Genetic diversity and population structure of *Atractylodes japonica* K$_{OIDZ}$. in Korea

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한국내 삽주의 유전적 다양성과 집단구조

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ABSTRACT : The study of genetic diversity was carried out in *Atractylodes japonica* K$_{OIDZ}$.. Although this species has been regarded as medically important one, there is no report on population structure in Korea. Starch gel electrophoresis was used to investigate the allozyme variation and genetic structure of eight Korean populations of this species. Of the 15 genetic loci surveyed, nine (60.0%) was polymorphic in at least one population. Genetic diversity was high at the species level ($H_{es}=0.144$), whereas, that of the population level was relatively low ($H_{ep}=0.128$). Nearly 87% of the total genetic diversity in *A. japonica* was apportioned within populations. The sexual reproduction, high fecundity, and perennials are proposed as possible factors contributing to high genetic diversity. The indirect estimated of gene flow based on Gst was 1.69.

Key words : allozyme variation, *Atractylodes japonica*, genetic diversity

INTRODUCTION

The accumulation of allozyme data in higher plants has revealed causal relationships between allozyme diversity and the ecological and life history characteristics of a species (Hamrick & Godt, 1989). Various factors such as the breeding system, seed dispersal mechanism, and distribution pattern affect the genetic structure of plant populations (Wright, 1951; Loveless & Hamrick, 1988). For example, predominantly outcrossing species tend to maintain more genetic variation within their populations than predominantly self-pollinating species (Gottlieb, 1981). In general, geographically restricted or endemic species maintain fewer polymorphic loci, and fewer alleles per polymorphic locus than widespread congeneric species (Hamrick & Godt, 1989). However, widespread plant species that occur as small, isolated patches in specialized habitats are expected to maintain a low level of genetic variation

within the species and a high level of population divergence caused by genetic drift (Hamrick & Godt, 1989; Huh, 1999).

In this paper we investigated allozyme variation in Japanese and Korean populations of *Atractylodes japonica*. *A. japonica*, a widespread herb occurring throughout cooler, more temperate regions of the Northern Hemisphere is most abundant in the boreal and Artic areas. The plant height is 30–100 cm with red or white flowers. In this paper, we estimate the allozyme diversity maintained in natural populations of *A. japonica* and describe the genetic structure of populations in Korea. The results are compared with those of other species with similar ecological and life history characteristics.

MATERIALS AND METHODS

Sampling procedures and enzyme electrophoresis

The leaf samples were collected from eight natural populations of *A. japonica* (Fig. 1). More than 36 individuals were analyzed for each population.

The procedures for the homogenization of tissues, starch gel electrophoresis, and enzyme assays, followed the methods of Soltis et al. (1983). Young leaves were homogenized in Tris–HCl grinding buffer with PVP (pH 8.0) as described in Soltis et al. (1983). Electrophoresis was performed using 11.0% starch gels, and eight enzymes were assayed. Esterase (EST, EC 3.1.1.2), peroxidase (PER, EC 1.11.1.7), shikimate dehydrogenase (SKH, EC 1.1.1.25), and superoxide dismutase (SOD, EC 1.15.1.1) were resolved on system 9 of Soltis et al. (1983); isocitrate dehydrogenase (IDH, EC 1.1.1.42), malate dehydrogenase (MDH, EC 1.1.1.37), 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.43), and phosphoglucomerase (PGI, EC 5.3.1.9) were resloved on system 10 of Soltis et al. (1983).

For the enzymes resolved in more than one zone of activity, the most anodally migrating isozyme was designated as '1', and other subsequent isozymes were sequentially numbered. The alleles of isozyme 1, 2, 3, and so on were designated sequentially as 'a', 'b', and so on, respectively.

