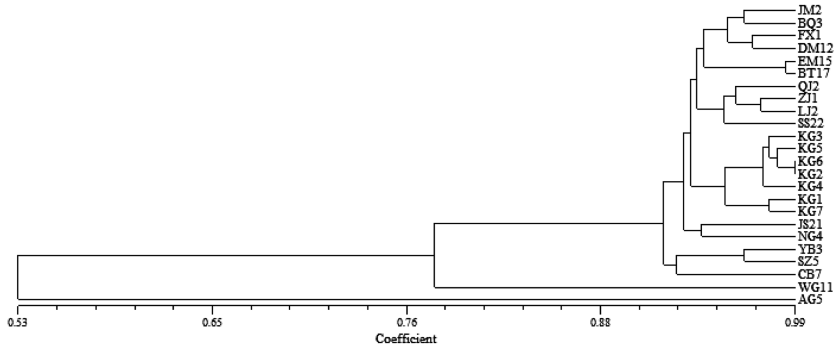

Fig. 2. Dendrogram of 24 ginseng accessions clustered based on SRAP Marker.

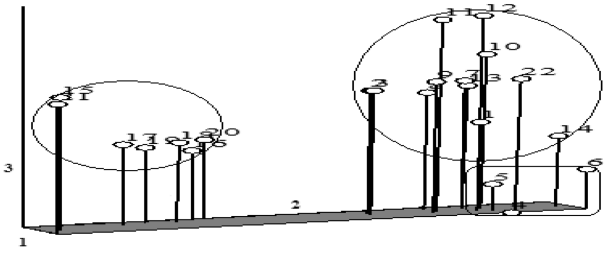

Fig. 3. Principal component analysis based on SRAP patterns in cultivated ginseng, 1-22, 22 cultivated ginseng in Table 1.

the second group and the group third and forth included American ginseng and wild ginseng, respectively. The relationships observed in the UPGMA analysis are mirrored in the PCA (Fig. 3): about 80.45% of the total variation was described by the first three PCA and the genetic diversity structure was shown generally in line with the geographical structure of the species at small geographical scale. It is noticeable that PCA axis 1 explained 72.14% of the variation and clearly separates out China and Korean populations from sampling sites.

3. Population genetic diversity, differentiation (*GST*), and gene flow (*Nm*)

The Table 3 showed that the highest Nei's gene diversity index (*H*) was observed in Jian population ($H = 0.0726$), and the genetic diversity index of Fusong and Korea populations were 0.0668 and 0.0326, respectively. The Nei's gene diversity index (H_T) was 0.0863 at species level, and the average Nei's gene diversity index was $H_S = 0.0573$ within the population. Genetic differentiation coefficient between populations (*GST*) was 0.3410. The genetic variation between populations accounted for 34.1% of total variations. Shannon's information index in Ji'an population ($I = 0.1051$) was higher than in Fusong ($I = 0.0980$) and Korea ($I = 0.0485$); Shannon's information index at species level was 0.1358, and the average index value within population was 0.0838. The gene flow *Nm* was 0.9662 according to the genetic differentiation coefficient between populations ($GST = 0.3410$). In agreement with the analysis from Nei's diversity statistics, AMOVA analysis showed significant ($p < 0.01$) variation among populations (30%), which was lower than the within population (70 %) genetic variation (Table 4).

4. Genetic identity and genetic distance between populations

To quantitatively assess genetic differentiation between populations, Nei's genetic identity *I N* and genetic distance *D* were calculated (Table 5). Population genetic identity ranged from 0.9474-0.9692 and the genetic distance varied from 0.0313to 0.0541. This indicates that the similarity degree

Table 3. Summary of genetic variations for populations.

