Journal of Life Science 2006 Vol. 16. No. 5. 740~745

Comparison of Genetic Diversity and Relationships of Genus *Kalopanax* Using ISSR Markers

Man Kyu Huh*

Department Molecular Biology, Dong-eui University, Busan 614-714, Korea

Received April 17, 2006 / Accepted July 25, 2006

Inter simple sequence repeat (ISSR) markers were performed in order to analyse the phylogenetic relationships of four taxa of Castor-aralia (*Kalopanax pictus*): *K. pictus*, *K. pictus* var. *magnificus*, *K. pictus* var. *maximowiczii*, and *thornless K. pictus*. The 11 primers were produced 64 reproducible ISSR bands. Analysis of ISSR from individual plants of Korean *K. pictus* resulted in 41 polymorphic bands with 64.1%. When species were grouped by four taxa, within group diversity was 0.115 (H_S), while among group diversity was 0.467 (G_{ST}) on a per-locus basis. The estimated gene flow (*Nm*) for *K. pictus* var. *maximowiczii* and *K. pictus* var. *magnificus* were very higher than *K. pictus*. It is suggested that the isolation of geographical distance and reproductive isolation among *K. pictus* populations may have played roles in shaping the population structure of this species. In phenetic tree, ISSR markers are very effective in classifying natural populations as well as taxon levels of genus *Kalopanax* in Korea.

Key words – Simple sequence repeat (ISSR), *Kalopanax pictus*

Introduction

The genus *Kalopanax* (Araliaceae) consists of one species, *Kalopanax pictus* and several varieties, distributed in temperate regions of East Asia[14]. Typical populations of this genus are small and distributed in patches. *K. pictus* can be classified as a narrow habitat species as it is usually found on subsites of several mountains, where it is found at elevations of 100-1,800 m above the sea level in Korea[12]. Especially, thornless *K. pictus* variety is an endemic to Cheongsong province in Korea. One of the most striking features between both taxa was spine (or thorn) which is sharp and stiff outgrowth of a stem. *K. pictus* is covered with many spines, whereas thornless castor cultivar "Cheongsong" (thereafter var. *cheong*) do not show this trait. *K. pictus* var. *magnificus* is tomentose on lower side than on the upper side of leaves. *K. pictus* var. *maximowiczii* is more lobed leaves than other *K. pictus* taxa and has many white hairs on leaves.

Although these taxa grows mountains with fertile soil, they are extensively cultivated as medicinal plants for wind-damp impediment pain, limp legs, aching lumbus and knees, and puffy swelling of the legs[9].

Although molecular and biochemical approaches are now increasingly being applied to address the taxonomic and phylogenetic relationships within plant species in Korea, no population genetic studies of genus *Kalopanax* have been conducted. In addition, the taxonomy of laver has processed mainly through morphological characteristics and morphological analysis[11]. However, some morphological characteristics are restricted their resolving power among very closely related taxa because of the small number of variables available or ecological variation. Efficient methods to clarify the taxonomic status of several species are much needed.

In recent years, a number of PCR-based DNA markers, such as RAPD (random amplified polymorphic DNA), SSR (simple sequence repeats) and ISSR (inter simple sequence repeats), have been widely used to investigate genetic diversity and populations genetic structure[2,20,22]. ISSR primers anneal directly to simple sequence repeats and thus, unlike SSR markers, no prior knowledge of target sequences is required ISSR[7]. Also, the sequences that ISSR targets are abundant throughout the eukaryotic genome and evolve rapidly; consequently ISSR may reveal a much higher number of polymorphic fragments per primer than RAPDs[2,4]. In addition, studies have indicated that ISSRs produce more reliable and reproducible bands compared with RAPDs because of the higher annealing temperature and longer sequence of ISSR primers[19,24]. Thus, ISSRs have proved to be useful in population genetic studies, especially in detecting clonal diversity and fingerprinting closely related individuals[2].

