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Original Research Article

Genetic Diversity and Population Genetic Structure of *Cephalotaxus koreana* in South Korea

Kyung Nak Hong*, Young Mi Kim, Yu Jin Park and Jei Wan Lee

Division of Forest Genetic Resources, Korea Forest Research Institute, Suwon 441-350, Korea

Abstract - The Korean plum yew (*Cephalotaxus koreana* Nakai) is a shade-tolerant, coniferous shrub. The seeds have been used as a folk medicine in Korea, and an alkaloid extract (HTT) is known to have anticancer properties. We estimated the genetic diversity of 429 trees in 16 populations in South Korea using 194 polymorphic amplicons from seven combinations of AFLP primer-restriction enzymes. The average number of effective alleles and the percentage of polymorphic loci were 1.37 and 79.4%, respectively. Shannon's diversity index and the expected heterozygosity were 0.344 and 0.244, respectively. We divided 16 populations into four groups on the UPGMA dendrogram and the PCA biplot. The first two principal components explained 84% of the total genetic variation. Genetic differentiation between populations explained 14% of total genetic variation, and the remaining 86% came from difference between individuals within populations, as determined by an analysis of molecular variance (AMOVA). However, the genetic differentiation did not correlate with the geographic distance between populations from the Mantel test. The Bayesian statistics, which are comparable to Wright's F_{ST} and Nei's G_{ST}, were $\theta^{I} = 0.406$ and $\theta^{II} = 0.172$, respectively. The population genetic diversity was slightly lower, and the strength of genetic differentiation was much weaker, than the average of those plants having similar life histories, as assessed using arbitrary marker systems. We discuss strategies for the genetic conservation of the plum yew in Korea.

Key words - Genetic differentiation, AFLP, Cluster analysis, Bayesian inference, Conservation

Introduction

There is still no agreement on the taxonomy of the genus *Cephalotaxus*, but it is generally presumed to consist of seven to nine species (Lang *et al*., 2011). In Korea, two species and one variety, including *C. koreana*, *C. harringtonia*, and *C. harringtonia* var. *nana* are registered (KPNI Committee, 2008), although only two species, *C. harringtonia* and *C. koreana*, were acknowledged as native species (Tripp, 1995). Otherwise, *C. koreana* was classified as a variety or subspecies of *C. harringtonia*, and *C. harringtonia* var. *nana* was designated as a species (Zhang *et al*., 2000; Lang *et al*., 2013). *C. harringtonia* var. *nana* with creeping was treated as a female of *C. koreana* (Lee, 1974), or every plum yew in Korea was classified as *C. harringtonia* (Chang *et al*., 2011).

Korean plum yews (*Cephalotaxus koreana* Nakai) are distributed in Korea, China, and Japan (Lee, 1974). They grow at rocky sites in valleys or at the feet of mountains at 100-1,300 m above sea level, at 37-38° latitude in Korea, except for Jeju Island and Ulleung Island. They are mainly dioecious or rarely monoecious, and have 2n = 24 chromosomes (Chang *et al*., 2011). Their flowers bloom in April, and the ripening of seeds with edible sweet arils occurs from September to October in the following year. Around 80% of their seeds germinate in a nursery bed, and clonal propagation by cuttings or layerings is also possible (Lee, 1974).

The seeds of the Korean plum yew have been used as a folk medicine in Korea (Kim and Jeong, 2008). Alkaloids and terpenoids extracted from *Cephalotaxus* spp. are valuable medical resources (Abdelkafi and Nay, 2012; Jung *et al*., 2010). Homoharringtonine (HTT) is an alkaloid from *C. harringtonia*, and it has an effect against hematological cancer, and it is being developed as a commercial medicine in the USA (Sisodiya, 2013). Natural homoharringtonine was superior to a synthetic one in anticancer essays (Abdelkafi and Nay, 2012), and its content was significantly different between plum yew populations in Korea (Jung *et al*., 2005). *Cephalotaxus* spp. are now rare and endangered worldwide (Farjon *et al*.,

*Corresponding author. E-mail : honeutal@forest.go.kr

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1993). The Korean plum yew also is designated as one of the specific plants whose habitats are threatened regionally in Korea (Oh *et al*., 2011).

Amplified fragment length polymorphism (AFLP) is a dominant marker having limitations to estimate precisely heterozygosity, and it requires rather complicated techniques, high-purity template DNAs, and a DNA sequencer. However, its reproducibility is higher than that of other arbitrary markers, such as randomly amplified polymorphic DNAs (RAPD) (Mba and Tohme, 2005). AFLP, unlike microsatellite markers, is easily applicable to non-model species with a lack of sequence information, and it generates many polymorphic markers per assay (Campbell *et al*., 2003). Thus, AFLPs have been useful for studies of genetic variation in plant species. Most studies of population genetic diversity using AFLP employed more than 100 loci, and optimally 200 to 600 loci, to obtain qualified results (Rieseberg *et al*., 2012; Nybom, 2004). A large number of loci are more efficient than a large number of individuals for estimating genetic diversity and genetic distance (Nei and Roychoudhury, 1974; Nei, 1978). Even with an unknown level of genetic diversity in a population, a total of 20 to 30 individuals using five to 10 microsatellite loci was an adequate sample size to assess genetic diversity (Pruett and Winker, 2008). For AFLP, at least 10 times more dominant markers should be used to attain the same efficiency of codominant markers as simple sequence repeats (SSR) (Mariette *et al*., 2002). However, it is not difficult to obtain more than 100 AFLP markers; therefore, AFLPs could be easily applied to non-model plant species.

