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Comparison of Genetic Diversity and Population Structure of *Kalopanax pictus* (Araliaceae) and its Thornless Variant Using RAPD

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ABSTRACT : *Kalopanax pictus* is a long-lived woody species mostly distributed in East Asia. *K. pictus* has been regarded as medically and ecologically important species in Korea. Thornless castor aralia variant, local name "Cheongsong" is an endemic to Cheongsong province in Korea. Random amplified polymorphic DNA (RAPD) was used to investigate the genetic variation and structure of Korean populations of two species. A high level of genetic variation was found in six *K. pictus* populations. Twelve primers revealed 49 loci, of which 29 were polymorphic (59.2%). Nei's gene diversity for *K. pictus* and *K. pictus* variant were 0.119 and 0.098, respectively. Mean of genetic diversity in *K. pictus* was higher than average values for species with similar life history traits. The asexual and sexual reproduction, perennial habitat, and longevity are proposed as possible factors contributing to high genetic diversity. An indirect estimate of the number of migrants per generation (*Nm* = 0.857) indicated that gene flow was not extensive among Korean populations of *K. pictus*. It is suggested that the isolation of geographical distance and reproductive isolation between *K. pictus* and *K. pictus* variant populations may have played roles in shaping the population structure of this species.

Key words : *Kalopanax pictus*, thornless castor aralia, genetic variation, genetic structure

INTRODUCTION

It is now widely appreciated that information of genetic variation and population structure is of critical importance to the conservation of threatened taxa (Holsinger & Gottlieb, 1991; Allnutt *et al.*, 2003). Genetic analyses can provide valuable insights into the process influencing extinction (Allnutt *et al.*, 2003) and genetic data are used to define units for conservation management and for inferring changes in population structure and dynamics (Moritz, 1995; Newton *et al.*, 1999).

The genus *Kalopanax* (Araliaceae) consists of one species, *Kalopanax pictus* and several varieties, distributed in temperate regions of East Asia (Lee, 1993). 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. Especially, *K. pictus* variant 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" (therefore *K. pictus* variant) does not this trait. In addition, castor cultivar has a thinner outer-bark (8.82 ㎜) when compared with thorned type trees (13.95 ㎜) (Kim *et al.*, 2002, 2004).

The species has been shown to be androgynous, long-lived perennial, and insect-pollinated with predominantly out-crossing (Lee, 1993). The species is diploid (2n = 48) with yellowish green flowers (Sun *et al.*, 1988; Kim *et al.*, 2004). Although this species grows high mountains with fertile soil, it is also extensively cultivated as a medicinal plant for muscle relaxant. *K. pictus* is also an economically important species in Korea, as the stems have been used for valuable household goods. The barks and roots are important in medicinal practice. In addition, until past several years, most parts of Korean forests had been disturbed by cutting trees and shrubs for firewood in rural areas (Huh, 1999; Huh & Huh, 2001). Now most of Korean forests are revegetated naturally and artificially. Despite the importance of knowledge concerning genetic variation for providing information for conservation purposes, detailed studies of the levels and distribution of genetic variation as well as population structure are not available for most woody taxa in Korea (Huh & Huh, 2001).

Random amplified polymorphic DNA (RAPD) has been

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useful in determining genetic relationships among closely related species (Beebe *et al.*, 2000). RAPD analysis is quick, robust, requires minimal preliminary work (Stewart & Excoffier, 1996). We expected that the RAPD analyses assess the amount and structure of genetic diversity within and among populations and we successfully assess the genetic relationships among the local populations of genus *Kalopanax* in Korea.

The objectives of study are to estimate the level of genetic diversity in the interspecies and are it possible to detect the pattern of differentiation and speciation in Korean *Kalopanax* species using RAPD makers? In addition, we compared the genetic diversity and structure of both species, and plant species having similar life-history characteristics.