***Corresponding author**
Tel : +82-51-890-1529, Fax : +82-51-890-1521
E-mail : mkhuh@deu.ac.kr

The objectives of this study are to estimate the level of genetic diversity in the interspecies and to detect the pattern of differentiation and speciation in Korean *Kalopanax* species using ISSR makers.

Materials and Methods

Plant materials

Leaf tissues were collected from five natural populations of *K. pictus*, one population of *K. pictus* var. *cheong*, two populations of *K. pictus* var. *magnificus* and two populations of *K. pictus* var. *maximowiczii* in Korea (Fig. 1). Typical populations of *K. pictus* var. *magnificus* and *K. pictus* var. *maximowiczii* are small and distributed in patches. In Korea, *K. pictus* trends to occur in small, isolated populations, restricted to a small number of isolated sites. I found only two natural populations of each *K. pictus* var. *magnificus* and *K. pictus* var. *maximowiczii* which maintain effective population sizes during three years (2003-2005). More than 30 plants (one leaf per plant) were sampled from each population. *Acanthopanax sessiliflorus* was used as an outgroup.

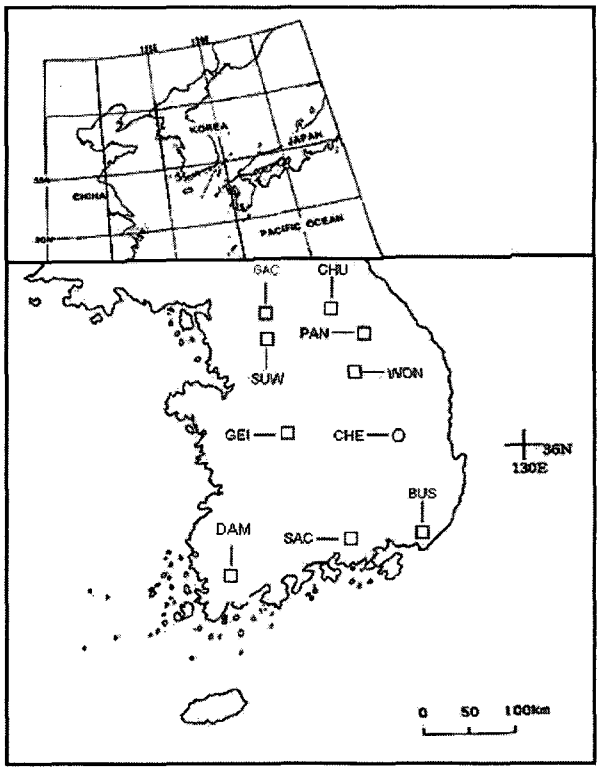

Fig. 1. Collection sites of populations for ISSR analysis. GAC: Gacheon, Gyuongki-do, CHU: Chuncheon, Gangwon-do, PAN: Paengchang, Gangwon-do, SUW: Suwon, Gyeongki-do, WON: Woenju, Gangwon-do, DAM: Damyang-gun, Cheonlanam-do, GEI: Mt. Georeoung, Chungcheongnam-do, CHE: Cheongsong-gun, Gyeongsangbuk-do, SAC: Sacheon, Gyeongsangnam-do, BUS: Kumjeong-gu, Busan-ci.

Genomic DNA isolation and ISSR analysis

DNA was extracted using the NucleoSpin Plant (Macherey-Nagel Inc., Valencienner, Germany) according to the manufacturer's protocol. To analyze the DNA of individuals, I selected eleven decamer primers that produced ISSR bands in a preliminary test. ISSR primers supplied by Bioneer Technologies Inc. were used for the analysis. The ISSR analysis was carried out using the following mixture: genomic DNA (1 ng/$\mu\ell$) 5 $\mu\ell$, primer (5 μM) 2 $\mu\ell$, dNTPs (250 μM total) 2 $\mu\ell$, *Taq*-polymerase (5 U/$\mu\ell$) 0.2 $\mu\ell$, 10× buffer 2.5 $\mu\ell$, distilled water 13.3 $\mu\ell$, for a total of 25 $\mu\ell$ reaction mixture. The *Taq*-polymerase and other reagents were purchased from BIONEER (KOREA). Amplification reactions were carried out on the DNA Thermal Cycler (BIOMETRA) subjected to an initial two minutes at 94℃, followed by 35 cycles of 30 seconds at 94℃, 30 seconds at 50℃, 60 seconds at 72℃, and a final 5 minutes at 72℃. The amplification products were separated by electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and photographed under UV light using Alpha Image TM (Alpha Innotech Co., USA).