We estimated the genetic diversity and relationships of *C.koreana* populations in Korea using an AFLP marker system to obtain information on the *in situ* conservation of their genetic resources.

Materials and Methods

Sample collection

We chose 16 populations that have a sufficient sample size ($n \geq 30$, except for some populations; Table 1) through literature searches and field surveys. The needles of 429 plum yews from the 16 populations were collected, and their growth was measured. We also collected the needles of 30 *Torreya nucifera* Sieb. et Zucc. in Jeju Island as an outgroup. Total DNA was isolated using the DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA).

Table 1. Population locations and growth performances of the samples in *Cephalotaxus koreana* populations in Korea[z]

Population	Place-name	Tree form	Tree height(m)	N	Latitude and Longitude	Protection status
Gwacheon	Mt. Gwanak	S	0.24	11	37° 26′ 29″ 126° 58′28″	-
Danyang	Mt. Sobaek	S	< 0.5	30	36° 59′ 10″ 128° 28′59″	NP
Geumsan	Mt. Daedun	S	< 0.5	28	36° 08′ 19″ 127° 18′15″	PP
Dalseong	Mt. Biseul	S	< 0.5	30	35° 43′ 03″ 128° 30′53″	CP
Daegu	Mt. Palgong	S	< 0.5	13	36° 00′ 13″ 128° 41′22″	PP
Mungyeong	Mt. Juheul	S	< 0.5	30	36° 46′ 20″ 128° 04′52″	NP
Geochang	Mt. Gibaek	S	< 0.5	30	35° 41′ 38″ 127° 46′24″	NP
Hapcheon	Mt. Kaya	S + T	< 0.5	30	35° 48′ 14″ 128° 05′54″	NP
Yeonggwang	Mt. Bulgap	T	1.87	30	35° 12′ 14″ 126° 33′04″	-
Jangseong	Mt. Naejang	S	< 0.5	30	35° 26′ 19″ 126° 53′02″	NP
Haenam	Mt. Duryun	S + T	0.99	30	34° 28′ 46″ 126° 36′52″	PP
Jinan	Mt. Unjang	S	0.69	30	35° 53′ 15″ 127° 21′28″	-
Kwangyang	Mt. Paegun	S	< 0.5	30	35° 06′ 33″ 127° 36′05″	-
Gurye	Mt. Jiri	S + T	3.6	30	35° 15′ 39″ 127° 31′47″	NP
Hwasun	Mt. Gaecheon	S + T	< 0.5	30	34° 54′ 17″ 126° 55′18″	NM
Wando	Dumun-ri	T	5	17	34° 21′ 50″ 126° 38′ 34″	NM

[z]S or T = shrub or tall tree in tree form, N = number of samples, NP = national park, PP = provincial park, CP = county park, NM = natural monument by the law.

AFLP PCR

AFLP analysis carried out using a slightly modified protocol of Vos *et al*. (1995). The genomic DNA (0.5 ㎍) was digested with each 10 units of *Eco*RI and *Mse*I endonuclease (New England Biolabs, Ipswich, MA, USA) in a total volume of 25 ㎕ for 2 h at 37℃. Five ㎕ of the digestion reaction was mixed with 15 ㎕ of ligation solution, which contained 5 pmole of *Eco*RI adaptor, 50 pmole of *Mse*I adaptor (Applied Biosystems, Foster City, CA, USA), and 100 units of T4 DNA ligase (New Engalnd Biolabs). The mixture was incubated for 3 h at 37℃ and was diluted 10-fold with H_2O.