MATERIALS AND METHODS

Plant materials

Leaf tissues were collected from five natural populations of *K. pictus* and one natural population of *K. pictus* variant (castor aralia) in Korea (Fig. 1). Detailed information about location for natural populations is not provided because of the sensitive nature of the populations (site information is available from the authors upon request for scientific study). In Korea, *K. pictus* trends to occur in small, isolated populations, restricted to a small number of isolated sites. We found only one natural population of *K. pictus* variant which maintains effective population sizes during five years (1999~2003). More than 26 plants (one leaf per plant) except *K. pictus* variant were sampled from each population.

Genomic DNA isolation and RAPD analysis

DNA was extracted using the plant DNA Zol Reagent (Life Technologies Inc., Grand Island, New York, USA) according to the manufacturer's protocol. Sixty arbitrarily chosen 10-mer primers, the kit D (OPD-01 to 20), of Operon Technologies (Alameda, Co.) were used. All the reactions were repeated twice and only reproducible bands were scored for analyses. To analyze the DNA of individuals, we selected the decamer primers that produced RAPD bands in both taxa in a preliminary test.

Amplification reactions were performed in 0.6 ㎖ tubes containing 25 ㎕ of the reaction buffer; 10 ㎜ Tris-HCl, pH 8.8, 50 ㎜ $MgCl_2$, 100 ㎛ each of dATP, dCTP, dGTP, dTTP, 0.2 ㎜ primer, 2.1 units Taq DNA polymerase, and 25 ng of genomic DNA. 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). A 100 bp ladder DNA marker (Pharmacia) was used in the end of for the estimation of fragment size.

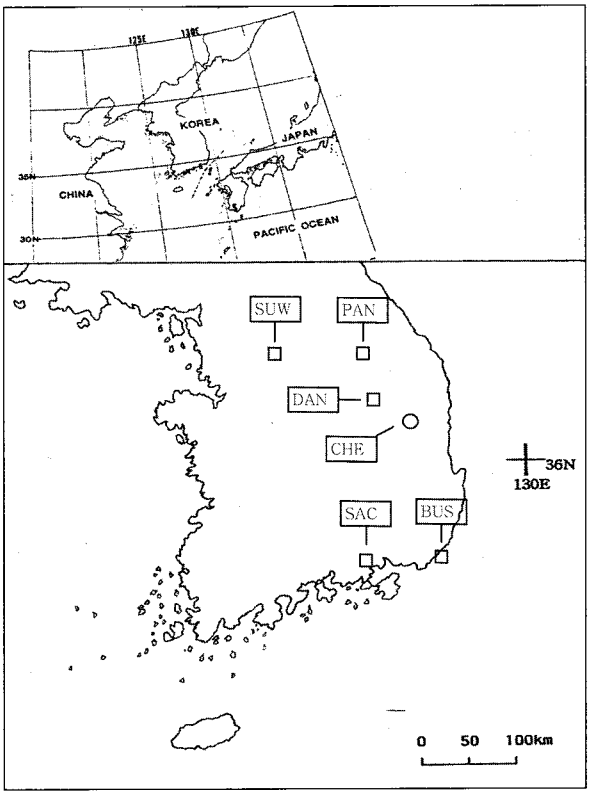

Fig. 1. Collection sites of populations for RAPD analysis. □: *K. pictus*, ○: *K. pictus variant*. PAN: Paengchang, Gangwon-do, SUW: Suwon, Gyeongki-do, DAN: Danyang-gun, Chugcheongbuk-do CHE: Cheongsong-gun, Gyeongsangbuk-do, SAC: Sacheon, Gyeongsangnam-do, BUS: Kumjeong-gu, Busan-ci.

Data analysis

All monomorphic and polymorphic RAPD bands were scored and only unambiguously scored bands were used in the analyses. Each polymorphic RAPD 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 (Yeh *et al.*, 1999). The percentage of polymorphic loci (*P*p), mean number of alleles per locus (*A*), effective number of alleles per locus (A_E), Nei's (1973) gene diversity (*H*), and Shannon's Information index (*I*) (Lewontin, 1972).

The degree of polymorphism was quantified using phenotypic diversity (Bowman *et al.*, 1971):

$$H_O = -\Sigma pi \log pi$$

where pi is the frequency of a particular phenotype *i* (King & Schaal, 1989).

