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Genetic Diversity and Phenetic Relationships of Genus Oxalis in Korea Using Random Amplified Polymorphic DNA (RAPD) Markers

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We evaluated the phenetic relationships within six taxa of genus *Oxalis* L. in Korea with random amplified polymorphic DNA (RAPD) markers. Ten primers produced 125 bands for six taxa, and the mean number of bands per primer was 12.5. Across the six taxa, 121 (96.8%) bands were polymorphic, and only four were monomorphic. The mean number of RAPD phenotypes across the six taxa varied from 3.6 (*O. stricta* and *O. corymbosa*) to 4.8 (*O. corniculata* for. *rubrifolia*). In a simple measure of intraspecies variability according to the percentage of polymorphic bands, *O. stricta* and *O. corymbosa* exhibited the lowest variation (28.8%), and *O. corniculata* for. *rubrifolia* showed the highest (38.4%). A mean of 32.7% of the loci was polymorphic within taxa. The total interspecies genetic diversity (H_T) and intraspecies genetic diversity (H_S) was 0.362 and 0.122, respectively. On a per-locus basis, the proportion of total genetic variation due to differences among species (G_{ST}) was 0.663. This indicates that about 66.3% of the total variation was among species. The node of *O. stricta* and *O. corniculata* for. *rubrifolia* was strongly supported, with a high bootstrap value in the NJ tree and sistered with *O. corniculata*. According to RAPD analysis, the number of chromosomes was not congruent with a phenetic relationship.

Key words : Genetic diversity, genus *Oxalis*, phenetic relationships, polymorphic, random amplified polymorphic DNA (RAPD)

Introduction

Oxalis is by far the largest genus in the wood-sorrel family [17]. There are some 850 different species of them, according to the Encyclopedia Britannica [11]. The genus occurs throughout most of the world, except for the polar areas. Some species of the genus are economically important and cultivated as crops [1]. For example, edible, somewhat similar to a small potato, have long been cultivated for food in Colombia and elsewhere in the northern Andes Mountains of South America [3]. The leaves of scurvy-grass sorrel (*Oxalis enneaphylla*) were eaten by sailors travelling around Patagonia as a source of vitamin C to avoid scurvy. In India, creeping wood sorrel (*Oxalis corniculata*) is eaten only seasonally, starting December/January. The leaves of common wood sorrel (*Oxalis acetosella*) may be used to make a lemony-tasting tea when dried.

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Classification of species has been at the heart of all plant systematics. The classification process generally tries to arrange plants into a logical form and doing so to sort the species in some evolutionary manner. Genus *Oxalis* L. is a taxonomically problematic group because of variations of morphological characters (even within a species) [10] and difficulty in defining specific boundaries [16].

Oxalis L. reaches major diversity in southern Africa [20] and South America [11] especially in arid desert and mountain environments. Members of section *Carnosae* Reiche are typical components of the flora of the subtropical desert belt along the South American Pacific coast and their diversity is centered in the Atacama coastal desert between 24°S and 30°S with about sixteen endemic species [5, 11]. Within South Africa, the main diversity center is located in the Cape Town-Hottentot's Holland area, while two secondary centers are found in the Clanwilliams-Nieuwoudtville areas [15].

In Korea, Lee [8] and Lee [9] have provided detailed taxonomic species of the genus *Oxalis*. Their classifications of species do not match each other. This was a problem for many plant sytematicists who had few examples of species available and used this limited number to describe the species. Koo et al. [7] have well studied the systematic relationships of the five Korean *Oxalis* species by the nucleotide

sequences (ITS). Their work included a detailed analysis of nine individuals and an understanding of the phonetic relationships of this genus. However, they omitted one species and it is necessary to perform extensive work to fully understand the within species variation.