Preselective amplification was conducted in a 10 ㎕ reaction volume, using 3 ㎕ of the ligation mixture, 1 pmol each of *Mse*I + C and *Eco*RI + A preselective primers (Applied Biosystems), 0.2 mM dNTPs, 1 x PCR buffer containing 2 mM $MgCl_2$, and 2 units of *Taq* DNA polymerase (RBC Bioscience Corp., Taipei, Taiwan). The PCR was run for 29 cycles of 15 s at 94℃, 30 s at 60℃, and 1 min at 72℃, increasing the ramp time by 1 s for every extension and keeping the last extension for 2 min at 72℃. The pre-amplification product was diluted 10-fold with H_2O for selective amplification. The selective amplification was performed using a 12 ㎕ reaction volume, including 4 ㎕ of diluted pre-amplification product, a selective primer combination of 2.5 pmol of *Mse*I-primer and 0.5 pmol of *Eco*RI-primer tagged with a fluorescent dye (Applied Biosystems), 0.22 mM dNTPs, 1 × PCR buffer containing 2 mM $MgCl_2$, and 1 unit of *Taq* DNA polymerase (RBC Bioscience Corp.). Seven selective-primer combinations were used (Table 1). The PCR started with 12 cycles of 10 s at 94℃, 30 s at 65℃, decreasing by 0.7℃ for each subsequent annealing step, and 1 min at 72℃, followed by 25 cycles of 10 s at 94℃, 30 s at 56℃, and 1 min at 72℃, increasing the ramp time by 1 s for every extension and keeping the last extension for 2 min at 72℃.

A solution including 3 ㎕ of selective amplification products, 11.64 ㎕ of deionized formamide, and 0.36 ㎕ of GeneScan 500 ROX dye size standard was denatured for 5 min at 95℃, cooled on ice, and analyzed by automatic electrophoresis in the ABI 3130xl Genetic Analyzer (Applied Biosystems). We scored the peaks between 100 and 350 base pairs in GeneMapper version 4.0 (Applied Biosystems) and exported data on the peak height of all scored fragments with >100 relative fluorescence units (rfus).

Statistical analysis

Estimation of genetic diversity and analysis of molecular variance (AMOVA) were conducted using the GenAlEx program (Peakall and Smouse 2006). For Bayesian clustering, we followed Pritchard *et al*. (2000) using the STRUCTURE program, and estimated the optimal number of groups (Evanno *et al*., 2005). We estimated the parameters with a burn-in of 100,000 and a Markov Chain Monte Carlo (MCMC) repetition of 100,000 per run. The average heterozygosity within each population was estimated with the Bayesian computation, including a MCMC with a burn-in of 50,000 and a sampling run of 250,000 iterations using the Hickory program (Holsinger *et al*., 2002). Population-specific Wright's *F* statistics for each population were estimated with Approximate Bayesian Computation using the ABC4F program (Foll *et al*., 2008).

Results

Genetic diversity of *Cephalotaxus koreana*

A total 194 polymorphic amplicons were harvested from 429 individual trees in 16 populations using seven combinations of AFLP primer-restriction enzymes. By adding 30 samples of one *Torreya nucifera* population, we obtained 208 polymorphic amplicons. The average number of effective alleles and the percentage of polymorphic loci were 1.373 and 79.4%, respectively (Table 2). The average Shannon's diversity index (*I*) and the expected heterozygosity were 0.344 and 0.224, respectively. The genetic diversity (*I*) of *Chephalotaxus* was lower than that of *Tilia amurensis* (0.416; KFRI, 2013), using AFLP, and *Taxus cuspidata* (0.478; Kwon and Kim, 2002) and *Torreya nucifera* (0.353; Hong *et al*., 2000) using inter-simple sequence repeats (ISSRs) in South Korea. The genetic diversity was higher in Danyang, Jinan, and Youngkwang, and lowest in Gwacheon (Table 2). However, using Baysian inference, the expected heterozygosity was higher in Hwasoon, Gwacheon, and Danyang. The average inbreeding coefficient within populations was as high as 0.861, and that in Wando was exceptionally low, 0.315.

Table 2. Genetic diversity index with AFLP markers of *Cephalotaxus koreana* populations in Korea[z]