H_O can be calculated and compared for different populations (Paul *et al.*, 1997). Let

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

be the average diversity over the n different species and let

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

be the diversity of species calculated from the phenotypic frequencies p in all the species considered together (Paul *et al.*, 1997). Then the proportion of diversity presented within species, H_{POP}/H_{SP}, can compared with that of between species $(H_{SP}-H_{POP})/H_{SP}$.

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 (Nei & Li 1979). 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). Homogeneity of variation among species was tested by Bartlett's statistics.

Genetic differentiation measured by G_{ST} among species was also calculated. Furthermore, gene flow (Nm) between the pairs of species was calculated from G_{ST} values by $Nm = 0.5(1/G_{ST}-1)$ (McDermott & McDonald, 1993). To elucidate the organization of the variation in *K. pictus*, genetic variation was examined by partitioning of the total genetic diversity (H_T) to within (H_S) and among (D_{ST}) population components using Nei's (1973) genetic diversity statistics. A measure of differentiation among populations, relative to the total diversity was calculated at each locus as $G_{ST} = D_{ST}/H_T$. Weir & Cockerham's (1984) estimates of Wright's F_{ST} (=G_{ST}) were computed for variable loci with FSTAT ver. 1.2 (Goudet, 1995).

A genetic distance matrix was used to construct a dendrogram with the unweighted pair group method with arithmetic average (UPGMA) method in the neighbor algorithm of the Phylogeny Inference Package (PHYLIP ver. 3.57; Felsenstein, 1993).

RESULTS

A high level of genetic variation was found in the six *K. pictus* populations. Twenty-nine of 49 loci (59.2%) showed polymorphism in at least one population, while the remaining 20 loci were monomorphic in all populations. An average of 59.2% of the loci was polymorphic within populations, with individual-population values ranging from 22.5 to 32.7% (Table 1).

Across populations, the average number of alleles per locus (A) was 1.592, varying from 1.225 for the population with the lowest number of alleles to 1.327 for that with the highest number (Table 1). The effective numbers of alleles per locus (A_E) for *K. pictus* and *K. pictus* variant were 1.212 and 1.162, respectively. Mean gene diversity within populations for *K. pictus* and *K. pictus* variant were 0.119 and 0.098, respectively. Population PAN had the highest expected diversity (0.137), populations BUS and CHE the lowest (0.098). Shannon's information index (I) for *K. pictus* and *K. pictus* variant were 0.173 and 0.147, respectively.

The genetic frequencies detected with nine polymorphic primer combinations were calculated and used in estimating phenotypic diversity (H_O) within populations (Table 2). An average of H_O was 1.065 across the populations, varying from 0.977 for the lowest H_O and 1.129 for the highest.

Table 1. Measures of genetic variation for RAPD generated interspecies. 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.	N_p	P_p	A	A_E	H	I
K. pictus						
BUS	11	22.5	1.225	1.180	0.098	0.141
SAC	14	28.6	1.286	1.181	0.108	0.161
DAN	15	30.6	1.306	1.218	0.124	0.181
PAN	16	32.7	1.327	1.246	0.137	0.199
SUW	14	28.6	1.286	1.236	0.127	0.182
Mean	14	28.6	1.286	1.212	0.119	0.173
K. pictus variant						
CHE	13	26.5	1.265	1.162	0.098	0.147
Total	29	59.2	1.592	1.288	0.183	0.283
t-test		ns	ns	*	ns	ns

*$p<0.05$, ns: not significant ($p>0.05$).

Table 2. Estimates of phenotypic diversity (H_O) within species of *K. pictus* and *K. pictus* variant

Primer	BUS	SAC	DAN	PAN	SUW	CHE
OPD05	0.693	0.693	0.693	0.693	0.693	0.935
OPD06	0.693	0.935	1.028	0.974	0.884	0.693
OPD07	1.438	1.522	1.592	1.730	1.700	1.367
OPD08	1.771	1.768	1.782	1.786	1.786	1.792
OPD09	1.099	1.099	1.314	1.264	1.332	1.386
OPD10	1.576	1.512	1.041	0.997	1.364	1.782
OPD11	1.593	1.681	1.723	1.763	1.779	1.542
OPD12	0.240	0.625	0.691	0.662	0.673	0.973
OPD14	0.668	1.074	0.598	0.816	0.562	0.816
Mean	0.977	1.091	1.046	1.068	1.077	1.129

Table 3. Partitioning of the genetic diversity into within and among six populations of both *K. pictus* and *K. pictus* variant.