Data analysis

All monomorphic and polymorphic ISSR bands were scored and only unambiguously scored bands were used in the analyses. Each polymorphic ISSR band was given a score of 1 for presence or 0 for absence. Several standard genetic parameters were estimated using the computer program, POPGENE ver. 1.31[25] and AMOVA[3]. The percentage of polymorphic loci (*Pp*), mean number of alleles per locus (*A*), effective number of alleles per locus (A_E), Nei's[17] gene diversity (*H*), and Shannon's Information index (*I*) were calculated with POPGENE[15].

The degree of polymorphism was quantified using phenotypic diversity[1]:

$Ho = - \sum p_i \log p_i$

where p_i is the frequency of a particular phenotype i [13].

H_O can be calculated and compared for different populations[18]. Let

$H_{POP} = 1/n \sum H_O$

be the average diversity over the n different populations and let

$H_{SP} = - \sum p \log p$

be the diversity of species calculated from the phenotypic frequencies p in all the populations considered together[18]. Then the proportion of diversity presented within populations, H_{POP}/H_{SP}, can be compared with that of between populations, $(H_{SP} - H_{POP})/ H_{SP}$.

The estimation of genetic similarity (GS) between genotypes was based on the probability that an amplified fragment from one individual will also be present in another [17]. GS = 2 x Number of shared fragment between A and B / (Number of fragment in A + Number of fragment in B). GS was converted to genetic distance (1-GS).

Nei's gene diversity formula (H_T, H_S, and G_{ST}) were used to evaluate the distribution of genetic diversity within and among populations[17]. Genetic differentiation measured by G_{ST} among populations was also calculated. Furthermore, gene flow (Nm) between the pairs of populations was calculated from G_{ST} values by $Nm = 0.5(1/G_{ST} 1)$[16].

Cluster analyses

A phylogenetic tree was constructed by the neighbor-joining (NJ) method[21] based on the genetic distance using the NEIGHBOR program in PHYLIP version 3.57[5].

Results

From the eleven decamer primers used for a preliminary ISSR analysis, all primers produced good amplification products both in quality and variability. Overall, 64 fragments were generated among the tested *K. pictus* array (Table 1). Three unique alleles which showed in one species loci were found in *K. pictus*, whereas none was specific to var. *cheong*. The presences of two and one fixed alelles specific to var. *magnificus* and var. *maximowiczii*, respectively, allow for a molecular identification of these taxa based on ISSR markers.

In a simple measure of intrapopulation variability by the percentage of polymorphic bands, PAN population exhibited the lowest variation (35.9%). GAC population showed the highest (43.8%) (Table 2). The number of alleles per locus (A) was 1.813 across all populations, ranging from 1.375 for the population with the lowest mean number of alleles to 1.438 for the population with the highest mean. The effective numbers of alleles per locus (A_E) for *K. pictus* taxa was 1.420. Mean gene diversity with in populations (H) was 0.254. The var. *maximowiczii* had the highest gene diversity (0.188), while var. *cheong* and var. *magnificus* had the lowest (0.159). Shannon's information index (I) was 0.389. Wilcoxin's signed-rank test, however, there were not significant differences for measures of genetic parameters except H ($p > 0.05$).