Population	*A*e	*I*	*He*	%*P*	*hs*	PS-F_{IS}	PS-F_{ST}
Gwacheon	1.199 (0.021)	0.201 (0.017)	0.125 (0.012)	53.85%	0.296 (0.004)	0.953 (0.893 ~ 1.000)	0.753 (0.587 ~ 0.947)
Danyang	1.537 (0.028)	0.435 (0.019)	0.298 (0.014)	79.81%	0.279 (0.004)	0.855 (0.704 ~ 1.000)	0.329 (0.252 ~ 0.405)
Geumsan	1.435 (0.023)	0.400 (0.017)	0.263 (0.012)	82.21%	0.249 (0.004)	0.977 (0.950 ~ 1.000)	0.096 (0.044 ~ 0.144)
Dalseong	1.354 (0.025)	0.333 (0.018)	0.215 (0.013)	75.00%	0.294 (0.004)	0.993 (0.985 ~ 1.000)	0.121 (0.074 ~ 0.166)
Daegu	1.374 (0.027)	0.328 (0.019)	0.217 (0.014)	67.31%	0.265 (0.005)	0.976 (0.945 ~ 1.000)	0.340 (0.262 ~ 0.419)
Mungyeong	1.342 (0.022)	0.333 (0.017)	0.214 (0.012)	75.48%	0.205 (0.007)	0.980 (0.955 ~ 1.000)	0.135 (0.071 ~ 0.196)
Geochang	1.324 (0.021)	0.325 (0.017)	0.206 (0.012)	77.40%	0.274 (0.004)	0.998 (0.996 ~ 1.000)	0.104 (0.055 ~ 0.147)
Hapcheon	1.294 (0.021)	0.298 (0.017)	0.188 (0.012)	73.08%	0.264 (0.004)	0.997 (0.993 ~ 1.000)	0.172 (0.107 ~ 0.234)
Yeonggwang	1.504 (0.028)	0.418 (0.019)	0.283 (0.014)	83.17%	0.259 (0.005)	0.889 (0.756 ~ 1.000)	0.210 (0.150 ~ 0.272)
Jangseong	1.349 (0.023)	0.335 (0.018)	0.216 (0.012)	75.00%	0.273 (0.004)	0.977 (0.947 ~ 1.000)	0.151 (0.084 ~ 0.207)
Haenam	1.281 (0.019)	0.303 (0.015)	0.187 (0.011)	78.37%	0.217 (0.005)	0.999 (0.997 ~ 1.000)	0.076 (0.044 ~ 0.105)
Jinan	1.505 (0.027)	0.426 (0.018)	0.287 (0.013)	82.69%	0.265 (0.004)	0.952 (0.894 ~ 1.000)	0.155 (0.094 ~ 0.207)
Kwangyang	1.301 (0.020)	0.307 (0.017)	0.195 (0.012)	71.15%	0.270 (0.006)	0.989 (0.975 ~ 1.000)	0.239 (0.173 ~ 0.302)
Gurye	1.273 (0.022)	0.283 (0.016)	0.174 (0.011)	78.85%	0.274 (0.005)	0.997 (0.993 ~ 1.000)	0.338 (0.253 ~ 0.410)
Hwasun	1.442 (0.026)	0.383 (0.019)	0.257 (0.014)	74.04%	0.303 (0.004)	0.783 (0.579 ~ 1.000)	0.552 (0.414 ~ 0.729)
Wando	1.461 (0.026)	0.392 (0.019)	0.265 (0.014)	71.63%	0.271 (0.006)	0.315 (0.039 ~ 0.616)	0.583 (0.436 ~ 0.762)
Mean	1.373 (0.006)	0.344 (0.005)	0.224 (0.003)	74.94%	0.266	0.861	0.256

[z]*A*e = number of effective alleles, *I* = Shannon's diversity index, *He* = expected heterozygosity, and %*P* = percentage of polymorphic loci. Population heterozygosity (*hs*) and population-specific F_{IS} and F_{ST} estimated by Bayesian methods. The values in parenthesis were the standard deviation and the highest posterior density interval at 90% credible level.

Genetic relatedness between *Cephalotaxus* populations

We divided the 16 populations into four groups based on their genetic relationships in the unweighted pair group method with arithmetic mean (UPGMA) dendrogram, putting the *Torreya nucifera* population on Jeju Island as an outgroup (Fig. 1). Its criterion was a genetic distance of 0.07. This grouping pattern was the same as that of the principal component analysis (PCA) (Fig. 2). The first and the second principal components in the PCA scatterplot explained 84.0% of the total genetic variation. The first axis (the 1st PC) explained 66% of the variation, and it classified three groups (Group I, Group IV, and the others), and the second axis explained 18% of the variation, and divided the others into Group II and Group III. The grouping supported 11.3% of the total genetic variation in the hierarchical AMOVA, 5.5% was among-population variation, and 83.2% was within-population variation. The genetic differentiation did not correlate with the geographic distance between populations (Mantel's r =

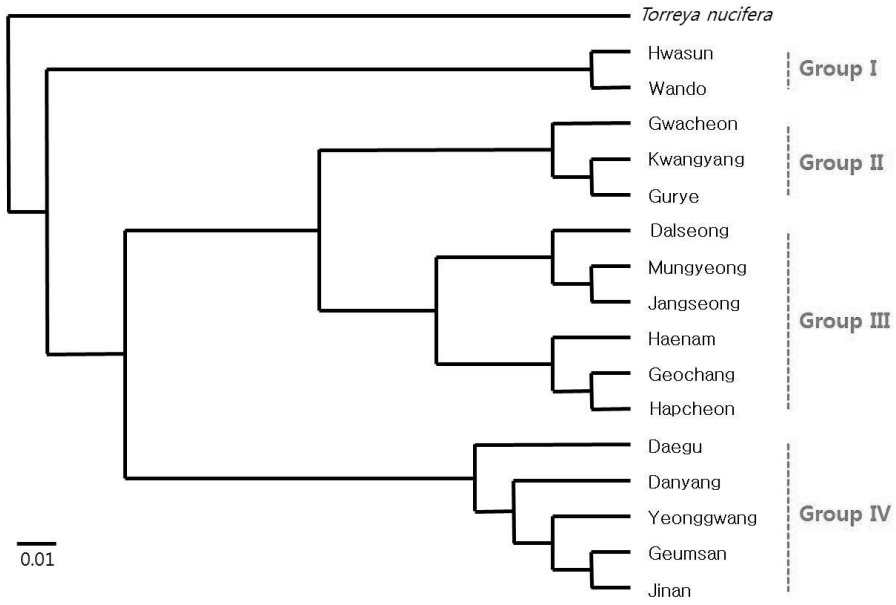

Fig. 1. Dendrogram based on Nei's genetic distance by UPGMA method of *Cephalotaxus koreana* populations in Korea.