Population	*Sample size*	*Na*	*Ne*	*H*	*I*
Specie level	21	1.3284	1.1368	0.0863	0.1358
Jian	6	1.1791	1.1325	0.0726	0.1051
Fusong	8	1.1791	1.1201	0.0668	0.0980
Korea	7	1.0945	1.0583	0.0326	0.0485

Populations Observed number of alleles (Na) Effective number of alleles (Ne)
Nei's gene diversity (H) Shannon's information index (I)

Table 4. Summary of nested analysis of molecular variance (AMOVA).

Source	f	SS	MS	Est.Var.	%
P					
Among regions	1	25.190	25.190	1.256	16
< 0.01					
Among pops	1	13.256	13.256	1.109	14
< 0.01					
Within pops	18	101.744	5.652	5.652	71
< 0.01					
Total	20	140.190		8.017	100

Table 5. Nei's unbiased measures of genetic identity and genetic distance.

Pop	Jian	Fusong	Korea
Jian	***	0.9692	0.9474
Fusong	0.0313	***	0.9563
Korea	0.0541	0.0447	***

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

was higher between populations and the genetic distance was smaller. The similarity degree between Jian and Fusong was the highest and the genetic identity was 0.9692; the genetic distance (0.0541) between Ji'an and Korea was the largest, genetic differentiation was the highest.

DISCUSSION

SRAP has been proved to be a method with a high resolution for detection of genetic variations among and within populations of ginsengs. With 16 primer pairs, we could differentiate 24 SRAP-phenotypes among 24 plants studied. In the present study, SRAP revealed low genetic diversity ($H_T = 0.175$) in ginsengs. On one hand, ginseng depends on self-pollination to propagate. This mode of reproduction lacks genetic recombination and thus results in low level of genetic diversity. On the other hand, long-term artificial selection can also reduce the genetic diversity. When people select landraces, they usually focus on the forms with high economic values, for example, special root shape and high yield, etc. As a result, substantial genetic variation from ancestral gene pool is lost during domestication.

A phenogram representing varieties relatedness, based on the analysis of SRAP loci, showed four major clusters separated at 92% similarity. No clear separation according to their geographical origins was observed. American ginseng and ginsengs spanned the two extremes in the dendrogram at a similarity index value of 0.52.

The genetic structure of plant populations reflects the interactions of various factors, including the long-term evolutionary history of the species (shifts in distribution, habitat fragmentation, and population isolation), genetic drift, mating system, gene flow and selection (Schaal *et al*., 1998). In the present study, there was high level of genetic differentiation ($G_{ST} = 0.341$) among populations. The result is concordant with the reported by Kim and Cho (2003). This could be attributed to random genetic drift and differential selection pressure (by the environment). According to Slatkin (1981, 1985), Caccone (1985) and Waples (1987) *Nm* values can be grouped into three categories: high ($Nm > 1.000$), intermediate, ($0.250 - 0.990$), and low ($0.000 - 0.249$). In this study, the relatively high genetic differentiation and intermediate level of gene flow ($Nm = 0.962$) detected strongly indicate that genetic drift has greatly affected the genetic composition of individual populations. The lower gene flow among populations could be attributed to geographical, social or cultural isolation/barrier inbreeding mode and limited use of ginseng seeds purchased from one location for cultivation in another place. Inbreeding mode usually reduces gene exchange (gene flow) both between different individuals and populations, leading to significant differentiation between populations (Slatkin, 1987).

Information on current levels of genetic diversity of germplasm at gene bank is essential for designing appropriate strategies for future conservation. According to the results of the present study, the high genetic differentiation among the populations suggests that all the populations should be well represented by more samples *ex situ*. Furthermore, the high genetic diversity within some populations (Jian, Fusong and Korea) indicate that the most effective strategy for

preserving genetic variation would be to conserve a large number of individuals for more diverse population(s).

In summary, the present study shows that SRAP markers are important for genetic diversity study in ginsengs. The results also provide important foundations for future conservation and improvement programs of ginseng. The results from gene flow estimates suggest that there could be a possibility of sampling plants with the same genetic constitution from different regions. Therefore, representing a population by as many collections as possible would be the best approach.

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