The phenotypic frequency of each band was calculated

Table 1. List of decamer oligonucleotide utilized as primers, their sequences, and associated fragments

No	No. Sequence (5′ to 3′)	No. of fragments
ISSR-04-01	-(AG)8G-	6
ISSR-04-02	-(CT)8G-	4
ISSR-04-03	-(CA)8RG-	3
ISSR-04-04	-(TC)8RA-	0
ISSR-04-05	-G(GA)2G(GA)2G(GA)2-	10
ISSR-04-06	-(GA)8GT-	9
ISSR-04-07	-(GA)8CG-	6
ISSR-04-08	-(GA)8TC-	7
ISSR-04-09	-GCGA(AC)8-	5
ISSR-04-10	-GCGA(CA)8-	7
ISSR-04-11	-CCGG(AC)8-	7
Total		64

Table 2. Measures of genetic variation for genus *Kalopanax*. The number of polymorphic loci (Np), percentage of polymorphism (Pp), mean number of alleles per locus (A), effective number of alleles per locus (A_E), gene diversity (H), and Shannon's information index (I)

Pop.	Np	Pp	A	A_E	H	I
K. pictus						
BUS	24	37.5	1.375	1.277	0.155	0.226
SAC	25	39.1	1.391	1.299	0.165	0.238
DAM	25	39.1	1.391	1.315	0.171	0.245
PAN	23	35.9	1.359	1.277	0.152	0.219
SUW	26	40.6	1.406	1.308	0.172	0.249
Mean	24.6	38.4	1.384	1.295	0.163	0.235
K. pictus variant						
CHE	24	37.5	1.375	1.283	0.159	0.230
K. pictus var. *maximowiczii*						
GAC	28	43.8	1.438	1.352	0.193	0.276
CHU	27	42.2	1.422	1.330	0.182	0.262
Mean	27.5	43.0	1.430	1.341	0.188	0.269
K. pictus var. *magnificus*						
GEI	24	37.5	1.375	1.283	0.157	0.228
WON	25	39.1	1.391	1.286	0.161	0.234
Mean	24.5	38.3	1.383	1.285	0.159	0.231
Total	52	81.3	1.813	1.420	0.254	0.389
SD	-	-	0.393	0.350	0.177	0.243

and used in estimating genetic diversity within populations. Total genetic diversity (H_T) of var. *cheong* (0.215) was higher than those of other taxa and showed significant difference (paired *t*-test) (Table 3). The interlocus variation of population genetic diversity (H_S) was low (0.167). Although the average number of individuals exchanged between populations per generation (*Nm*) was estimated to be moderate (0.958), there were there were significant differences for four taxa ($p < 0.05$).

An assessment of the proportion of diversity present within populations, H_{POP}/H_{SP}, indicated that about 25.5% the total genetic diversity for four *K. pictus* taxa was among populations (Table 4). Thus, the three quarters of genetic variation resided within populations.

A similarity matrix based on the proportion of shared fragments (GS) was used to establish the level of relatedness among four *K. pictus* taxa (Table 5). The estimate of GS ranged from 0.979 between var. *magnificus* and var. *maximowiczii* to 0.789 between var. *cheong* and var. *magnificus*.

Table 3. Estimates of genetic diversity of *K. pictus*. Total genetic diversity (H_T), genetic diversity within populations (H_S) proportion of total genetic diversity partitioned among populations (G_{ST}), and gene flow (*Nm*)

Taxa	H_T	H_S	G_{ST}	*Nm*
K. pictus	0.178	0.163	0.085	5.384
K. pictus variant	0.215	0.115	0.467	0.571
var. *maximowiczii*	0.194	0.188	0.036	13.500
var. *magnificus*	0.168	0.159	0.054	8.714
Total	0.254	0.167	0.343	0.958

Table 4. Partitioning of the genetic diversity into within and among populations of *K. pictus* taxa

Taxa	H_{POP}	H_{SP}	H_{POP}/H_{SP}	$(H_{SP}-H_{POP})/H_{SP}$
K. pictus	0.555	1.767	0.314	0.686
K. pictus variant	0.255	1.767	0.144	0.856
var. *maximowiczii*	0.300	1.767	0.170	0.830
var. *magnificus*	0.280	1.767	0.158	0.842
Total	0.451	1.767	0.255	0.746

Table 5. Genetic identity (above diagonal) of four *K. pictus* taxa based on ISSR and genetic distances (below diagonal) at species level