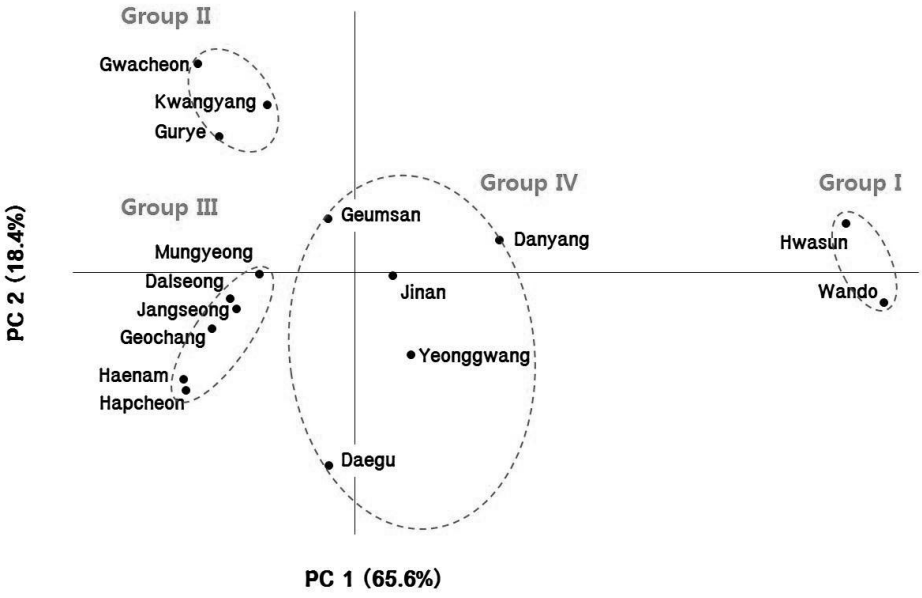

Fig. 2. Biplot from principal component analysis (PCA) with the genetic distance matrix of *Cephalotaxus koreana* populations using AFLP markers in Korea. The 1st principal component (PC 1) and the 2nd (PC 2) explained 65.6% and 18.4% of total variation, respectively.

0.208, P > 0.10), and this was also confirmed from the results of the cluster analysis (UPGMA) and the PCA.

Bayesian clustering was conducted under Hardy-Weinberg equilibrium and the linkage equilibrium for 16 populations. With the optimal number of populations (K = 2) by Evanno *et al*. (2005) method, one group was represented by Wando, Hawsoon, etc., and the other included Gwacheon, Gurye, etc. (Table 3). We could not find any biogeographical trend in the discrimination of the groups because the proximal populations were showing similar genetic architecture, such as the results of the clustering analysis (Fig. 1). The optimal number of populations using the STRUCTURE program represents the

Table 3. Posterior distribution probabilities for each population from Bayesian clustering with the given number of clusters (K) using STRUCTURE (Pritchard *et al*., 2000) of *Cephalotaxus koreana* populations in Korea

Population	K = 2		K = 4				
	CL[z] I	CL II	CL I	CL II	CL III	CL IV	SD[y]
Gwacheon	0.091	0.909	0.090	0.766	0.058	0.086	0.463
Danyang	0.765	0.235	0.718	0.172	0.067	0.043	0.435
Geumsan	0.467	0.533	0.434	0.291	0.140	0.135	0.317
Dalseong	0.308	0.692	0.240	0.364	0.178	0.218	0.211
Daegu	0.556	0.444	0.418	0.054	0.083	0.444	0.205
Mungyeong	0.444	0.556	0.386	0.273	0.046	0.295	0.142
Geochang	0.350	0.650	0.300	0.169	0.173	0.358	0.210
Hapcheon	0.302	0.698	0.208	0.153	0.244	0.395	0.145
Yeonggwang	0.667	0.333	0.556	0.185	0.185	0.074	0.232
Jangseong	0.377	0.623	0.311	0.304	0.130	0.256	0.084
Haenam	0.299	0.701	0.227	0.084	0.251	0.439	0.094
Jinan	0.621	0.379	0.553	0.193	0.106	0.147	0.080
Kwangyang	0.350	0.650	0.320	0.543	0.022	0.115	0.146
Gurye	0.183	0.817	0.160	0.498	0.058	0.285	0.104
Hwasun	0.916	0.084	0.902	0.035	0.019	0.044	0.189
Wando	0.952	0.048	0.944	0.002	0.051	0.002	0.344
Mean	0.478	0.522	0.423	0.255	0.113	0.209	0.130

