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Study of Genetic Diversity and Taxonomy of Genus *Sorbus* in Korea Using Random Amplified Polymorphic DNA

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Genus *Sorbus* is a long lived woody species. Plants of this genus are primarily distributed patchy throughout Asia and Europe. *Sorbus commixta* is primarily distributed throughout Europe. Eastern Asian *Sorbus* species are regarded as very important herbal medicines in Korea and China. Random amplified polymorphic DNA (RAPD) was used to investigate the genetic variation and phylogenetic analysis of four species of this genus in Korea. Although some Korean populations of these species were isolated and patchily distributed, they exhibited a high level of genetic diversity. Twenty-six primers revealed 205 loci, of which 128 were polymorphic (62.4%). *S. commixta* showed the highest diversity (0.165), whereas *S. aucuparia* showed the lowest diversity (0.109). The estimated gene flow (*Nm*) was low high among intra-species (mean *Nm* = 0.755). A similarity matrix based on the proportion of shared fragments (GS) was used to evaluate relatedness among species. The estimate of GS ranged from 0.786 to 0.963. The molecular data allowed us to resolve well-supported clades in Korean taxa and European species. An addition, especially, species-specific markers for genus *Sorbus* by RAPD analysis may be useful in germ-plasm classification and agricultural process of several taxa of this genus.

Key words – *Sorbus*, RAPD, genetic variation, phylogenetic analysis

Introduction

Genus *Sorbus* is known as about 100 species within the subfamily Rosaceae comprising diploid species and distributes mainly from temperate zones to subarctic zones of The Northern Hemisphere. The genus *Sorbus* in Asia is compose of three species and 12 variants [10]. Species of this genus have been utilized from the example by various usage of coronal shape, industry, edibility, medical use, and so on. Many plants of genus *Sorbus* had been used from the example for good materials for old men's stick. *S. commixta* can be classified as narrow habitat species because they are usually found on subsites of several mountains, at elevations of 500 to 1,000 m. This long-lived perennial has white flowers and are a bisexual reproductive system, being predominantly out-crossed via insect-pollination. *Sorbus aucuparia* is mostly distributed in Europe and America [3]. This species is introduced in Korea about one hundred years ago which today is widely appreciated ornamental and landscape architecture.

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Specially, nutrition effects had been known as water in which medicine has been dissolved that is in discharge of phlegm, bronchial catarrh and several diseases such as body weakness and research about abstraction of material and so on, separation is reported on several kind of flavonoids, carotene and antioxidant [7,12].

Although molecular and biochemical approaches are now increasingly being applied to address the taxonomic and phylogenetic relationships within the animals and plants in Korea, no population genetic studies of genus *Sorbus* have been conducted. In addition, the taxonomy of genus *Sorbus* has processed mainly through morphological characteristics and ecological study [3].

Random amplified polymorphic DNA (RAPD) is used widely in core anbury liver distinction that use amplification of random gene [1]. Because RAPD analysis is quick than other molecule creature school register techniques and because preliminary knowledge about rank need seldom much, expense of an experiment is inexpensive than other techniques, is used widely owing in PCR development in stalk classification techniques [17]. Therefore, we achieved a judged experiment to be useful analyzing genus of *Sorbus* inside classification in the Korea by RAPD analysis.

Also, the purpose of this research was to compare with other flora that verify genetic variety of genus *Sorbus* taxa and degree of differentiation whether is possible by RAPD in Korea. Therefore, the objectives of this study were to estimate how much genetic diversity is maintained in genus *Sorbus* and to describe how population-specific markers, which may be useful in germ-plasm conservation is distributed among populations.

Materials and Methods

Plant materials and DNA extraction

All of the 60 plants were collected from five populations in Korea (Fig. 1, Table 1). One young leaf per mature tree ($\geq$ 5 yr) was sampled. Seven or eight plants were randomly collected from each population. In addition, European species, *Sorbus aucuparia*, of the same genus was provided for the outgroup and used to compare the phylogenetic relationship.

The genomic DNA of the samples including outgroup was extracted from fresh leaves using the plant DNA Zol Kit (Life Technologies Inc., Grand Island, New York, U.S.A.) according to the manufacturer's protocol.

RAPD analysis

Forty arbitrarily chosen 10-mer primers, the kit C (OPC01 to 20) and the kit D (OPD01 to 20) of Operon Technoligies (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 twenty-six decamer primers that produced RAPD bands in a preliminary test.

Amplification reactions were performed in 0.6 ml tubes containing 25 ul of the reaction buffer; 10 mM Tris-HCl, pH 8.8, 50 mM $MgCl_2$, 100 uM each of dATP, dCTP, dGTP, dTTP, 0.2 mM 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 Polaroid 667 film. A 100 bp ladder DNA marker (Pharmacia) was used in the end of for the estimation of fragment size.

