Relationship of the Korean *Lycoris* (Amaryllidaceae) using the RAPDs analysis

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For six taxa and their populations of the Korean *Lycoris*, RAPDs analysis was performed to illustrate their systematic relationship using the UPGMA method. Six taxa were clustered into three groups. *L. squamigera*, *L. flavesceus*, *L. sanguinea* var. *koreana*, and *L. chejuensis* showed close relationships, while *L. chinensis* var. *sinudata* and *L. radiata* each revealed the independent lineage. These results were congruent with the previous cluster analysis based on morphology, cytology and palynology except the inclusion of *L. sanguinea* var. *koreana* in the first group. Populations within each species were clustered at low genetic distance, but populations of *L. radiata* were clustered at relatively high genetic distance, hence implying genetic polymorphism. RAPDs were useful in elucidating the relationship of *Lycoris* plants

Key words: *Lycoris*, RAPDs, relationship, UPGMA

The genus *Lycoris* of Amaryllidaceae has a distribution limited to East Asia (Lawrence, 1951; Kurita, 1987; Tae and Ko, 1996a). For *Lycoris*, its morphology (Creech, 1952; Tae and Ko, 1995b), cytology (Nishiyama, 1928; Inariyama, 1953; Kurita, 1978; Tae and Ko, 1991, 1996d), palynology (Inariyama, 1931; Koyama, 1976; Lee and Kim, 1987; Tae and Ko, 1996a), anatomy (Tae and Ko, 1996b), leaf epidermis (Tae and Ko, 1995a), taxonomy (Traub, 1957, 1958; Tae and Ko, 1996c) and phylogeny (Tae and Ko, 1997) have been investigated. Traub (1957, 1958) classified it into two subgenera, *Symmanthus* and *Lycoris*, based on the presence of white on the midrib of leaves. But Tae and Ko (1997) divided ten taxa of the genus *Lycoris* into four groups through clustering analysis, while into three groups through cladistic analysis. Hence, such

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differences raised the necessity to examine their systematic relationship on the molecular level.

Since Williams *et al.* (1990) had revealed the usefulness of polymorphisms among randomly amplified products by using polymerase chain reaction (PCR) with arbitrary primers as genetic markers for soybean and corn individuals, Demeke *et al.* (1992) adopted RAPDs to investigate the relationship of *Brassica*, and obtained very good agreement with the classical *Brassica* relationship. Adams and Demeke (1993) analysed the systematic relationship to 44 taxa of *Juniperus* using RAPDs, and insisted that RAPDs were useful to *Juniperus* taxonomy ranging from sectional to varietal levels. RAPDs have been also used by others, that is, Cho *et al.* (1994) for taxonomic analysis of *Brassica*, Marsolais *et al.* (1993) for determination of the origin of interspecific lilac hybrids, Takemori *et al.* (1994) for confirming the somatic hybrids of the potato. In order to investigate the systematic relationship of *Lycoris*, the preliminary experiments of RAPDs were performed, and they resulted in closer relationships between populations within the same species than interspecific relationship. Therefore, this study focused on illustrating the systematic relationship using RAPDs to six taxa of *Lycoris*, comparing the result with the classical relationships (Traub, 1957, 1958; Tae and Ko, 1996c) and the clustering and cladistic analyses (Tae and Ko, 1997), and evaluating the usefulness of RAPDs for the *Lycoris* systematics.

Materials and Methods

The leaf tissues were stored at -80°C until DNA extraction. The treated six taxa (*L. chinensis* var. *sinuolata*, *L. squamigera*, *L. radiata*, *L. flavesceus*, *L. chejuensis*, *L. sanguinea* var. *koreana*) of *Lycoris* were collected from various locations in Korea (Table 1), and five individuals were randomly sampled from each population for DNA extraction.

DNA EXTRACTION: DNA was extracted from leaf tissue using the CTAB procedure of Doyle and Doyle (1987) with the addition of a phenol extraction.