Species	H_{POP}	H_{SP}	H_{POP}/H_{SP}	$(H_{SP}-H_{POP})/H_{SP}$
K. pictus	1.052	1.196	0.880	0.120
K. pictus variant	1.129	1.196	0.944	0.056
Total	1.065	1.196	0.890	0.110

Table 4. 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).

	H_T	H_S	G_{ST}	Nm
K. pictus	0.183	0.115	0.368	0.857
K. pictus variant	0.193	0.083	0.571	0.376

Although the Korean populations were isolated and patchily distributed, they maintained a high level of genetic diversity. The same trend was observed in the average diversity (H_{SP}) (Table 3). The mean H_{SP} was 1.196.

Total genetic diversity values (H_T) for *K. pictus* and *K. pictus* variant were 0.183 and 0.193, respectively (Table 4). Interlocus variation in the within-population genetic diversity (H_S) for *K. pictus* and *K. pictus* variant were 0.115 and 0.083, respectively. On a per locus basis, the proportion of total genetic variation due to differences among populations (G_{ST}) for *K. pictus* and *K. pictus* variant were 0.368 and 0.571, respectively. This indicated that about 36.8% and 57.1% of the total variation were among populations. The estimate of gene flow, based on G_{ST}, was very low among Korean populations of *K. pictus* ($Nm = 0.857$ and 0.376). Values of genetic distance (D) were below 0.269. Genetic identity values among pairs of populations ranged from 0.764 to 0.995.

Clustering of *K. pictus* populations was performed based on the genetic distances (Fig. 2). The dendrogram showed two distinct groups *K. pictus* and *K. pictus* variant populations were well separated each other. The trees also show genetic differentiation among local populations for both *K. pictus* species.

DISCUSSION

K. pictus maintains high levels of genetic diversity in species than does the average plant species. For example, its

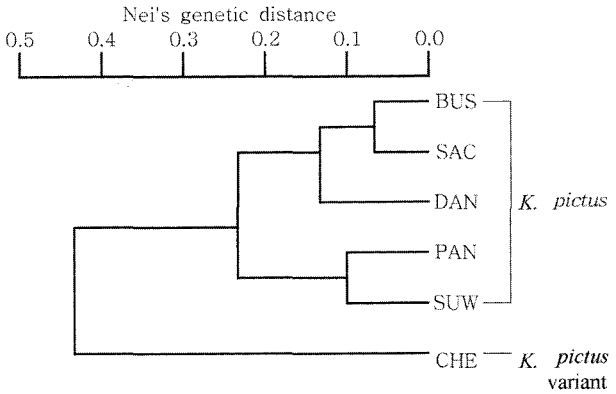

Fig. 2. A phenogram showing the relationships among six populations of *K. pictus* and *K. pictus* variant, based on data of genetic distance obtained by RAPD. See Fig. 1 for locality information of populations.

genetic diversity of 0.183 is higher than that for temperate-zone species (0.146), dicots (0.136), species with a sexual reproduction mode (0.151), and long-lived woody habit (0.177) (Hamrick & Godt, 1989). The percentage of polymorphic loci at the species level was 59.2%. This value is also higher than the average for species with temperature-zone distributions (48.5%), dicots (44.8%), and species with a sexual reproduction mode (51.6%), but lower than those with a long-lived woody (64.7%) (Hamrick & Godt, 1989).

The relatively high level of genetic variation found in *K. pictus* is consistent with several aspects of its biology. First, the breeding system of species is an important determinant of variability at both the species and population levels. *K. pictus* performs both asexual and sexual reproduction. The ability to regenerate by root or stump sprouting may explain the high level of genetic diversity within populations (Huh, 1999). Sexual reproduction could act to enhance genetic variation and asexual reproduction could maintain the enhanced genetic variation (Bayer, 1990). The asexual reproduction assures the stabilization and persistence of a phenotype that is well adapted to the immediate environment (Huh, 1999). Second, a perennial and/or long-lived species generally maintains relatively higher levels of variation than do annuals (Loveless & Hamrick, 1984). The observation of annual rings in *K. pictus* revealed that the plants were at least 20~30 years old. As individuals of *K. pictus* are long-lived, opportunities for the accumulation of mutations should be high (Ledig, 1986).

