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Morphological Analysis on the *Kalopanax pictus* (Araliaceae) of Korean Populations

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Morphological characteristics of *Kalopanox pictus* Nakai were studied to examine population differentiation of this species. Based on a phenogram of using 23 morphological characteristics, differentiation of regions were distinct. Collections of 138 specimens from nine populations served as operational taxonomic unit (OTU's) were examined for phenotypic similarity and morphological variation using clustering (Ward's minimum variance method) and principal component analysis (PCA). The first three principal components were responsible for 77.0% of the total variance. Principal component 1 explained 52% of the total variance and was contributed to by the number of palmately parted, the number of pinnately lobed, and width between two lateral lobe apex.

Key words – Morphological characteristics, *Kalopanox pictus*, phenogram

Morphological differentiation of species within their geographic distribution has been of considerable interest in the study of the evolution of species [17]. Morphological data from variables measured on a continuous scale are important in generating and testing evolutionary hypotheses and taxonomic hierarchies. Divergence among populations may occur as a result of microevolutionary changes in isolated populations in different nature in time. These changes in isolated populations in different environments produce individuals with different ecological tolerances to physical factors, resulting in the differentiation of divergence ecotypes. The actual geographical distribution of a species may be reflective of these changes over geological time. Differentiation between populations has received much attention in recent years using different approaches and many distinct taxa [1,2,13].

The genus *Kalopanox* consists of one species, *Kalopanox pictus* (Araliaceae) which distributes throughout the world [7]. The species is distributed in temperate regions, with centers of diversity in East Asia. Typical populations are small and distributed in patches. *K. pictus* can be classified as a narrow habitat species because it is usually found on subsites of several Korean mountains, at elevations of 300-400 m. This species is long-lived perennial and has yellow-green flowers [15]. The species is a polygamo-monoecious diploid species (2n = 48), being predominantly out-crossed via wind-pollination [6,7]. Although plants grow high in the mountains on fertile soil, they are also extensively cultivated as a medicinal muscle relaxant. *K. pictus* is also an economically important for it stems, which historically were used for wooden shoes with clogs. Household goods are also valued because of the figured heartwood.

Until recently, much of the Korean forest has been disturbed by the cutting of trees and shrubs for firewood in rural areas[3,4]. Most sites are now being revegetated both naturally and artificially[4]. Although it is important to gain knowledge of the genetic variation for conservation purposes, detailed information on the level and distribution of this variation, as well as population structure, are not available for most woody taxa in Korea [3].

As *K. pictus* has been domesticated from wild relatives, it is expected that valuable cultivars may be found in these cultivators [5]. For a better characterisation and comparison of *K. pictus* germplasm diversity available in Korea, it is vital to use a morphological approach of population differences of this germplasm from wild populations.

Although there is much interesting the medicine and economic, only a handful of studies do not have explored basic of the population biology and ecology of *K. pictus.*

Consequently, there is a need to study the investigation of the nature to better understand the morphological characteristic of Korean *K. pictus* species. The objective of this paper is to; 1) compare the differentiation of *K. pictus,* 2) determine the overall geographical distribution patterns for the Korean species, and analyse morphological variation within and among Korean populations.

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Materials and Methods

Collecting sites

Samples of *K. pictus* were collected from nine populations in Korea. Table 1 lists the collection sites used in this study, along with three-letter abbreviations, and population sizes.

Morphological characteristics

The measurements of quantitative characters and observation of qualitative characters were taken on each of total individuals directly from their natural habitats from during 2002 and 2003. Table 2 was shown lists of the morphological characteristics. The measurements made on plants were arranged in a data matrix indicating the means of plant characteristics for each population.