The random amplified polymorphic DNA (RAPD) markers are DNA fragments from PCR amplification of the genomic DNA's random segments with single primer of arbitrary nucleotide sequence [24]. It is a relatively easy, inexpensive and rapid technique because of its simplicity and requirement for minimal amounts of genomic DNA [13]. Thus, RAPD markers have popular means for identification and authentication of plant and animal species because these marker techniques may generate relatively high numbers of DNA markers per sample and are technically simple [2]. The improved RAPD can improve the resolution of the PCR products and its repeatability [18]. The methods have been used extensively in genetic analysis of prokaryotes and eukaryotes though the marker system has certain disadvantages such as reproducibility [6].

In this paper, RAPD analysis of the intraspecific molecular variation patterns of *Oxalis* in Korea is first presented. We analyzed intra- and interspecific phylogenic relationships within genus *Oxalis* in Korea and to compare with the results of previous studies of this genus.

Materials and Methods

Sample materials

Five species and one form were selected to represent main lineages within genus *Oxalis*; *O. corniculata*, *O. stricta*, *O. corniculata* for. *rubrifolia*, *O. acetosella*, *O. obtriangulata*, and *O. corymbosa* (Table 1). *Geranium koraiense* Nakai was used as an outgroup species in this study.

The genomic DNA of the samples 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. The concentration of DNA samples was adjusted to 20 μg/ml.

RAPD analysis

Forty arbitrarily chosen primers of Kit A (OPA-01 to 20) and Kit B (OPB-01 to 20) (Operon Technologies, Alameda, CA) were used. From the primers used for a preliminary RAPD analysis, ten primers of them produced good amplification products both in quality and variability.

Amplification reactions were performed in 0.6 ml tubes containing 2.5 μl of the reaction buffer, 10 mM Tris-HCl (pH 8.8), 1.25 mM each of dATP, dCTP, dGTP, dTTP, 5.0 pM primer, 2.5 units Taq DNA polymerase, and 25 ng of genomic DNA. The samples amplified for 45 cycles. The amplification products were separated by electrophoresis on 2.0% agarose gels and 2.5 μl (500 μg/ml) of 100 bp ladder DNA marker (Pharmacia, Piscataway, NJ) was used in the end of the gel for the estimation of fragment size. The gel was stained with ethidium bromide and photographed under UV light using Alpha Image TM (Alpha Innotech Co., USA). All experiments were repeated twice and only reproducible bands were scored for analyses.

Statistical analyses

All 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 (0) of the band, respectively.

The following genetic parameters were calculated using a POPGENE computer program (ver. 1.31) developed by Yeh et al. [25]: the percentage of polymorphic loci (P_P), mean numbers of alleles per locus (A), effective number of alleles per locus (A_E) and gene diversity (H) [14].

Nei's gene diversity formulae (H_T, H_S, and G_{ST}) were used to evaluate genetic diversity within and among populations [14]. H_T is the expected heterozygosity of an individual in an equivalent random mating total interspecies. H_S is the expected heterozygosity of an individual in an equivalent

Table 1. The codes, color of petal, number of chromosome, and population location of genus *Oxalis*

Codes	Scientific name Species	Color of petal	Chromosome (2n)
COR	*Oxalis corniculata* L.	Yellow	24, 28, 44, 48
STR	*Oxalis stricta* L.	Yellow	18~24, 24, 28
RUB	*Oxalis corniculata* for. *rubrifolia* (Makino) H. Hara	Yellow (central is a red circle)	48
ACE	*Oxalis acetosella* L.	White	22, 22~24, 30
OBT	*Oxalis obtriangulata* Max.	Whitish yellow	18
COY	*Oxalis corymbosa* DC.	Purplish pink	14, 28

random mating total intraspecies. The G_{ST} coefficient corresponds to the relative amount of differentiation among populations. Furthermore, gene flow (Nm) between the pairs of species was calculated from G_{ST} values by $Nm = 0.5(1/G_{ST} - 1)$ [12].

Nei's genetic identity and genetic distance between genotypes were based on the probability that an amplified fragment from one individual will also be present in another [14].

A phenetic relationship was constructed by the neighbor-joining (NJ) method [19] in PHYLIP version 3.57 [4] using MEGA5 program [22].