Data analysis

A locus was considered polymorphic if two or more alleles were detected, and the frequency of the most common allele was less than 0.99. Several standard genetic parameters were estimated using a computer program developed by Loveless and Schnabel (Edwards & Sharitz, 2000). The percentage of polymorphic loci (P), mean number of alleles per locus (A), effective number of alleles per locus (A_E), number of alleles per polymorphic locus (A_P), and gene diversity (H_E) were estimated from the data (Hamrick et al., 1992). Observed heterozygosity (H_O) was compared with the Hardy–Weinberg expected value using Wright's fixation index (F) (Wright, 1965). These indices were tested for deviation from zero by x^2-statistics following Li & Horvitz (1953). Nei's gene diversity formulae (H_T, H_S, D_{ST}, and G_{ST}) were used to evaluate genetic diversity within and among populations (Nei, 1973, 1977). The G_{ST} coefficient corresponds to the relative amount of differentiation among populations. In addition, x^2-statistics were used to detect significant differences in allele frequencies among populations for each locus (Workman & Niswander, 1970). Nei's genetic identity (I) was calculated for each pairwise combination of populations (Nei, 1972). PC-SAS program (SAS Institute Inc., 1989) was used to conduct a cluster analysis on Nei's genetic distances via the unweighted pairwise groups method arithmetic average (UPGMA).

The genetic structure within and among populations was also evaluated using Wright's (1965) F–statistics, F_{IT}, F_{IS} and F_{ST}. The F_{IT} and F_{IS} coefficients measure excesses of homozygotes relative to the panmictic expectations in the entire samples and within populations, respectively. Deviations of F_{IT} and F_{IS} from zero were tested using x^2-statistics (Li & Horvitz, 1953). In the context of multiallelic loci F_{ST} is denoted as G_{ST} (Nei, 1973). The estimate of Nm (the number of migrants per generation) was based on G_{ST} (Wright, 1951). The absolute population differentiation (D_M) was calculated using Nei's (1973) statistics. The correlation between geographical and genetic distances was evaluated using the modified Mantel's test (Smouse et al., 1986).

RESULTS

A high level of genetic variation was found in the *A. japonica* populations. Nine of the 15 loci examined (60.0%) showed polymorphisms in at least one population, while the remaining six loci (*Idh-2, Mdh-1, Per-3, Pgi-2, Sod-1,* and *Sod-2*) were monomorphic in all populations. The percentage of polymorphic loci within populations ranged from 26.7% to 46.7% with an average of 38.3% (Table 1). The majority of the polymorphic loci maintained three (*Est-1, Idh-1, Mdh-2, Per-2,* and *Pgd-2*) or two alleles (*Est-2* and *Pgi-1*), while the remaining two loci maintained four alleles (*Per-1* and *Skd*). The average number of alleles per locus (*A*) was 1.64 on average, varying from 1.53 for the PAE population to 1.87 for the JEC population. The number of alleles per polymorphic locus (*A$_P$*) was 2.69 across the populations. The mean genetic diversity within populations (*H$_{EP}$*) was 0.128. The JEC population had the highest genetic diversity (0.160), whereas the GAN population had lowest (0.092). The observed significant positive *F$_{IS}$* value for the eight populations, 0.410, indicated that there was a significant deficit of heterozygotes in these populations (Table 2). Total

genetic diversity values (*H$_T$*) varied from 0.018 (*Est-2*) to 0.565 (*Skd*), giving an average 0.239 over all polymorphic loci. The absolute measure of genetic differentiation among populations (*D$_M$*) was very low (0.028). On a per locus basis, the proportion of total genetic variation due to differences among populations (*G$_{ST}$*) ranged from 0.037 for *Idh-1* to 0.380 for *Per-2*, with a mean of 0.129, indicating that about 13% of the total allozyme variation was among populations. Analysis of fixation indices, calculated for all polymorphic loci in each population, showed a slight deficiency of heterozygotes relative to Hardy-Weinberg expectations (Table 3).

The estimate of gene flow based on *G$_{ST}$* was moderate among the eight populations (*Nm*=1.69).

Genetic identity values (I) among pairs of populations range from 0.920 to 0.996 (Table 4). Values of genetic distance (D) were below 0.10.

The correlation between genetic distance and geographic distance was high (*r*=0.65, *p*<0.05), for the Korean populations, indicating that geographically close populations tended to be genetically similar. The genetic relationships among the populations can be seen in the dendrogram, consisting of eight populations were recognized(Fig. 2).