Table 1. Localities for populations of *K. pictus* as source for morphological analyses

Code	Localities	Sample size
UNI	Uongyang-gun, Gyeongsangnam-do	17
SAC	Sacheon-si, Gyeongsangnam-do	16
BUS	Goijeong-gu, Busan-si	10
DAM	Damyang-gun, Cheonranam-do	20
SAM	Samcheok-si, Gangwon-do	16
DAN	Danyang-gun, Chungcheongbuk-do	15
CHE	Cheongyang-gun, Chungcheongbuk-do	12
PAN	Pyuangchang-gun, Gangwon-do	17
GAC	Gacheon-si, Gyeonggi-do	15

Data analysis

The main trends in variation between localities (populations) were summarized using univariate and multivariate analyses. Measurements of each particular trait were compared using one-way analysis of variance (ANOVA) with site of origin as a factor. Whenever a significant difference was found ($P<0.05$), multiple comparison tests were performed fellowing SYSTAT procedures [14]. Bartlett's test was applied to test the homogeneity of variance [18] and transformations of variables were performed when data were not homoscedastic. For those variables in which transformations were enable to make the data meet the assumptions of ANOVA, we used a non-parametric method: the Kruskal-Wallis test, analogous to a single classification ANOVA [18].

Pearson correlation coefficients were calculated using the Statistical Analysis System [18] to determine correlations between morphological characteristics.

Table 2. List of 23 descriptive characteristics used in the morphological analysis

Acronym	Characteristic derivation	Unit or Category
LOL	Number of lobe of leaf	ea
ACL	Angel of central lobe of leaf	degree
AP1	Angel of first palmatified	degree
AP2	Angel of second palmatified	degree
AP3	Angel of 3rd palmatified	degree
WIL	Width of leaf (maximum)	mm
WML	Width between both first lobe apex (middle)	mm
WSL	Width between both 3rd lobe apex (minimum)	mm
LEL	Length of leaf	mm
LTR	Rate of length/width (leaf)	ratio
LCA	Length from basal sinus to central lobe apex	mm
LSA	Length from basal sinus to second lobe apex	mm
L3A	Length from basal sinus to 3rd lobe apex	mm
L4A	Length from basal sinus to 4th lobe apex	mm
MAG	Major groove of leaf	mm
MMG	Middle groove of leaf	mm
MIG	Minor groove of leaf	mm
NS1	Number of serrulate at palmatified (1st margin)	ea
NS2	Number of serrulate at palmatified (2nd margin)	ea
NS3	Number of serrulate at palmatified (3rd margin)	ea
NS4	Number of serrulate at palmatified (4th margin)	ea
NS5	Number of serrulate at palmatified (5th margin)	ea
NS6	Number of serrulate at palmatified (6th margin)	ea

Multivariate principal component analyses (PCAs) were conducted to detect differences among populations considering several characters simultaneously of variances using the Statistical Analysis System [18], and nine populations were used in this analyses. The data were standardized for each characteristic, with the raw data matrix transformed such that each characteristic had a mean of zero as a standard deviation [14]. The first three principle components were extracted by the PCA analyses. Pair-group analysis was utilized using an unweighted method (UPGMA) of agglomerating clustering based an Euclidian distance similarity matrix [14]. This cluster analysis was performed with morphological phenogram among populations based on *quantitative variation* [11].

Results

The major morphological characteristics of *K. pictus* which can be used as identification keys are presented in Table 2. Our analysis of variance, calculated from 23 morphological characteristics in each population, showed a slight heterogeneity of variance. For example, thirteen characteristic values were homoscedastic, of which ten values departed significantly from zero ($P<0.05$). The number of lobe of leaf (LOL) were shown a significant difference among nine populations ($P<0.05$) (Table 4). The length from basal sinus to centrol lobe apex (LCA) has the highly significant positive correlations with the length of from basal sinus to second lobe apex (LSA), 3rd lobe apex (L3A), and 4th lobe apex (L4A).

The multivariate principal component analyses (PCAs) were performed based on the 23 morphological characteristics (Table 3). The first three principal component were responsible for 77.0% of the variance. Principal component 1 explained 52% of the total variance and was contributed to by the number of palmately parted, the number of pinnatedly lobed, and width between two lateral lobe apex. The second component accounts for 12.2% of the total variation and is mostly concerned with length of leaf (LEL and LTR).