Results

From the ten decamer primers used for a preliminary RAPD analysis, ten primers of them produced good amplification products for six taxa of genus *Oxalis* in quality and variability, while the remaining primers did not amplified or showed smear banding patterns (Table 2). Ten primers produced 125 bands for six taxa and mean number of bands per primer was 12.5. A total of 121 (96.8%) of these bands were polymorphic and only four bands were monomorphic across six taxa. The remaining fragments were monomorphic in all taxa.

The mean number of RAPD phenotypes across six taxa varied from 3.6 (*O. stricta* and *O. corymbosa*) to 4.8 (*O. corniculata* for. *rubrifolia*) (Table 3).

In a simple measure of intraspecies variability by the percentage of polymorphic bands, *O. stricta* and *O. corymbosa* exhibited the lowest variation (28.8%) and *O. corniculata* for. *rubrifolia* showed the highest (38.4%) (Table 4). A mean of 32.7% of the loci was polymorphic within taxa.

Table 2. List of decamer oligonucleotides utilized as primers, their sequences, and associated polymorphic fragments amplified in genus *Oxalis*

No. of primer	Sequence (5'->3')	No. of fragments	No. of polymorphic bands
OPA -04	AATCGGGCTG	16	15
OPA -05	AGGGGTCTTG	12	12
OPA -08	GTGACGTAGG	18	17
OPA -09	GGGTAACGCC	13	13
OPA -10	GTGATCGCAG	17	17
OPA -14	TCTGTGCTGG	13	12
OPA -17	GACCGCTTGT	11	11
OPB -01	GTTTCGCTCC	10	10
OPB -02	TGATCCCTGG	8	8
OPB -06	TGCTCTGCCC	7	6
Total	-	125	121

Table 3. The number of RAPD phenotypes in six taxa of *Oxalis* detected by each of the 10 primers

Loci	COR	STR	RUB	ACE	OBT	COY
OPA -04	7	5	6	8	4	5
OPA -05	3	3	4	6	3	2
OPA -08	6	6	6	3	6	4
OPA -09	2	2	3	3	7	5
OPA -10	2	3	6	7	7	6
OPA -14	4	2	7	2	5	5
OPA -17	2	5	5	3	6	3
OPB -01	5	3	7	2	3	2
OPB -02	3	5	3	2	1	1
OPB -06	4	2	1	5	4	3
Mean	3.8	3.6	4.8	4.1	4.6	3.6

Table 4. Measures of genetic variation for genus *Oxalis*

Taxa	Np	Pp	A	A_E	H	I
COR	38	30.4	1.304	1.206	0.118	0.173
STR	36	28.8	1.288	1.191	0.110	0.164
RUB	48	38.4	1.384	1.244	0.144	0.214
ACE	41	32.8	1.328	1.212	0.124	0.184
OBT	46	36.8	1.368	1.216	0.131	0.198
COY	36	28.8	1.288	1.174	0.104	0.157
Mean	40.8	32.7	1.327	1.207	0.122	0.182

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).

Mean number of alleles per locus (A) ranged from 1.288 to 1.368 with a mean of 1.327. *O. corniculata* for. *rubrifolia* showed the highest and *O. stricta* and *O. corymbosa* did the lowest. The effective number of alleles per locus (A_E) ranged from 1.174 to 1.244 with a mean of 1.207. The phenotypic frequency of each band was calculated and used in estimating genetic diversity (H) within taxa. As the typical populations of wild *Oxalis* were small, isolated, and patchily distributed for natural populations, they maintained a moderate level of genetic diversity for polymorphic primers. The total H was 0.122 across species. Shannon's index of phenotypic diversity (I) of *O. corniculata* for. *rubrifolia* (0.214) was highest of all taxa and *O. corymbosa* was the second (0.157).