Table 1. Summary of allozyme variation within eight populations of *Atractylodes japonica*

Pop[†]	*N*[‡]	*P*	*A$_P$*	*A*	*A$_E$*	*H$_{OP}$* (SD)	*H$_{EP}$* (SD)
GAN	50	26.67	3.00	1.53	1.14	0.061(0.011)	0.092(0.040)
GAP	36	40.00	2.67	1.67	1.22	0.068(0.011)	0.124(0.050)
EUI	36	40.00	2.50	1.60	1.23	0.077(0.012)	0.136(0.048)
HAM	50	33.33	2.80	1.60	1.23	0.062(0.010)	0.120(0.049)
JEC	50	46.67	2.67	1.87	1.32	0.077(0.012)	0.160(0.061)
AND	40	40.00	2.60	1.67	1.23	0.076(0.012)	0.137(0.050)
PAE	44	33.33	2.43	1.53	1.20	0.067(0.011)	0.116(0.048)
TAE	42	46.67	2.69	1.67	1.22	0.083(0.012)	0.136(0.045)
Mean		38.33		1.64	1.22	0.071(0.004)	0.128(0.017)
Species		60.00	3.00	2.20	–		0.144

Percentage of polymorphic loci (*P*), mean number of alleles per polymorphic population (*A$_P$*), mean number of alleles per locus (*A*), effective number of alleles per locus (*A$_E$*), observed heterozygosity (*H$_{OP}$*), and genetic diversity (*H$_{EP}$*).
† : Abbreviation codes as in Fig. 1. ‡ : Number of individuals examined.

Table 2. Estimates of genetic diversity statistics for nine polymorphic loci in eight populations

Locus	H_T	H_S	D_{ST}	D_M	F_{IS}	F_{IT}	G_{ST}
Mdh-2	0.304	0.283	0.021	0.024	0.330	0.376	0.069
Est-1	0.296	0.284	0.011	0.013	0.400	0.423	0.038
Est-2	0.018	0.017	0.002	0.002	0.339	0.394	0.084
Skd	0.565	0.518	0.047	0.054	0.589	0.623	0.084
Per-1	0.102	0.069	0.033	0.038	0.469	0.642	0.326
Per-2	0.209	0.130	0.079	0.091	0.457	0.664	0.380
Idh-1	0.319	0.307	0.012	0.014	0.422	0.444	0.037
Pgd	0.275	0.264	0.011	0.013	0.322	0.350	0.041
Pgi-1	0.066	0.060	0.007	0.008	0.361	0.425	0.099
Mean	0.239	0.215	0.025	0.028	0.410	0.482	0.129

Total genetic diversity (H_T), genetic diversity within populations (H_S), among populations (D_{ST}), absolute population differentiation (D_M), deviations of genotype frequencies from Hardy-Weinberg expectations within individual population (F_{IS}), over all populations (F_{IT}), and proportion of total genetic diversity partitioned among populations (G_{ST}).

Table 3. Wright's fixation indices and chi-square test for eight populations of *Atractylodes japonica*

Pop.	Mdh-2	Est-1	Est-2	Skd	Per-1	Per-2	Idh-1	Pgd	Pgi-1
GAN	0.450*	0.433*	–	0.753***	0.479**	0.650***	0.286	0.466**	–
GAP	0.455*	0.311	–	0.567***	–	0.525**	0.372*	0.365**	–
EUI	0.319	0.579***	0.351	0.558***	–	–	0.297	0.372*	–
HAM	0.272	0.361*	–	0.549***	–	–	0.694***	0.328*	–
JEC	–	0.333	–	0.452**	–	–	0.337	0.253	–
AND	0.348	0.435**	–	0.641***	–	0.256	0.542***	–	0.444**
PAE	0.276	0.405*	–	0.641***	–	–	0.445**	0.207	–
TAE	0.186	0.348*	–	0.645***	–	0.281	0.281	0.391**	0.310

*, **, and *** indicate significance at the 0.05, 0.01, and 0.001 levels, respectively.

Table 4. Nei's unbiased genetic identity values of among *Atractylodes japonica* (above diagonal) and genetic distances among populations (below diagonal)

Pop.	GAN	GAP	EUI	HAM	JEC	AND	PAE	TAE
GAN	–	0.951	0.930	0.938	0.911	0.932	0.920	0.932
GAP	0.050	–	0.989	0.992	0.976	0.987	0.986	0.990
EUI	0.072	0.012	–	0.995	0.991	0.994	0.995	0.992
HAM	0.064	0.008	0.005	–	0.990	0.990	0.994	0.995
JEC	0.093	0.024	0.008	0.010	–	0.989	0.996	0.989
AND	0.070	0.014	0.006	0.010	0.011	–	0.992	0.994
PAE	0.084	0.016	0.005	0.006	0.004	0.008	–	0.989
TAE	0.071	0.010	0.008	0.005	0.011	0.061	0.011	–

Fig. 1. The location of eight populations of A. japonica.