Pop.	*K. pictus*	*K. pictus* variant	var. *maximowiczii*	var. *magnificus*
K. pictus	-	0.797	0.865	0.877
K. pictus variant	0.227	-	0.795	0.789
var. *maximowiczii*	0.146	0.230	-	0.979
var. *magnificus*	0.132	0.239	0.022	-

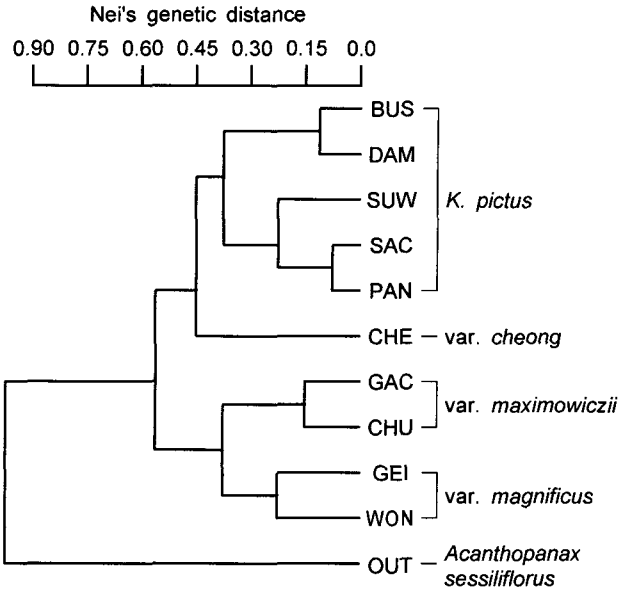

Fig. 2. A phenogram showing the relationships among ten populations of *K. pictus* taxa, based on data of genetic distance obtained by ISSR. OUT: outgroup.

Discussion

The most striking result emerging from this study is the similarity between *K. pictus* and var. *cheong*, in terms of genetic diversity and structure, although *K. pictus* was higher than those of var. *cheong*. To a large extent, the allelic composition of var. *cheong* represent subsets of *K. pictus*. For example, var. *cheong* was found to have fewer total bands (24 vs. 27), lower alleles (mean 1.375 vs. 1.384), and lower genetic diversity (mean 0.159 vs. 0.163) than *K. pictus*. A naturalized population of var. *cheong* is ultimately a product of both their biological characteristics and historical practices[8]. Var. *cheong* may be founded by a small sample of larger or moderate populations (i.e. founder effect). However, I consider that the other hypothesis still remained.

In addition, there is also the similarity between var. *magnificus* and var. *maximowiczii*. Genetic similarity (GS) value among pairs of var. *magnificus* and var. *maximowiczii* is 0.979.

Three unique alleles which showed in one species were found in *K. pictus*. Thus, a hypothesis suggests that identical spontaneous mutations in at least same contemporary times would have occurred, leading to the appearance of

var. *cheong*. Such an event is statistically highly improbable [6]. Another proposed hypothesis suggests that a recent tree introduced from China or Japan to Korea. However, the existence of several wild populations might invalidate this hypothesis.

The average percentage of polymorphic band was 43.0% for var. *maximowiczii* is higher than those of other taxa (Table 2). However, there is not significantly different from zero ($p > 0.05$). *A*, A_E, and *I* among four taxa are not significantly different from zero. These genetic diversity parameters indicated that *K. pictus* variety populations were not genetically much depauperate relative to its unknown presumptive progenitor and the speciation process has eroded the level of genetic variation of this species.