A total genetic diversity value (HT) was 0.362 (Table 5). Genetic diversity in the within- species (H_S) was low (0.122). On a per-locus basis, the proportion of total genetic variation

Table 5. Estimates of genetic diversity of genus *Oxalis*

Loci	H_T (SD)	H_S (SD)	G_{ST}	*Nm*
OPA -04	0.342 (0.101)	0.137 (0.106)	0.576 (0.333)	0.097 (1.609)
OPA -05	0.430 (0.123)	0.111 ().076)	0.683 (0.259)	0.538 (0.861)
OPA -08	0.415 (0.095)	0.110 (0.087)	0.705 (0.247)	0.483 (1.019)
OPA -09	0.415 (0.106)	0.106 (0.090)	0.705 (0.284)	0.914 (1.989)
OPA -10	0.407 (0.099)	0.115 (0.069)	0.663 (0.268)	0.635 (1.112)
OPA -14	0.378 (0.094)	0.141 (0.086)	0.578 (0.273)	0.741 (0.935)
OPA -17	0.231 (0.117)	0.135 (0.081)	0.350 (0.258)	1.715 (1.264)
OPB -01	0.306 (0.150)	0.129 (0.046)	0.470 (0.250)	1.085 (1.080)
OPB -02	0.359 (0.164)	0.121 (0.085)	0.543 (0.315)	1.054 (1.210)
OPB -06	0.437 (0.077)	0.205 (0.056)	0.497 (0.211)	0.804 (0.740)
Mean	0.362	0.122	0.663	0.254

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*).

due to differences among species (G_{ST}) was 0.663. This indicated that about 66.3% of the total variation was among species. The estimate of gene flow, based on G_{ST}, was very low among species (*Nm* = 0.254).

A genetic identity matrix based on the proportion of shared fragments was used to evaluate relatedness among species (Table 6). The genetic identities between species ranged from a minimum value of 0.557 between *O. corniculata* and *O. corymbosa* and the maximum value of 0.880 between *O. corniculata* and *O. corniculata* for. *rubrifolia*. Values of genetic distance were <0.584.

Table 6. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) in genus *Oxalis* using RAPD markers

Taxa	COR	STR	RUB	ACE	OBT	COY
COR	-	0.825	0.880	0.708	0.606	0.557
STR	0.192	-	0.847	0.743	0.610	0.574
RUB	0.128	0.166	-	0.737	0.602	0.563
ACE	0.346	0.297	0.305	-	0.682	0.627
OBT	0.501	0.494	0.508	0.383	-	0.806
COY	0.584	0.555	0.575	0.467	0.216	-

The taxon codes are the same as Table 1.

Clustering of taxa using the NJ algorithm was performed based on the matrix of calculated distances (Fig. 1). Three main clades were recognized: (1) *O. stricta*, *O. corniculata* for. *rubrifolia*, and *O. corniculata*, (2) *O. acetosella*, (3) *O. obtriangulata* and *O. corymbosa*. Phenetic relationships of taxa were related to color of petal, but not numbers of chromosome (Table 1, Fig. 1).

Discussion

In order to further evaluate the suitability of the morphological characters traditionally used in the taxonomy of *Oxalis*, selected morphological characters were mapped onto the combined plastid rbcL DNA sequence [16]. The evolutionary patterns encountered illustrate that some morpho-

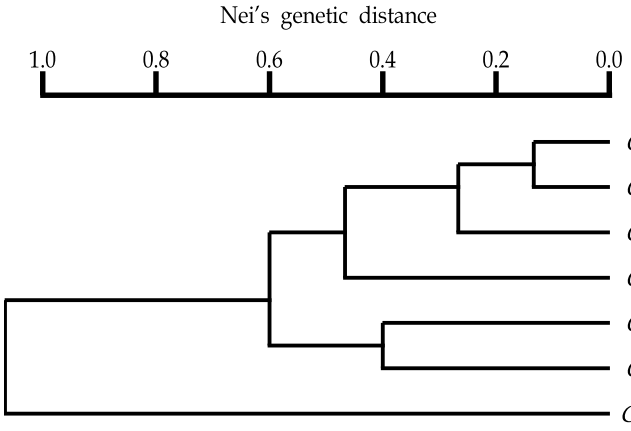

Fig. 1. A phenogram showing the relationships among eight species of genus *Oxalis* based on data of genetic distance obtained by RAPD. *Geranium koraience* is outgroup.