Nei's genetic distance

Fig. 2. A dendrogram showing the genetic relationships among eight populations of A. japonica, based on allozyme variation.
GAN : Ganghoa, Kyeonggi-do, GAP : Gapeng, Kyeonggi-do, EUI : Euiseong, Gyeongsangbuk-do, HAM : Hamyang, Gyeongsangnam-do, JEC : Jecheon, Chungcheonhbuk-do, PAE : Paengchang, Kwangwon-do, AND : Andong, Gyeongsangbuk-do, TAE : Taeback, Kwangwon-do.

DISCUSSION

Level of genetic variation

The level of genetic variation found in the Korean populations of A. japonica was high; the average percentage of polymorphic loci was 60.0, and the corresponding average gene diversity (heterozygosity) was 0.128 (Table 2). According to a review of plant allozyme literature by Hamrick & Godt (1989), the average percentage of polymorphic loci for short-lived herbaceous species was 41.3 at the species level (reviewed for N=152 species) and 28.0 at the population level (N=159). The mean genetic diversity (H_E) was 0.144 at the species level and 0. 128 at the population level. All the genetic diversity parameters of Korean populations of A. japonica were higher than those of species with similar ecological and life history characteristics. For example, the mean genetic diversity of 0.144 for Korean populations is higher than that of dicots (0.096), species with a regional geographical range (0.118), insect-pollinated, outcrossing species (0.124) and species that reproduce sexually (0.114) (Hamrick & Godt, 1989). Other measures of genetic variation confirm the Korean populations of A. japonica are more variable than ecologically comparable species.

Population structure of A. japonica

A natural local population of A. japonica on mountains consists of 10^2-10^3 individuals. Small population size is important when a population grow from a few founder individuals (Hartl & Clark, 1989). For example, an island population such as GAN (Ganghoa island) appears to have started from a very small number of founders. In the present study, a deficiency of heterozygotes from the Hardy-Weinberg expectation was detected (F_{is}=0.410) (Table 2). Analysis of fixation indices, calculated for all polymorphic loci in each population, showed also a slight deficiency of heterozygotes relative to Hardy-Weinberg expectations (Table 3). For example, all fixation indices were positive (46/46), of which 30 indices (65.0%) departed significantly from zero (p<0. 05). Consanguineous mating may cause it or this may be due partly to Wahlund's effect of subdivision of patchily
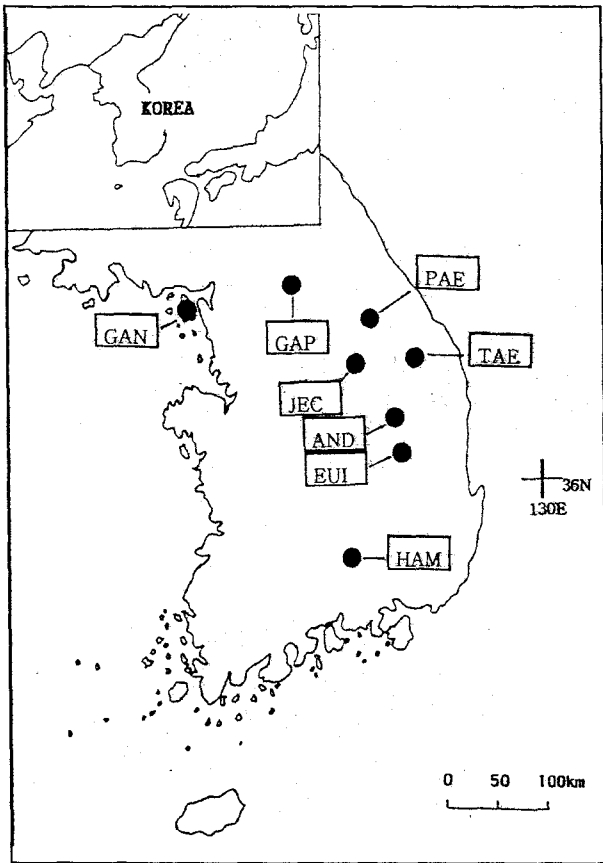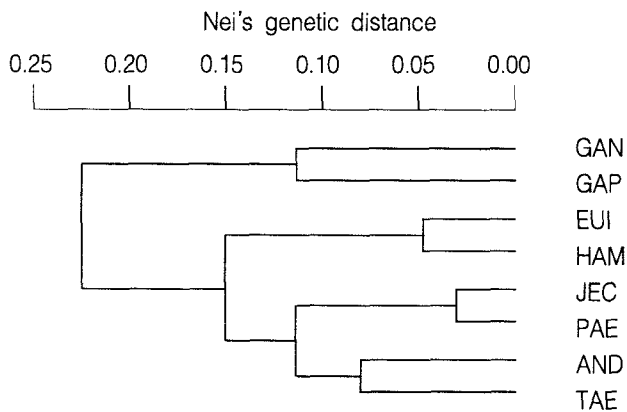
distributed natural populations(Wahlune, 1928). In natural populations of *A. japonica*, the allozyme variability was maintained within populations rather than among populations, judging from the observed G_{ST} values (0.129) (Table 2). Although we did not analyze further subdivision of a local population, we may suggest that allozyme variation in *A. japonica* populations is maintained in patchily distributed subpopulations or demes, either by random drift of neutral alleles or micro-environmental selection for adaptive alleles.