Genetic differentiation among populations is principally a function of natural selection, genetic drift, and gene flow via pollen and seed dispersal[8]. The most striking feature of these results was the relatively low degree of genetic differentiation recorded among populations, compared with results obtained for other woody species. For example, in a review of genetic variation in woody species based on allozyme analyses, the genetic variation in predominantly outcrossed wind-pollinated species was recorded averages <10% between populations[8]. For example, for *K. pictus*, about 8.5% of the total variation *K. pictus* was due to differences among populations (G_{ST} = 0.085). This low level of genetic differentiation also suggests that gene flow among the population is high (N_m = 5.38). The gene flow of var. *maximowiczii* and var. *magnificus* is higher than that of *K. pictus*. The correlation between gene flow and geographical distances is relative high in genus *Kalopanax* (Table 3). The gene flow of *K. pictus* may be explained in plant by the information about seed and pollen dispersal. For example, the periods of fruit maturation of *K. pictus* is from late October to early November, and matured fruits are transported by birds and rodents[10]. Fruiting of *K. pictus* is exceptionally low event that probably occurs one or two seeds in a drupe. Therefore, most populations have small population sizes and isolated each other.

Although I did not analyze further subdivision of a local population, I may infer that ISSR variation that resided mainly within *K. pictus* populations is maintained in patchily distributed subpopulations or demes, either by random drift of neutral alleles or micro- environmental selection for adaptive alleles. However, no great local differentiation of ISSR variation was observed.

In combination with random mating and low seed to juvenile survivorship, any amount of seed dispersal, even very short dispersal, could for any remaining reduction in near neighbor genetic structure between reproductive and juvenile stages.

In a phenetic tree based on allozyme variability, the position of the populations in the NJ tree and their geographical position did not almost completely matched in the Korean populations[10]. However, it is relevant to stress that ISSR markers which used allowed us to discriminate among all populations, even those that could not be distinguished on the basis of allozyme analysis. Thus, ISSR markers are very effective in classifying wild populations of genus *Kalopanax* in Korea.

Based on the available data, such as relatively high G_{ST} value, several populations of each group should be preserved, especially those with high variation. These populations could be used as a source of genetic diversity for the restoration of genetically poor populations[20]. In addition, I recommend that a desirable conservation population should be included at least 30 plants per population because high genetic diversity is observed with increasing population sizes (data not shown). It is assumed that if sufficient habitat is maintained to protect against environmental stochasticity, loss of genetic diversity is not an important concern[23]. However, I have failed to detect large actual populations except only two populations for var. *maximowiczii* and two populations var. *magnificus* examined in this study. Thus, these wild populations are imminent danger of becoming extinct if protection is not provided.

References

1. Bowman, K. D., K. Hutcheson, E. P. Odum and L. R. Shenton. 1971. Comments on the distribution of indices of diversity. *Stat. Ecol.* **3**, 315-359.
2. Esselman, E., J. L. Jiangquiang, D. J. Crawford, J. L. Winduss and A. D. Wolfe. 1999. Clonal diversity in the rare *Calamagrosis porteri* ssp. *insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and their simple sequence repeat (ISSR) markers. *Mol. Ecol.* **8**, 443-451.
3. Excoffier, L., P. E. Smouse and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondrial DNA restriction data. *Genetics* **131**, 479-491.
4. Fang, D. Q. and M. L. Roose. 1997. Identification of closely related citris cultivars with inter-simple sequence