In the two-dimensional plot constructed based on the morphological data derived from the PCA (Fig. 1), all the 23 characteristics are clearly separated into five or six classes. L3A, L4A, LCA, AP1, AP2, AP3, and ACL are classified one group. NS2 and NS3 are also classified one group. LOL, MAG, and NS1 are consisted of one group. WIL, NS5, and NS6 are same trend. LEL, NS4, MIG, MMG, WML, and LSA are formed a long ellipse. LTR showed a

Table 3. Testing the significance of differences among populations

Acronym	Mean+SE	SD	*F*	*t*	$P = 0.05$
LOL	6.73±0.70	3.18	4.83	4.83	$P < 0.05$
ACL	50.68±1.06	3.24	5.29	1.51	$P > 0.05$
AP1	40.14±0.81	1.51	10.13	2.68	$P > 0.05$
AP2	50.13±1.21	0.16	12.016	1.48	$P > 0.05$
AP3	45.65±0.91	1.84	1.84	2.10	$P > 0.05$
WIL	143.06±4.17	10.21	2.06	0.78	$P > 0.05$
WML	137.06±3.80	7.44	2.34	1.21	$P > 0.05$
WSL	67.23±2.09	4.25	2.56	1.49	$P > 0.05$
LEL	164.22±3.51	11.24	1.69	2.55	$P > 0.05$
LTR	1.14±0.03	0.10	2.05	2.28	$P > 0.05$
LCA	72.40±5.47	12.23	3.22	4.56	$P < 0.05$
LSA	56.20±4.72	7.30	4.11	3.65	$P < 0.05$
L3A	40.51±3.75	5.42	3.36	4.91	$P < 0.05$
L4A	27.66±3.20	5.37	3.45	4.88	$P < 0.05$
MAG	89.40±3.48	0.39	2.18	0.51	$P > 0.05$
MMG	60.30±4.93	0.45	3.25	1.32	$P > 0.05$
MIG	42.29±0.83	1.85	1.92	4.23	$P < 0.05$
NS1	20.15±1.55	1.34	2.43	2.17	$P > 0.05$
NS2	17.11±0.26	1.65	1.37	0.37	$P > 0.05$
NS3	13.03±1.10	1.48	2.87	2.73	$P < 0.05$
NS4	13.38±1.30	1.53	2.12	2.67	$P < 0.05$
NS5	5.90±0.91	2.89	5.65	2.96	$P < 0.05$
NS6	48.20±2.20	1.37	3.12	6.45	$P < 0.05$