[z]CL = cluster, [y]SD = standard deviation.

number of groups at the highest level for the given data; it is not a measure to designate biological populations themselves (Pritchard *et al*., 2000; Evanno *et al*., 2005). Occasionally, we needed to adjust the number according to a reasonable explanation. Setting the number to K = 4, which showed the second highest ΔK (data not shown) and was also the same as the number of groups in the clustering analyses (Fig. 1 and 2), we reallocated the 16 populations and counted the proportion of inferred ancestry of an individual (q). As a result, the average proportion ($\bar{q}$) for Cluster I was the highest, 0.423, and Wando and Hawsoon were the representatives (Table 3). The genotypes of Cluster II ($\bar{q}$ = 0.255) and Cluster III (0.113) were abundant in Gwacheon and in Haenam, respectively. The frequencies of the genotypes of Cluster IV (0.209) were relatively high in Daegu and Hapcheon. Additionally, the four genotypes were evenly distributed in Jangsung, Haenam, and Jinan (SD < 0.1).

Population genetic differentiation of *Cephalotaxus*

The genetic differences between populations explained 14% of the total genetic variation and the remaining 86% came from differences between individuals within populations, as assessed by AMOVA for the 16 populations of *C. koreana* (Table 4). The genetic differentiation of *C. koreana* populations (Φ_{ST} = 0.139) was higher than that of *Tilia amurensis* (Φ_{ST} = 0.105; KFRI, 2013), using AFLP, and those of *Taxus cuspidata* (Φ_{ST} = 0.092; Kwon and Kim, 2002) and *Torreya nucifera* (Φ_{ST} = 0.094; Hong *et al*., 2000) using ISSR in Korea. The distribution of genetic variation in *C. koreana* populations corresponded to the general trend that shrub species have a higher total genetic diversity and lower population genetic differentiation than tall tree species (Nybom and Bartish, 2000).

Table 4. Distribution of genetic variations in *Cephalotaxus koreana* populations in Korea from Analysis of Molecular Variance (AMOVA)

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variance
Among populations	15	2584.0	5.25	13.9%
Within populations	411	13389.9	32.58	86.1%
Total	426	15973.9	37.83	100.0%

Table 5. Coefficients of inbreeding (f) and genetic differentiation (θ^{I} and θ^{II}), and DIC statistics calculating under four alternative models in Bayesian approach (Holsinger *et al*., 2002) of *Cephalotaxus koreana* populations in Korea

Model	f	θ^{I}	θ^{II}	$\overline{D}$	$\hat{D}$	pD	DIC
full	0.977 (0.023)[z]	0.406 (0.016)	0.172 (0.005)	10711.9	8715.5	1996.4	12708.4
$f = 0$	-	0.304 (0.015)	0.118 (0.004)	10711.8	8653.0	2085.8	12770.6
$\theta = 0$	0.977 (0.022)	0.330 (0.020)	-	22220.9	22034.2	186.7	22407.5
f free	0.502 (0.292)	0.379 (0.026)	0.174 (0.014)	11287.2	8758.2	2529.0	13816.2

[z]Standard deviation in parenthesis.

We tested four alternative models to calculate the degree of population genetic differentiation and inbreeding coefficients using a Bayesian approach (Holsinger *et al*., 2002). The model adequacies between the full model and the $f = 0$ model were very similar, but the approximate number of parameters was smaller in the full model than in the $f = 0$ model (Table 5). Additionally, the device information criterion (DIC) of the full model was the smallest. Thus, we chose the full model as the optimum, which assumed both inbreeding and population differentiation. The Bayesian statistics, which are comparable to Wright's F_{ST} and Nei's G_{ST}, were $\theta^{I} = 0.406$ and $\theta^{II} = 0.172$, respectively (Table 5). The average inbreeding coefficient (f) for the total population was 0.977, which was higher than the estimate produced by the Approximate Bayesian Computation (ABC) method (f = 0.861; Table 2). The quantities of information from the data evenly supported the values of the inbreeding coefficient (I_E = 2.783 for f) and population differentiation (I_E = 2.745 for θ^{I}). The average population-specific genetic differentiation (0.256) was lower than that of the total population ($\theta^{I} = 0.406$), but the population-specific genetic differentiation in Gwacheon, Hawsoon, and Wando were very high (Table 3).