One of the most striking features of this study was not a significant difference between *K. pictus* populations and *K. pictus* variant population. For example, the average percentage of polymorphic band was 28.6% for *K. pictus* and 26.5% for *K. pictus* variant (Table 1). Genetic diversity of *K. pictus* (mean $H = 0.119$ and $I = 0.173$) is not significantly different from that of *K. pictus* variant (mean $H = 0.098$ and $I = 0.147$, p£æ0.05). These comparisons suggest that genetic diversity of *K. pictus* is not higher than those of *K. pictus* variant (one-tailed Wilcoxon's signed rank test) (Table 1). The comparison of banding patterns between *K. pictus* and *K. pictus* variant revealed that the four unique bands were found specific to *K. pictus*, whereas none to *K. pictus* variant. The most alleles of the *K. pictus* were a subset of wild populations. These genetic diversity parameters indicated that *K. pictus* variant populations were not genetically much depauperate relative to its presumptive progenitor and the speciation process has eroded the level of genetic variation of this species.

A naturalized population of *K. pictus* variant species is ultimately a product of both their biological characteristics and historical practices (Hagen & Hamrick, 1998). *K. pictus* variant species may be founded by a small sample of larger or moderate populations (i.e. founder effect). However, we consider that the other hypothesis still remained.

Genetic differentiation among populations is principally a function of natural selection, genetic drift, and gene flow via pollen and seed dispersal (Loveless & Hamrick, 1984). The most striking feature of these results was the relatively high degree of genetic differentiation recorded between 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 (Hamrick & Godt, 1989). For *K. pictus*, about 36.8% of the total variation *K. pictus* was due to differences among populations ($G_{ST} = 0.368$). This high level of genetic differentiation also suggests that gene flow among the population is low ($Nm = 0.857$). Although we did not analyze further subdivision of a local population, we may infer that genetic variation that resided mainly within wild populations is maintained in patchily distributed subpopulations or demes, either by random drift of neutral alleles or micro-environmental selection for adaptive alleles (Beebe *et al.*, 2000).

If an *Nm* value (0.857) can be considered similar to 1, and as a result, genetic drift should be a factor in *K. pictus* populations. Thus, the levels of gene flow we have calculated are not of sufficient magnitude to counterbalance genetic drift and may play a major role in shaping the genetic structure of the populations. 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

Table 5. Similarity matrix (above diagonal) of nine populations based on RAPD and genetic distances (below diagonal).

Pop.	BUS	SAC	DAN	PAN	SUW	CHE
BUS	-	0.995	0.972	0.973	0.963	0.756
SAC	0.006	-	0.981	0.983	0.980	0.764
DAN	0.029	0.020	-	0.994	0.980	0.772
PAN	0.028	0.017	0.007	-	0.989	0.764
SUW	0.037	0.021	0.020	0.013	-	0.766
CHE	0.267	0.269	0.258	0.269	0.267	-

late October to early November, and matured fruits are transported by birds and rodents (Huh, observation). 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.

For a conservation perspective, transplant from mountains to lowlands is not a great help to conservation of the narrow distributed species but an action of destruction of habitats. The conservation of rare species requires consideration of ecological and genetic factor (Neel & Ellstrand, 2003). Ecological factors such as mountain habitats (100~1,800 m above the sea level) and very fertile soil may be of primary importance in the preservation of most populations, artificial transplants cannot play an important role in determining as rare or endemic species conservation. The level of distribution of genetic variation among populations is of primary importance to the conservation of genetic diversity and the evolutionary potential of species (Hamrick & Godt, 1989). 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, such as populations PAN and SUW. These populations could be used as a source of genetic diversity for the restoration of genetically poor populations.

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