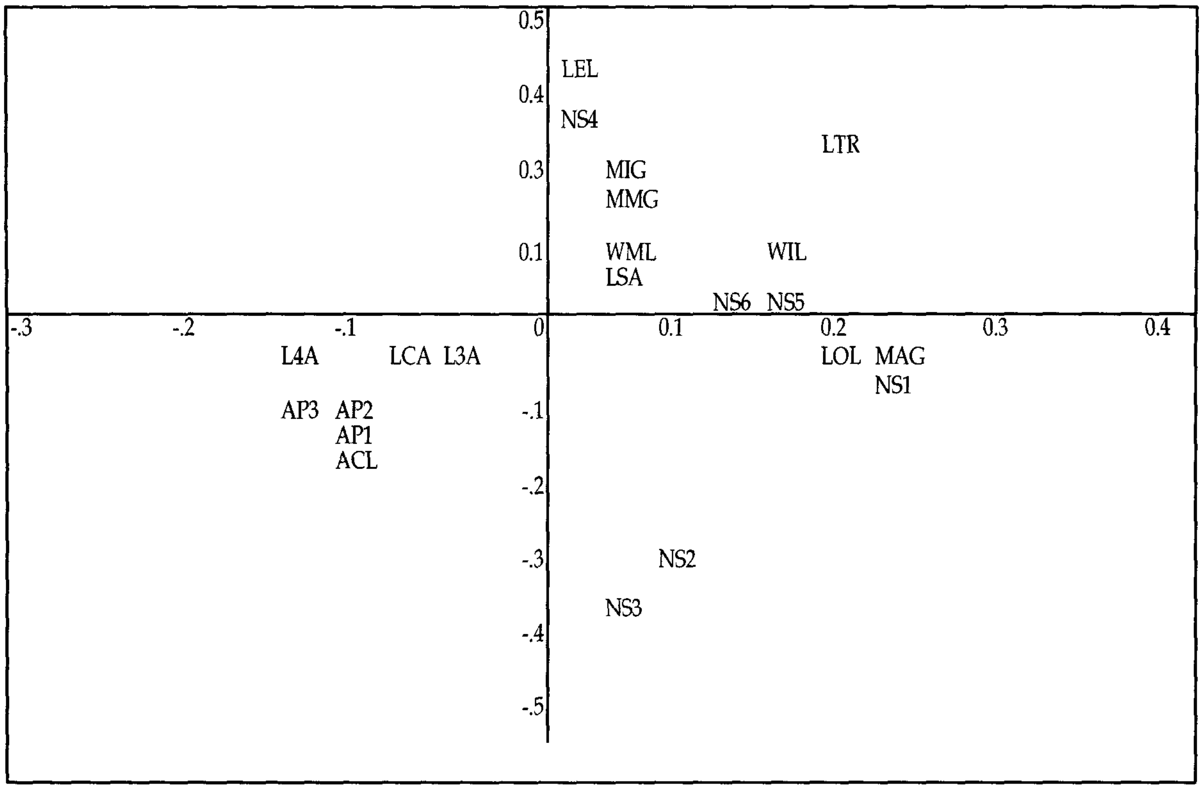

Fig. 1. Principal component analysis using 23 morphological characteristics of *K. pictus*.

Table 4. First three principle components composed of 100 OTUs derived from 23 morphological characteristics

Acronym	PC 1	PC 2	PC 3
LOL	0.232	-0.054	-0.053
ACL	-0.194	-0.175	0.074
AP1	-0.187	-0.136	0.065
AP2	-0.139	-0.104	0.202
AP3	-0.209	-0.138	0.035
WIL	0.207	0.111	0.066
WML	0.123	0.210	0.031
WSL	0.166	0.326	0.027
LEL	0.044	0.377	0.221
LTR	0.141	0.348	0.160
LCA	-0.107	0.079	0.105
LSA	0.076	0.059	0.116
L3A	-0.055	0.132	0.057
L4A	-0.102	-0.033	0.020
MAG	0.199	-0.067	0.045
MMG	0.022	0.104	0.431
MIG	0.020	0.139	0.279
NS1	0.224	-0.014	0.044
NS2	0.109	-0.303	0.344
NS3	0.008	-0.381	0.305
NS4	0.052	0.345	0.094
NS5	0.170	0.059	0.042
NS6	0.123	0.046	0.043
Eigen value	16.197	3.345	3.083
Proportion of variance	0.515	0.122	0.084
Cumulative variance	0.515	0.637	0.721

clear separation of the other characteristics.

The cluster analysis (Fig. 2) is concordant with the results obtained from the morphological data. Clustering of *K. pictus* populations, using UPGMA, was performed based

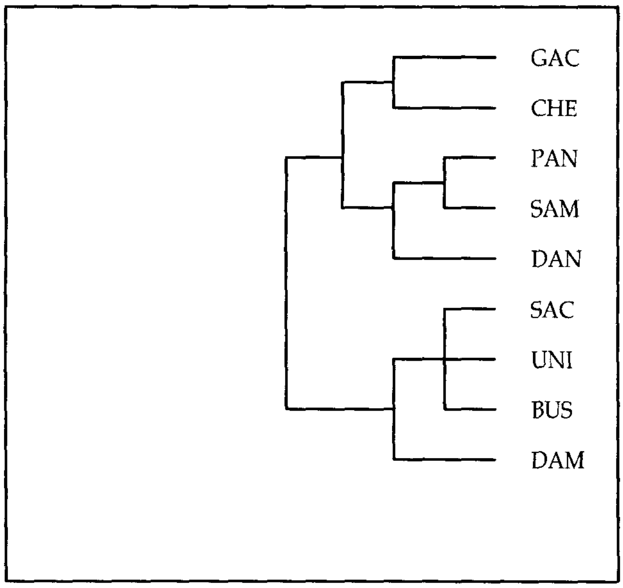

Fig. 2. Correlation phenogram (UPGMA) expressing phenetic similarity of *K. pictus* based on 23 morphological characteristics.

on morphological characteristics. The dendrogram showed two distinct groups: the northern part of Korea and the southern part of Korea. Many of the morphological characteristics studied among the nine populations were associated with latitude (Fig. 2). For example, the more northern a population group, the less the lobe of leaf but more serrulate at palmatified. However, most morphological variation except LOL and NS is significantly correlated with latitude.