markers. *Theor. Appl. Genet.* **95**, 408-417.
5. Felsenstein, J. 1993. PHYLIP (Plyogeny Inference Package) ver. 3.5s. Distributed by the author. Univ. Washington, Seattle, USA. http:// evolution.genetics.washington.edu/ phylip.
6. Gallois, A., J. C. Audran and M. Burrus. 1998. Assessment of genetic relation ships and population discrimination among *Fagus sylvatica* L. by RAPD. *Theor. Appl. Genet.* **97**, 211-219.
7. Godwin, I. D., E. A. B. Aiken and L. W. Smith. 1997. Application of inter simple sequence repeat (ISSR) markers to plant genetics. *Electrophoresis* **18**, 1524-1528.
8. Hagen, M. J. and J. L. Hamrick. 1998. Genetic variation and population genetic structure in *Trifolium pratense*. *J. Hered.* **89**, 178-181.
9. Jiao, S. D. 2003. *Ten Lectures of the Use of Medicinals*. pp. 711. Paradigm Publications, MA, USA.
10. Jung, S. D., H. W. Huh, J. H. Hong, J. S. Choi. H. S. Chun, K. H. Bang and M. K. Huh. 2003. Genetic diversity and population structure of *Kalopanax pictus* (Araliaceae). *J. Plant Biology* **46**, 255-262.
11. Jung, S. D., J. S. Sung and M. K. Huh. 2005. The study of the root cutting propagation of *Kalopanax pictus* Nakai. *J. Life Sci.* **15**, 21-26.
12. Kim, S. H., H. G. Chung and J. S. Chang. 2004. Characteristics and breeding of a new thornless castor Aralia cultivar "Cheongsong 2". *Plant Res.* **17**, 123-126.
13. King, L. M. and B. A. Schaal. 1989. Ribosomal DNA variation and distribution of *Rudbeckia missouriensis*. *Evolution* **42**, 1117-1119.
14. Lee, T. B. 1979. Illustrated Flora of Korea. Hyangmoonsa.
15. Lewontin, R. C. 1972. The apportionment of human diversity. *Evol. Biol.* **61**, 381-398.
16. McDermott, J. M. and B. A. McDonald. 1993. Gene flow in plant pathosystems. *Ann. Rev. Phytopathy.* **311**, 353-373.
17. Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA.* **701**, 3321-3323.
18. Paul, S. P., F. N. Wachira, W. Powell and R. Waugh. 1997. Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) O. Kuntze) revealed by AFLP markers. *Theor. Appl. Genet.* **941**, 255-263.
19. Qian, W., S. Ge and D. Y. Hong. 2001. Genetic variation within and among populations of a wild rice *Oryza granulata* from China detected by RAPD and ISSR markers. *Theor. Appl. Genet.* **102**, 440-449.
20. Rossetto, M., G. Jezeierski, S. D. Hopper and K. W. Dixon. 1999. Conservation genetics and clonality in two critically endangered eucalypts from the highly endemic south-western Australian flora. Biological *Conservation* **88**, 321-331.
21. Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **41**, 406-425.
22. Tani, N., N. Tomaru, Y. Tsumura, M. Araki and K. Ohba. 1998. Genetic structure within a Japanese stone pine (*Pinus pumola* Regal) population on Mt. Aino-dake in Central Honshu, Japan. *J. of Plant Research* **111**, 7-15.
23. Templeton, A. R., K. Shaw, E. Routman and S. K. Davies. 1990. The genetic consequences of habitat fragmentation. *Ann. Mo. Bot. Gard.* **771**, 13-27.
24. Tsumura, Y., K. Ohba and S. H. Strauss. 1996. Diversity and inheritance of inter-simple sequence repeat polymorphisms in douglas-fir (*Pseudotsuga menziesii*) and sugi (*Cryptonmeria japonica*). *Theor. Appl. Genet.* **92**, 40-45.
25. Yeh, F. C., R. C. Yang and T. Boyle. 1999. POPGENE version 1.31, Microsoft windows-based freeware for population genetic analysis, Edmonton, Canada.

초록 : ISSR을 이용한 음나무속 분류군의 유전적 다양성과 관련성 비교

허만규*
(동의대학교 분자생물학과)

ISSR 마크로 한국내 자생하는 음나무속 4분류군(음나무, 가시없는 음나무, 털음나무, 가는잎음나무)에 대해 유전적 다양성과 계통관계를 조사하였다. 64개의 재현성 높은 ISSR 밴드가 생성되었다. 음나무속의 각 개체별 분석에서 41개 밴드(64.1%)가 다형성을 나타내었다. 네 분류군을 통합하였을 때 그룹내 다양도는 0.115였고 그룹간 다양도는 0.467이였다. 종내 유전자 흐름(*Nm*)의 측정결과 음나무의 *Nm*값은 털음나무, 가는잎음나무에 비해 낮았다. 이는 지리적 거리에 따른 생식적 격리가 이 종의 집단구조를 형성하고 있다고 판단된다. 계통도 분석에서 ISSR 마크로 속수준의 네 분류군뿐만 아니라 집단까지도 잘 분리되어 본 연구에 사용한 마크가 분류에 효과적임이 규명되었다.