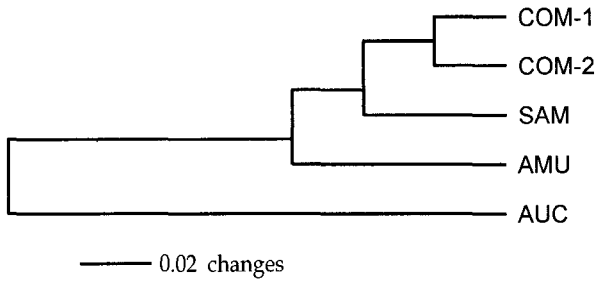

Fig. 1. A phylogenetic tree for genus *Sorbus* based on RAPD analysis.

Statistical analyses

The following genetic parameters were calculated using a POPGENE computer program (ver. 1.31) developed by Yeh et al. [18]: the percentage of polymorphic loci (*Pp*) mean numbers of alleles per locus (*A*) and per polymorphic locus (*Ap*); effective number of alleles per locus (*Ae*); and gene diversity (*H*), and Shannon index (*I*) [5]. Diversity at the level of population was calculated as described by Hartl and Clark [6]. Multiple tests to search for unique alleles were performed according to the sequential Bonferroni procedure [11]. To elucidate the organization of variability within populations, we examined the genetic variation by partitioning the total genetic diversity (H_T) to within- (H_S) and among- (D_{ST}) populations, using the genetic diversity statistics of Nei [13]. A measure of the differentiation among populations, relative to the total diversity, was calculated at each locus as $G_{ST} = D_{ST} / H_T$.

The indirect estimate of gene flow was then calculated. Estimate of the number of migrants per generation (*Nm*) was based on G_{ST} [16].

All monomorphic and polymorphic RAPD bands were scored by eye and only unambiguously scored bands were used in the analyses. Because RAPDs are dominant markers, they were assumed that each band corresponded to a single character with two alleles, presence (1) and absence

Table 1. Codes and geographic locations of four *Sorbus* taxa

Taxa	Localities	Code
Sorbus. aucuparia	Osan, Gyeonggi-do	AUC
S. amurensis	Ulleung-do, Gyeongsangbuk-do	AMU
S. sambucifolia var. *pseudogracilis*	Mt. Gebang, Pyeongchang, Gangwon-do	SAM
S. commixta	Mt. Giri, Sancheong-gun, Gyeongsangnam-do	COM-1
S. commixta	Mt. Gariwang, Jeongseon, Gangwon-do	COM-2

(0) of the band, respectively. The degree of polymorphism was quantified using Shannon's index of phenotypic diversity [2]:

$Ho = - pi \sum \log pi$

where pi is the frequency of a particular phenotype *i*. H_O can be calculated and compared for different populations [15]. Let

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

be the average diversity over the different populations and let

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

be the diversity calculated from the phenotypic frequencies p in all populations considered together [15]. Then the proportion of diversity present within populations, H_{POP}/H_{SP}, can compared with that of between populations (G_{ST}), $(H_{SP} - H_{POP})/H_{SP}$.

To determine the extent of genetic departure, we calculated the Nei genetic identity and genetic distance for each pairwise combination of populations [13]. 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[14]. GS was converted to genetic distance (1-GS) [9]. Homogeneity of variance among populations was tested by Bartlett's statistics. Genetic differentiation measured by G_{ST} among populations was also calculated. Furthermore, gene flow between the pairs of populations was calculated from G_{ST} values by $Nm = 1/4(1/G_{ST}-1)$ [16].

A genetic distance matrix was used to construct a dendrogram, using UPGMA (unweighted pair group method with arithmetic average) in the neighbor algorithm of the Phylogeny Inference Package (PHYLIP ver. 3.57) [4].

Results

From the 40 decamer primers used for a preliminary RAPD analysis, 26 primers produced good amplification products both in quality and variability. Overall, 205 polymorphic fragments were generated among the tested array (Table 3). In a simple measure of intra-species variability by the percentage of polymorphic bands, the population COM-2 exhibited the highest variation (41.5%). The European species showed the highest (29.3%) (Table 2).

The phenotypic frequency of each band was calculated and used in estimating genetic diversity (*I*) within populations (Table 3). The mean *I* of population COM-2 (0.242) was highest of all populations and showed significant difference (paired *t* test).