AMPLIFICATION CONDITIONS: Polymerase chain reactions (PCR) were performed in a volume of 50 μ l reaction solution containing 2.5mM MgCl₂, 250 μ M dNTPs, 10-mer random primer (Table 2), 50ng genomic DNA and 1 unit Taq DNA polymerase. Amplification was accomplished in Perkin Elmer Cetus DNA Thermal Cycler programmed for 40 cycles of 1 min. at 94°C, 1 min. at 35°C, 1 min. at 72°C. PCR products were analyzed by electrophoresis in 1.5% agarose gels, stained with ethidium bromide (EtBr), and visualized by illumination with UV light. As molecular

Table 1. Materials used in this study, and their collection sites.

Taxon	Korean name	Collection sites	Population abbreviation
<i>L. chinensis</i> Traub var.	Jin - No - Rang -	CB: Naejangsan	CHI
<i>sinuolata</i> K. Tae et S. Ko	Sang-Sa-Hwa		
<i>L. chejuensis</i> K. Tae et S. Ko	Che-Ju-Sang-Sa-Hwa	CJ: Sogwangso-ri CJ: Andok Valley	CHS CHA
<i>L. flavesceus</i> M. Kim et S. Lee	Buk - No - Rang - Sang-Sa-Hwa	CN: Paegyongsan KG: Kanghwa	FLP FLK
<i>L. radiata</i> (L'Hérit.) Herb.	Sok-San	CB: Sonunsan KN: Ssanggyersa CN: Paegyongsan	RAS RASs RAP
<i>L. squamigera</i> Maxim.	Sang-Sa-Hwa	TJ: Gisung-dong CN: Paegyongsan	SQG SQP
<i>L. sanguinea</i> Maxim. var. <i>koreana</i> (Nakai) Koyama	Baek-Yang-Got	CN: Paegyongsan	SKO

CN, Chollanamdo; CB, Chollabukdo; CJ, Chejudo; KN, Kyongsangnamdo; KG, Kyonggido; TJ, Taejeon

Table 2. Sequences of primers used in this study for the RAPDs

Primer (Code No.)	Sequence (5'→3')
A (497)	GCATAGTGCG
B (439)	GCCCCTTGAC
C (437)	AGTCCGCTGC
D (438)	AGACGGCCGC

All primers were purchased from the University of British Colombia.

weight marker, Bst E II digested Lamda DNA was used(Fig. 1).

DATA ANALYSIS: The RAPDs bands were scored as: 0= no band, 1= band present. Uncertain bands were excluded in data matrix (Table 3), from which genetic similarity coefficients (Table 4) were computed using NTSYS-pc (ver. 1.70, Rohlf, 1992). Similarity measures were prepared using UPGMA (Unweighted pair-group method using arithmetic average).

Fig. 1. Random amplified polymorphic DNA of *Lyxoris* in Korea. A, Primer code No. 497 (GCATAGTGCG); B, No. 439 (CCCCCTTGAC); C, No. 437 (AGTCCGCTGC); D, No. 438 (AGACGGCCGC); M, Bst E II digest of Lambda DNA; 1, *L. chinensis* var. *sinuolata* (Naejangsan); 2, *L. squamigera* (Gisung-dong); 3, *L. squamigera* (Paegyongsan); 4, *L. radiata* (Paegyongsan); 5, *L. radiata* (Ssanggyersa); 6, *L. radiata* (Sonunsan); 7, *L. flavescens* (Paegyongsan); 8, *L. flavescens* (Kanghwa); 9, *L. chejuensis* (Sogwangso-ri); 10, *L. chejuensis* (Andok Valley); 11, *L. sanguinea* var. *koreana* (Paegyongsan)