logical characters traditionally used in the classification of *Oxalis*. For example, morphologies (color of petal, the number of chromosome, the position and shape of ovary, locule number, numbers of ovules per locule and indumentum, the shape of fruit (capsule), the number of seeds, and the presence or absence of hairs on the epidermis of the cotyledons) have been used to separate genus *Oxalis*.

This finding suggests that morphological evolution of genus *Oxalis* was complex. In addition, morphological characteristics are restricted by their resolving power mainly because of the small number of variables available.

Several species in section Corniculatae share a base chromosome number x=5 with species in section Ripariae, while other species in the former have a x=6 [23]. In this study, phenetic relationships of taxa were related to color of petal, but not numbers of chromosome (Fig. 1). It was no one reason that the number of chromosome for species were or constant (Table 1).

RAPD analysis was applied to estimate the genetic variability in Japanese populations of *O. corniculata* [21]. They found that about 22% of the total variation was attributed to the variation component among populations. In this study, RAPD variation within species was 33.7%, while 66.3% among species (Table 5). OPA-04-16 locus and OPA-09-03 locus can be recognized as unique locus of *O. corymbosa*. Thus these loci can be used distinguish introduced species from natural Korean *Oxalis* species.

In the study with nuclear ribosomal DNA internal transcribed spacer sequences (ITS) [7], *O. corniculata* and *O. corniculata* for. *rubrifolia* were grouped into small clades, while *O. acetosella* and *O. obtriangulata* have distinct relationships. The results by RAPD were not in agreement with results obtained by ITS analysis. This is in agreement with the results of this study. In addition, additional molecular experiments such as AFLP (amplified fragment length polymorphism), microsatellites, and ITS (nuclear ribosomal DNA internal transcribed spacer sequences) are necessary to identify species. *Oxalis* is a taxonomically problematic group because of variations of morphological characters. Many botanists have had difficulty in defining specific boundaries and some have given different scientific names for same species. It is a problem for classification of *Oxalis* that plant encyclopedias in Korea have not match each other. Hybridization events are taking place in many foreign countries. It is necessary to establish the standard taxonomic keys for *Oxalis* quickly. This study can be contributed in information on the taxonomic research.

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초록 : RAPD마크를 이용한 한국 내 괭이밥속 식물의 유전적 다양성과 표현형 관계

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RAPD마크를 이용한 한국 내 괭이밥속(*Oxalis* L.) 식물의 유전적 다양성과 표현형 관계를 평가하였다. 10개의 시발체로 125개의 밴드를 얻었으며 시발체당 12.5개였다. 이들 밴드 중 121개(96.8%)는 다형성을 나타내었으며 단지 4개만 단형성을 나타내었다. 6개 분류군에서 RAPD 표현형의 평균은 3.6개(선괭이밥, 괭이밥)에서 4.8개(붉은괭이밥)였다. 종간 변이에서 선괭이밥과 자주괭이밥이 가장 낮은 변이를 나타내었으며(28.8%), 붉은괭이밥이 가장 높은 변이를 나타내었다(38.4%). 분류군 내 대립유전자좌위는 평균 32.7%였다. 종 간 전체 유전적 다양도와 종 내 유전적 다양도는 각각 0.362와 0.122였다. 종간 분화에 근거한 전체 변이의 몫(G_{ST})은 0.663이였다. 이는 전체 변이의 66.3%는 종간에 있음을 나타낸다. NJ tree에서 선괭이밥과 붉은괭이밥의 분지군은 높은 지지도를 가지며 괭이밥과 자매군을 형성하였다. 염색체의 수와 RAPD의 표현형적 관계와 일치하지 않았다.