Discussion

Population genetic differentiation of *C. koreana*

Cephalotaxus species are long-lived conifers in East Asia, and one species, or some subspecies, in Korea have been classified. *C. koreana* is a shade-tolerant shrub growing at the base of mountains in central and southern Korea. This is a wind-pollinated, dioecious species, and it is mainly outcrossed. Having an edible seed aril, seed dispersal is expected to occur via the activities of rodents or birds (Tripp, 1995). *C. harringtonia* var. *nana* in the lower crown, together with *Fraxinus lanuginosa* in the upper crown grows from the middle to the late succession stage in Japan (Nakagoshi and Wada, 1990). Additionally, one of six main vegetation types at the Baedudaegan in Korea is composed of *C. koreana* and *F. mandshurica* (Cho *et al*., 2004). Based on this information, we could compare the genetic parameters of *C. koreana* with those of plants (Nybom, 2004). The amount of genetic diversity of *C. koreana* populations using AFLP (He = 0.224) was slightly lower, and the strength of genetic differentiation ($\Phi_{ST} = 0.139$, $G_{ST} = 0.186$) was less than half the average, than those plants having a similar life history using arbitrary marker systems: long-lived perennials (He = 0.25, $\Phi_{ST} = 0.25$, $G_{ST} = 0.19$), outcrossing plants (He = 0.27, $\Phi_{ST} = 0.27$, $G_{ST} = 0.22$), seed dispersal by animal herbivores (He = 0.24, $\Phi_{ST} = 0.27$, $G_{ST} = 0.16$), and mid-successional mid (He = 0.21, $\Phi_{ST} = 0.39$, $G_{ST} = 0.27$) or late-successional (He = 0.30, $\Phi_{ST} = 0.23$, $G_{ST} = 0.22$) stages (Nybom, 2004).

The congener species of *C. koreana* were studied mainly in China. Both genetic diversity (He= 0.338, I = 0.530) and differentiation (Φ_{ST} = 0.259, G_{ST} = 0.251) by ISSR in *C. fortuneii* populations (Li *et al*., 2008) were higher than those in *C. koreana*. Those of *C. mannii* (He = 0.135, $G_{ST} = 0.123$; Xiang *et al*., 2001) and *C. oliveri* (He = 0.118; Chen *et al*., 2003), as assessed by isozymes, were lower, but the genetic differentiation of *C. mannii* ($\Phi_{ST} = 0.149$; Du *et al*., 2002) by RAPD was higher than in this study. As a few studies applied

different marker systems to the same samples, the level of genetic diversity using AFLP was similar to those of RAPD or ISSR, and it was higher than that of isozyme (Nybom and Bartish, 2000; Nybom, 2004). The genetic differentiations by isozyme, RAPD, or AFLP showed similar values, and were lower than that by ISSR.

Cephalotaxaceae and Taxacea, including the genera *Taxus* and *Torreya*, differentiated during the Jurassic period (149-179 myr ago) of the Mesozoic era (Cheng *et al*., 2000). Subsequently, both *Taxus* and *Torreya*, which are relatively closer than *Cephalotaxus*, went through similar evolutionary histories, which might have affected the genetic differentiation of their populations. In this study, *C. koreana* had a lower genetic diversity and greater genetic differentiation than those of *Taxus cuspidata* ($He = 0.316$, $I = 0.478$, $\Phi_{ST} = 0.092$) growing on mountaintops in Korea, as assessed by ISSR (Kwon and Kim, 2002). *Torreya nucifera* growing at the same sites as *C. koreana* showed a very similar level of genetic diversity ($I = 0.353$), but weaker genetic differentiation ($\Phi_{ST} = 0.094$) than *C. koreana* (Hong *et al*., 2000). *C. koreana* is a shrub that grows widely in central and southern Korea, but the tall-tree species (*T. cuspidata* and *T. nucifera*) are distributed narrowly (Lee, 1974; Chang *et al*., 2011). *T. cuspidata* was designated as a vulnerable species by the Korea Forest Service, and it grows mostly on mountaintops over 1,000 m above sea level. *T. nucifera* trees are distributed partly in temperate and warm-temperate regions below 35 degrees of latitude in Korea, and some of its natural habitats are severely threatened (Baek *et al*., 2013). Although geographic range did not have a significant effect on genetic diversity in arbitrary marker studies, endemic taxa showed somewhat lower values than narrow, regional, and widespread taxa (Nybom, 2004). The within-population genetic diversity of *C. koreana* was lower than those of *T. cuspidata* or *T. nucifera* in Korea, and this is in agreement with the common trends (Hamrick *et al*., 1992). However, the among-population genetic diversity of *C. koreana* was quite higher than both of these species, which did not follow the trends.

Speciation of the genus *Cephalotaxus* started in the Oligocene (33.88 ± 4.83 myr ago) of the Tertiary period (Hao *et al*., 2008), and fossils from the Miocene were found in the northern part of the Korean Peninsula (Kong, 1995). The molecular phylogeny of *Cephalotaxus* species showed a speciation process according to their ancestral distribution areas (Hao *et al*., 2008). Thus, we assumed that *C. koreana* had distributed widely in the Korean Peninsula, and then the distribution area shrank due to species competition and the cold climate after the last glacial maximum period (18,000 yr ago) in the Quaternary period. Currently, it is growing in parts of the temperate and warm-temperate regions (Kong, 2004). *Cephalotaxus* species seemed to have experienced a genetic bottleneck, which could have resulted in its high degree of heterozygosity. If this happened, a large bias in the fixation index could occur using AFLP, which was a dominant marker with disparate allele frequencies between the dominant ($p = 0.748$) and the recessive ($q = 0.252$) alleles in this study. Moreover, *Cephalotaxus* species are outcrossed conifers; thus, the fixation index of 0.977 (Table 2) was too high, which might not make biological sense.