Table 3. Data matrix for RAPD analysis of *Lycoris*

Taxa		CHI	SQG	SQP	RAP	RASs	RAS	FLP	FLK	CHS	CHA	SKO
bp												
1900		0	0	0	1	1	1	0	0	0	0	0
1640		0	0	0	1	1	1	0	0	0	0	0
1420		0	0	0	0	0	0	0	0	0	0	1
1250		0	1	1	0	0	0	1	1	0	0	1
1230		1	1	1	0	0	0	1	0	0	0	0
1220		1	0	0	0	0	0	0	0	0	0	0
1200		1	1	1	1	0	1	1	1	1	1	1
1020		0	0	0	1	1	0	0	0	0	0	0
720		1	0	0	1	1	1	1	1	1	1	1
710		0	0	0	0	1	0	0	0	0	0	0
700		1	1	1	0	0	1	1	1	1	1	1
690		1	1	1	1	1	1	1	1	1	1	1
665		0	0	0	1	1	0	0	0	0	0	0
570		1	1	1	1	1	1	1	1	1	1	1
525		1	1	1	1	1	1	1	1	1	1	1
520		1	1	1	0	0	0	1	1	1	0	1
380		0	0	0	1	1	1	0	0	0	0	0
350		0	0	0	1	1	1	0	0	0	0	0
300		1	0	0	0	0	0	0	0	0	0	0
270		0	1	0	0	0	0	0	0	0	0	0
200		0	0	0	1	1	1	0	0	0	0	0
175		0	0	0	1	0	1	0	0	0	0	0
165		1	1	1	1	1	1	1	1	1	1	1
160		1	1	1	0	1	1	1	1	1	1	1
135		0	0	0	0	1	1	0	0	0	0	0

Table 4. Genetic similarity matrix made from data matrix

	CHI	SQG	SQP	RAP	RASs	RAS	FLP	FLK	CHS	CHA	SKO
CHI	0.00000										
SQG	0.24418	0.00000									
SQP	0.19652	0.04766	0.00000								
RAP	0.77022	0.90904	0.86138	0.00000							
RASs	0.80472	0.94353	0.89588	0.18865	0.00000						
RAS	0.51704	0.60706	0.55941	0.18865	0.22314	0.00000					
FLP	0.13882	0.09531	0.04766	0.72672	0.76121	0.47353	0.00000				
FLK	0.19652	0.15302	0.10536	0.67906	0.71356	0.42588	0.04766	0.00000			
CHS	0.14384	0.21812	0.17046	0.61638	0.66088	0.37320	0.10034	0.05268	0.00000		
CHA	0.20273	0.29276	0.24510	0.56749	0.60199	0.31430	0.15923	0.11157	0.05889	0.00000	
SKO	0.24418	0.20067	0.15302	0.72672	0.76121	0.47353	0.09531	0.04766	0.10034	0.15923	0.00000

Results and Discussion

RAPDs analysis was performed in order to illustrate genetic relationships to six taxa of the Korean *Lycoris* and to populations of each species. The phenogram was prepared from the data matrix using UPGMA cluster (Fig. 2). The available bands were 27, which ranged from 135 to 1900 bp.

The treated six taxa including their populations were clustered into three groups (Fig. 2). The first group composed of *L. squamigera* (SQG, SQP), *L. flavescentis* (FLP, FLK), *L. chejuensis* (CHA, CHS) and *L. sanguinea* var. *koreana* (SKO) was clustered at 0.168 of genetic distance. The second group, *L. chinensis* var. *sinuolata* (CHI), and the third group, *L. radiata* (RAP, RASs, RAS) was also independently clustered at 0.195 and 0.206, respectively (Fig. 2). This clustering pattern is congruent with their phylogeny based on morphological characteristics, in fact that they are clustered into three groups like above except *L. sanguinea* var. *koreana* (Tae and Ko, 1996c, 1997). This result as well as morphological classification (Tae and Ko, 1996 c) and cladistic and clustering analyses based on morphological, cytological and palynological characteristics (Tae and Ko, 1997) did not support Traub's classification (1957, 1958), which was based on morphological characteristics, i.e., presence of white stripe on the midrib of leaves and arrangement of perianths. The presence of white stripe sometimes, however, is not distinct and the perianth arrangement is variable in certain taxa. Especially, the perianth of *L. squamigera* (SQG, SQP) of subgenus *Symmanthus* under his