Table 2. Lists of decamer oligonucleotide utilized as RAPD primers, their sequences, and associated polymorphic fragments amplified in *Sorbus*

Primer	Sequence (5′ to 3′)	No. of fragments detected
OPC01	TTCGAGCCAG	10
OPC02	GTGAGGCGTC	7
OPC03	GGGGGTCTTT	8
OPC04	CCGCATCTAC	6
OPC05	GATGACCGCC	10
OPC06	GAACGGACTC	11
OPC07	GTCCCGACGA	11
OPC08	TGGACCGGTG	7
OPC09	CTCACCGTCC	6
OPC10	TGTCTGGGTG	9
OPD01	ACCGCGAACG	7
OPD02	CGACCCAACC	13
OPD03	GTCGCCGTCA	9
OPD04	TCTGGTGAGG	6
OPD06	ACCTGAACGG	11
OPD07	TTGGCACGGG	4
OPD08	GTGTGCCCCA	6
OPD10	GGTCTACACC	4
OPD11	AGCGCCATTG	5
OPD12	CACCGTATCC	8
OPD13	GGGGTGACGA	12
OPD15	CATCCGTGCT	6
OPD16	AGGGCGTAAG	11
OPD17	TTTCCCACGG	9
OPD19	CTGGGGACTT	7
OPD20	ACCCGGTCAC	2

Table 3. Genetic diversity for all loci among *Sorbus* taxa by RAPD markers

Taxa	N_P	P_P	*A*	*Ae*	*H*	*I*
AUC	60	29.3	1.293	1.182	0.109	0.163
AMU	73	35.6	1.356	1.226	0.135	0.200
SAM	62	30.2	1.302	1.190	0.114	0.169
COM-1	68	33.2	1.332	1.209	0.124	0.185
COM-2	85	41.5	1.415	1.289	0.165	0.242
Mean	72.8	35.5	1.355	1.231	0.136	0.201

As the typical populations of *S. commixta*, *Sorbus sambucifiolia* var. *pseudogracilis* and *S. amurensis* were small, isolated, and patchily distributed for natural populations, they maintained a low level of genetic diversity (H_T = 0.215) (Table 4).

An assessment of the proportion of diversity present within species, *Hpop*/*Hsp*, indicated that about 5.9% the

Table 4. Estimates of genetic diversity statistics and polymorphic loci in genus *Sorbus* by RAPD

Locus	H_T	H_S	G_{ST}	*Nm*
OPC01	0.205	0.181	0.125	3.618
OPC02	0.434	0.398	0.084	5.444
OPC03	0.391	0.297	0.223	3.016
OPC04	0.382	0.193	0.496	0.509
OPC05	0.312	0.281	0.113	5.913
OPC06	0.266	0.164	0.300	2.940
OPC07	0.411	0.336	0.189	4.911
OPC08	0.459	0.276	0.369	5.692
OPC10	0.460	0.420	0.084	6.134
OPD01	0.437	0.490	0.203	4.184
OPD02	0.266	0.180	0.283	3.425
OPD03	0.336	0.277	0.195	6.950
OPD04	0.402	0.343	0.155	3.131
OPD06	0.458	0.258	0.406	4.447
OPD07	0.342	0.206	0.350	1.600
OPD08	0.320	0.236	0.248	6.201
OPD11	0.371	0.285	0.211	7.174
OPD12	0.372	0.163	0.496	1.143
OPD13	0.294	0.190	0.338	2.535
OPD15	0.344	0.280	0.175	4.047
OPD16	0.362	0.267	0.256	9.795
OPD17	0.418	0.313	0.276	3.184
OPD19	0.392	0.270	0.272	6.021
Mean	0.215	0.129	0.399	0.755

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*) between the pairs of populations.

Table 5. Partitioning of the genetic diversity into within and among genus *Sorbus* taxa by RAPD

Primer	H_{SP}	H_{POP}	H_{POP}/H_{SP}	$(H_{SP}-H_{POP})/H_{SP}$
OPC01	2.295	2.217	0.966	0.034
OPC02	1.867	1.809	0.969	0.031
OPC03	2.052	1.994	0.972	0.028
OPC04	1.780	1.721	0.967	0.033
OPC05	2.292	2.287	0.998	0.002
OPC06	2.329	2.259	0.970	0.030
OPC07	2.318	2.256	0.973	0.027
OPC08	1.854	1.756	0.947	0.053
OPC09	1.792	1.792	1.000	0.000
OPC10	2.116	2.011	0.950	0.050
OPD01	2.116	1.507	0.712	0.288
OPD02	2.500	2.443	0.977	0.023
OPD03	2.077	1.985	0.955	0.045
OPD04	1.617	1.521	0.940	0.060
OPD06	2.177	1.831	0.841	0.159
OPD07	1.231	0.985	0.800	0.200
OPD08	1.776	1.676	0.944	0.056
OPD10	1.404	1.329	0.946	0.054
OPD11	1.597	1.533	0.960	0.040
OPD12	1.597	1.533	0.960	0.040
OPD13	2.406	2.290	0.952	0.048
OPD15	1.719	1.630	0.948	0.052
OPD16	2.290	0.000	0.000	1.000
OPD17	2.046	1.915	0.936	0.064
OPD19	1.909	1.772	0.928	0.072
OPD20	0.693	0.693	1.000	0.000
Mean	1.917	1.804	0.941	0.059