Population-based F_{ST} values by Approximate Bayesian Computation (Holsinger *et al*., 2002) indicated a similar level of genetic divergence for most populations, except for those of Wando, Hwasun, and Gwacheon, which had significantly higher population-specific F_{ST} values than the others. Wando, having only tall tree-type individuals, had a very low inbreeding coefficient and a high population-specific differentiation value (Table 1). Hwasoon, having both a shrub type and a tall tree type, and Gwacheon, with the northernmost population, had high population-specific differentiation, but not interbreeding values. In Korea, taxonomists once classified trees of the genus *Cephalotaxus* in Korea into one, two, or three species. A tall tree-type was classified as *C. harringtonia* ('Keun-Gae-Bi-Za-Na-Mu' in Korean; KPNI Committee, 2008), and the key traits of *C. harringtonia* var. *nana* ('Nun-Gae-Bi-Za-Na-Mu' in Korean) were presumed to be the sexual characteristics of individual trees (Lee, 1974). However, tall tree-typed plum yews were commonly growing with the shrub type (Table 1), and we could not find any genetic difference between the tall- and the shrub-typed trees in the study (data not shown). There has been no scientific consensus regarding the morphological description of the genus *Cephalotaxus* (Chang *et al*., 2011; Lang *et al*., 2011, 2013), and we did not have any conviction to re-classify the samples into other species other than *C. koreana*. Thus, we identified the samples as one species, *C.*

koreana, following Lee (1974).

Genetic conservation of *C. koreana*

There needs to be conservation activities not only for rare species, but also for common species, as common species are frequently experiencing population declines due to habitat destruction, fragmentation, climate change, and ill-managed restoration efforts (Kramer and Havens, 2009). Most species in the genus *Cephalotaxus* have an economic value due to their medicinal activities, but their numbers have declined, mostly due to human disturbances (Farjon *et al*., 1993). Additionally, *C. koreana* is in a similar situation (Oh *et al*., 2011). Although we did not ascertain the correlation between HTT contents and genetic diversity in this study (data not shown), the HTT contents are significantly different in each population (Jung *et al*., 2005). Furthermore, *C. koreana* is a subsidiary member of the six main vegetation types in the Baekdudaegan Mountains of Korea; therefore, its genetic resources should be protected through *in situ* conservation actions.

Based on the inference that high genetic diversity may commonly increase the fitness of a population (Jump *et al*., 2008; Pertoldi *et al*., 2007), the information on population genetic structure can help to set up an *in situ* conservation plan for the genetic resources of *Cephalotaxus* species. We divided 16 populations into four groups according to their genetic relationship shown in the dendrogram (Fig. 1) and the PCA plot (Fig. 2), and we chose candidate forests for *in situ* conservation from every group, considering the distribution of genetic diversity. The genetic diversity of *C. koreana* populations as a whole was high in Danyan, Jinan, Youngkwang, and Geumsan (Table 2), but these populations belonged to the same group. Thus, it would be better to choose one population per group, with a priority for *in situ* conservation by taking 'local adaptation (Leimu and Fischer, 2008)' into consideration. On the basis of the groups, Wando in Group I, Kangyang in Group II, Jangseong in Group III, and Danyang in Group IV had the highest values of genetic diversity in each group. Wando and Danyang showed a high proportion of Cluster I genotypes (Table 4). Kwangyang had a high proportion of Cluster II genotypes, and the genotypes of Jangseong were distributed quite evenly among the four clusters based on the Bayesian inference results (Table 3). Because protected areas are designated by law, we should also know their forest management states, including social information on ownership, potential risks, cooperation with villagers, etc. (Wilshusen *et al*., 2002). Because the populations in Danyang, Jangseong, and Wando are protected via the designation of natural parks or natural monuments (Table 1), the highest priority might be given to the Kwangyang population in Group II.

We should consider not only genetic information, but also demographic approaches, for the conservation of a target species (Oosetermeijer *et al*., 2003). Although *C. koreana* is distributed along the Baeckdudaegan mountain ridge (Cho *et al*., 2004), the number of individuals has been decreasing locally. We could collect only 11 individuals in the Gwacheon population, which is known as the northernmost population in Korea (Table 1), from several field surveys. One needs to consider the *ex situ* conservation of the population in Mt. Gwanak because its genetic architecture was unique (population-specific F_{ST} = 0.753; Table 3), and the population size was extremely smaller than the others.

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