Fig. 2. UPGMA cluster phenogram of *Lycoris* based on RAPD data. Symbols and their collecting site of OTUs are as the followings; CHI, *L. chinensis* var. *sinuolata* (Naejangsan); SQG, *L. squamigera* (Gisung-dong); SQP, *L. squamigera* (Paegyongsan); RAP, *L. radiata* (Paegyongsan); RASs, *L. radiata* (Ssanggyersa); RAS, *L. radiata* (Sonunsan); FLP, *L. flavesceus* (Paegyongsan); FLK, *L. flavesceus* (Kanghwa); CHS, *L. chejuensis* (Sogwangso-ri); CHA, *L. chejuensis* (Andok Valley); SKO, *L. sanguinea* var. *koreana* (Paegyongsan)

classification shows actinomorphic arrangement before full bloom but they are changed into zygomorphic arrangement after anthers are opened (Tae and Ko, 1996c).

For four primers, different populations of *L. squamigera*, *L. flavesceus*, *L. chejuensis* and *L. radiata* showed different patterns of bands (Fig. 1-A, B, C), but for others, showed the same patterns (Fig. 1-D). Hence they have very low genetic variations within species. Populations of *L. radiata* (Paegyongsan, Ssanggyersa and Sonunsan) were independently clustered at 0.189 and 0.206 of genetic distance, which meant that *L. radiata* was genetically polymorphic, compared with above three species. Independences of *L. radiata* and *L. chinensis* var. *sinuolata* were coincident with morphological and phylogenetic results of Tae and Ko (1995b, 1996c, 1997). Among the treated taxa, *L. radiata* was a cytologically unique species of which chromosome number

was $2n=33$ ($x=11$, triploid), and all of its chromosomes basically consisted of only R-shaped chromosome, although B chromosomes and V-shaped chromosomes were sometimes occurred (Tae and Ko, 1996d). In particular, the fact that the populations of RASs (Ssanggyersa) and RAP (Paegyongsan) were firstly clustered may be due to the difference of habitats (inland vs. seashore).

In conclusion, RAPDs of *Lycoris* plants revealed that *L. squamigera*, *L. flavesceus*, *L. sanguinea* var. *koreana*, and *L. chejuensis* were closely related taxa, while *L. chinensis* var. *sinuolata* and *L. radiata* were the independent lineage in their systematic relationship. However, for *L. sanguinea* var. *koreana* it is necessary to investigate its possibility as an independent group with respect to flower color, perianth arrangement and karyotype. RAPDs was thought to be very useful in illustrating the relationship of *Lycoris* plants.

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RAPDs 분석에 의한 한국산 상사화속의 유연관계

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적 요

한국산 상사화속 6분류군을 대상으로 UPGMA 유집방법에 의한 RAPD분석을 수행하여 이들의 계통학적 유연관계를 분석하였고, RAPD 분석방법의 유용성을 파악하였다. 이 결과 6분류군은 크게 3 group을 형성하였고, 첫 번째 group인 *L. squamigera*, *L. flavescens*, *L. sanguinea* var. *koreana* 및 *L. chejuensis*는 분류군간에 유연관계가 매우 가깝고, 두 번째 group인 *L. chinensis* var. *sinudata*와 세 번째 group인 *L. radiata*는 각각 독립적으로 유집되었다. 이러한 결과는 *L. sanguinea* var. *koreana*가 첫 번째 group에 유집된 것을 제외하고는 이전의 형태학적 및 유집분석 결과와 유집형태에 있어 일치하였다. 각 분류군내의 군집들은 낮은 유전적 거리지수로 유집되었으나, *L. radiata*는 군집간의 유전적 거리지수가 비교적 높게 나타나 유전적으로 다형질성을 보였다. RAPDs 분석은 상사화속 식물의 유연관계를 파악하는데 유용한 분석방법으로 생각된다.

주요어: 상사화속, RAPDs, 유연관계, 비가중산술법

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