Discussion

K. pictus has been known for thousands of years as a medicine and vegetative plant. During this time, it has been subjected to selection by man for characteristics that have perfected it as a cultivated crop plant.

The larger number of populations (cultivars) did not differ from each other in most morphological characteristics. However, some morphological characteristics of nine populations were distinct. Although 83.8% of the total morphological variation was within populations, one of the most striking features of this study was existed a significant difference among populations. The average percentage of morphological differences was 16.2%.

The number of lobe of palmatified leaf, minor groove of palmatified leaf, and length from basal sinus to central lobe apex were those which were considered likely to be useful in the subsequent intensive examination of *K. pictus* (Table 3).

Similar results have already been published on *K. pictus based* on isozyme analysis [5]. They detected a considerable amount of variability within and between the cultivars and natural populations of Korea. In this study morphological characteristics were used to explore the organization of morphological variation within and among nine populations of this species.

In a phylogenetic tree based on morphological variability, the position of the populations in the tree and their geographical position matched almost completely in the populations.

Phenotypic plasticity is an important "buffering" mechanism for individuals in the face of environmental variation when a population has a within gene flow [8,12]. Because of the limited number of plants available, phenotypic plasticity could not be assessed quantitatively in the present study. Phenotypic of quantitative traits are subject to environmental influence, *K. pictus* showed the plastic response to environmental variations. The heterogeneous nature of the nine populations, and the low species richness, along with the degree of habitat destruction and fragmentation among populations ought to be an obvious indicator of the need to protect the remaining forest vestiges.

Although *K. pictus* is distributed in a wide geographic range, it is ecologically restricted, growing in high 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 *K. pictus* in general maintain less genetic variation than do species with continuous, abundant populations growing on broad-niched mainland habitats ("habitat generalists") [10]. 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.

The transplanting of materials from the mountains to the lowlands does not greatly aid in the preservation of this narrowly distributed species, but rather it results in the destruction of habitat. Conservation of rare species requires that ecological and genetic factors be considered [9]. Specific environmental conditions, such as a mountain habitat (400 to 500 m above sea level) and very fertile soil, may be of primary importance in the preservation of most populations. Therefore, artificial transplants cannot play a crucial role in determining the conservation of rare or endemic species. Moreover, the degree to which genetic variation is distributed among populations is critical to preserving genetic diversity and the evolutionary potential of a species[2].

In addition, we 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 [16]. However, we have failed to detect large actual populations (>20 plants) except nine populations for *K. pictus* examined in this study. Thus, these natural populations are imminent danger of becoming extinct if protection is not provided.

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초록 : 한국 음나무(두릅과) 집단의 형태적 분석

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한국내 분포하는 음나무(*Kalopanox pictus* Nakai)의 집단간 분화를 연구하기 위해 형태적 특성을 조사하였다. 23개 형태 형질에 근거하여 지역간 분화는 현저하였다. 아홉 집단에서 138 표본으로 유효한 분류학적 단위(OTU)를 표현형 유사도와 형태적 변이를 조사하였으며 주성분 분석을 실시하였다. 첫 세 주성분 요소가 전체 변이의 77.0%에 관련이 있었다. 이 중 첫 번째 주성분 요소가 전체 변이의 52%에 기여하였는데 이에 해당되는 형질은 장상복엽의 장상렬의 수, 그리고 장상렬의 폭이다. 형태적 변이에 근거한 군집분석에서 우리나라 남부와 중부지방의 두 그룹으로 나누어졌다.