Table 6. Genetic identity (upper diagonal) of *Sorbus* taxa and genetic distances (low diagonal) based on RAPD analysis

Taxa	AUC	AMU	SAM	COM-1	COM-2
AUC	-	0.802	0.801	0.786	0.801
AMU	0.220	-	0.914	0.920	0.912
SAM	0.222	0.090	-	0.935	0.942
COM-1	0.241	0.084	0.067	-	0.963
COM-2	0.222	0.092	0.060	0.037	-

total genetic diversity was among species (Table 5). Thus, the majority of genetic variation (94.1%) resided within species. The average number of individuals exchanged between populations per generation (*Nm*) was estimated to be very low (0.059).

Many loci including OPC02-01 locus can be recognized as unique loci of *S. aucuparia* (data not shown). Many loci including OPC01-09 locus were not shown in *S. aucuparia*. Thus these loci can be used distinguish Korean genus *Sorbus* from *S. aucuparia*.

Clustering of radish populations, using the NJ algorithm, was performed based on the matrix of calculated distances (Fig. 1). The phylogenic tree showed Korean populations were well separated each other. The tree also shows genetic differentiation among local populations for Korean species.

Discussion

Given the proliferation of genetic markers, comparisons between techniques are inevitable. However, there is a need technique is best suited the issues being examined. In this study, RAPDs were used to determine the genetic relationships among populations and the results compared to pedigree relationships where there were available [2].

A striking feature of this study is the lacking of intra-species variation. 40.0% of genetic variation was found among species and about 60.0% within species (Table 4). It was shown that most of the genetic variation (94.1%) resided within populations (Table 5). The populations in

Korea are less differentiated than the other wind-pollinated outcrossing species [5]. Common life history traits, such as wind dispersal or animal dispersal of both pollen grains and seeds, high reproductive capability, similar longevity and successional behavior, could more readily account for most of the homology in the population genetics of these species, and most likely for the low differentiation observed at the intra-specific level. These factors can reduce the effect of geographic isolation for genetic divergence.

The *S. commixta* and *S. aucuparia* were morphologically distinguished from each other [3,8]. One of the most striking features between both taxa was length and diameter of winter buds. However, the resolving power of morphological characteristics is restricted, mainly because of the small number of characters available. Efficient methods to clarify the taxonomic status of several species are much needed. The molecular data allowed us to resolve well-supported clades in Korean taxa and European species. Especially, species-specific markers for genus *Sorbus* by RAPD analysis may be useful in germ-plasm classification and agricultural process of several taxa of this genus.

In addition. by current categories for threatened taxa in Korea, all species belonging to genus *Sorbus* are not considered threatened. The probability of extinction of any single population is high, since the populations or populations of natural *S. commixta* are so small. Ecological management of these populations will be necessary to preserve species. Many populations of genus *Sorbus* are decline in population size because of the habitat loss by such road-structuring, land development business in a low mountain, and human activities such as over-gathering medicinal plants. Taking the circumstances into this consideration, some populations showed the highest genetic diversity by DNA fingerprinting, thus these populations may be recommend for in-situ conservation.

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초록 : RAPD에 의한 한국내 마가목속 식물의 유전적 다양성과 분류학적 연구

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마가목 속(genus *Sorbus*)은 아시아와 유럽에 분포되어 있는 목본이다. 유럽마가목(*Sorbus commixta*)은 유럽에 일차적으로 분포한다. 마가목 속 식물은 한국과 중국에서 약용으로 쓰인다. 한국내 마가목 속 식물에 대해 RAPD에 의한 유전적 변이와 계통관계를 조사하였다. 한국 내 마가목 속 식물은 비록 패치분포를 보이나 높은 유전적 다양도를 가지고 있었다. 26 시발체로 205 유전자좌위를 얻었으며 그 중 128 개(62.4%)는 다형성을 나타내었다. 마가목(*S. commixta*)이 가장 높은 유전적 다양도를 나타낸 반면(0.165), 유럽마가목(*S. aucuparia*)이 가장 낮은 유전적 다양도를 나타내었다(0.109). 종간 세대를 통해 이주하는 유전자수(*Nm*)는 매우 낮았다(평균 $Nm = 0.755$). 분류군간 유사도는 0.786에서 0.963사이에 있었다. 이 분자마크(RAPD)로 한국내 분류군과 유럽종간 구분이 잘 되었다. 또한 종 특이 마크로 종 동정에 이용할 수 있었으며, 종의 보전이나 생식질 보전에 기초로 이용될 수 있을 것으로 사료된다.