In general, species or taxa with widespread geographic distribution maintain a higher level of genetic diversity than those with narrow or endemic distributions (Hamrick & Godt, 1989). Species with discrete populations in patchy distributions have relatively lower levels of variation within populations than species with more continuously distributed populations (Huh, 1999). Although *A. japonica* is distributed in a wide geographic range, it is ecologically restricted, growing in mountains in East Asia. Therefore, local populations are isolated each other, and they are discretely distributed. Furthermore, as discussed above, each local population is subdivided, consisting of many subpopulations. Species with a relatively narrow niche, and with discrete, isolated populations ("habitat specialists") like A. japonica in general maintain less genetic variation than do species with continuous, abundant populations growing on broad-niched mainland habitats ("habitat generalists") (Nevo et al., 1984). This probably implies that the population structure below the local population level may be critical, along with the biological characteristics of the species itself, to determine the level of variation.

Usually, plant species with high fecundity maintain high genetic diversity (Huh, 2001). *A. japonica* has high seed production; an individual plant produces hundreds of seeds in the field. It has many strong spikes in leaves. The spikes may limit the access of animals to the fruits. In addition, the seed coat of this species is very hard for animals to digest. Thus, many seeds are deposited in the soil as a seed bank, where they may contribute to later generations.

The phylogenetic tree shown in Fig. 2 clearly distinguishes eight populations. The correlation between genetic distance and geographical distance is relatively high. The positions of the populations in the tree almost completely match the corresponding geographical positions. The one exceptional, the western population (GAN) is relatively small and maintained less genetic variation than the other populations, probably due to (having suffered) a founder effect.

Although *A. japonica* is not seem to be in a genetic extinction vortex, the conservation of medically important species requires consideration of ecological and genetic factors. The long-term genetic potential of species is affected by many factors, including local population size, levels of gene flow among populations, the total number of reproducing individuals, and extinction of local populations (Lande, 1988). This study indicates that *A. japonica* maintains high levels of genetic variation within populations and is a genetically diverse species. Nevertheless, some populations are under threat from overexploiting for medicine and destruction by individuals.

국 문 적 요

한국내 분포하는 다년생 초본인 삽주 집단의 유전적 다양도와 집단구조를 조사하기 위해 전분 전기영동으로 분석하였다. 15 대립유전자좌위당 9개 좌위에서 다형현상(60.0%)을 보였으며, 유전적 다양도는 종수준에서 0.144로 높은 반면 집단수준이 이보다 약간 낮았다. 삽주의 유전적 다양도는 대부분 집단내에 존재하였고 유사한 생활양식을 가진 다른 식물종에 비해 높았다. 그 이유로는 유성생식, 다년생, 다산 등에 기인한다. 집단간 분화는 약 13%였고 지리적 거리와 유전적 거리의 상관은 높았다($r=0.65$). 그럼에도 불구하고 일부 격리된 집단은 유효집단크기를 가지지 못하여 이형접합체의 결여가 유의성을 보여 다양도가 높은 집단의 보존이 요망된다.

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