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Identification and Classification of the Genus *Lycoris* Using Molecular Markers

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Abstract. To evaluate the germplasm of the genus *Lycoris*, 80 samples of *Lycoris* species and unidentified accessions were collected from various sources in the United States, China, Japan, and Korea for identification and classification using polymorphic bands generated by the random amplified polymorphic DNA (RAPD) method. Twenty three-random 10-mer primers were initially used to screen and select primers for polymorphisms using DNA from 6 selected *Lycoris* accessions. Using six selected primers, all accessions, divided into three groups, were subjected to amplification for a preliminary identification: group A of 27 accessions of *L.* × *albiflora* Koidzume, *L. anhuiensis* Xu & Fan, *L. aurea* Herbert, *L. caldwellii* Traub, *L. chejuensis* K.H. Tae & S.C. Ko, *L. flavescens* var. *flavescens* M. Kim & S. Lee, *L. chinensis* var. *chinensis* Traub, *L. chinensis* Traub var. *sinuolata* K.H. Tae & S.C. Ko, *L. elsiae* Traub, *L. flavescens* var. *flavescens* M. Kim & S. Lee, and two unknowns, group B of 27 accessions of *L.* × *haywardii* Traub, *L.* × *houdyshelii* Traub, *L. incarnata* Comes ex C. Spreng, *L. longituba* var. *longituba* Y. Hsu & G.J. Fan, *L. radiata* (L'Hérit) var. *radiata* Herb., *L. radiata* (L'Hérit) var. *pumila* Herb., and *L. sanguinea* var. *koreana* Nakai, and group C of 28 accessions of *L.* × *rosea* Traub & Moldenke, *L. sanguinea* Maxim. var. *sanguinea*, *L. sanguinea* Maxim. var. *kiusiana* (Makino), *L. sanguinea* var. *koreana* Nakai, *L. shaanxiensis* Xu & Hu, *L. sprengeri* Comes ex Baker, *L. squamigera* Maxim., *L. straminea* Lindley, and *L. traubii* Hayward. Variations observed with *L. aurea* Herbert could be attributed to the difference in geographical origins or misidentification. All accessions collected from Korea, *L. chejuensis* K.T. Tae & S.C. Ko (K6, K8, K11 and S17) were grouped together with *L. chinensis* var. *chinensis* Traub (S8) and *L. flavescens* var. *flavescens* (S16). *Lycoris longituba* (JW11) and *L. radiata* var. *pumila* (S14) that were not clustered with other *L. longituba* var. *longituba* accessions (B6 and S6) and *L. radiata* var. *pumila* (D1, HM4, and D8) may have been mislabeled in the trade. *Lycoris radiata* var. *radiata* could be divided into three or possibly four sub-groups, two accessions (Y1 and JW1) being distinctly related to other accessions (K1, K10, B7, B8 and HM5). Clustering of *L.* × *rosea, L. sanguinea, L. sprengeri, L. squamigera*, and *L. traubii* were variable within accessions of a given species. Based on results from the preliminary analyses, *L.* × *albiflora* (O4 and S13), *L. anhuiensis* (S5), *L. aurea* (HM1), *L. caldwellii* (JW10), *L. chejuensis* (K11), *L. chinensis* var. *chinensis* (S7); *L. chinensis* var. *sinuolata* (S8), *L. elsiae* (JW16), *L. flavescens* var. *flavescens* (S16), *L.* × *haywardii* (B5), *L.* × *houdyshelii* (D3), *L. incarnata* (HM6), *L. longituba* var. *longituba* (S6), *L. radiata* var. *radiata* (K1), *L. radiata* var. *pumila* (D8), *L.* × *rosea* (B1), *L. sanguinea* var. *sanguinea* (S2), *L. sanguinea* var. *kiusiana* (S3), *L. sanguinea* var. *koreana* (K3), *L. shaanxiensis* (JW3), *L. sprengeri* (B3), *L. squamigera* (H6), *L. straminea* (JW18), *L. traubii* (L1 and S12), and unidentified accessions from Korea (K2 and K5) were selected for a final identification and classification. Clustering of unidentified accession K2 collected from Cheju Island, Korea, together with *L. incarnata* (HM6), karyotype (2n=29+B=30=3M+1M+1m+5T+20A), and flower morphology of K2 indicated that K2 was identified as *L. incarnata* native to Cheju Island. Clustering of *Lycoris* species based on RAPD polymorphic bands generally agrees with the taxonomical treatments based on the morphological and phenological observations.

Additional key words: Amaryllidaceae, germplasm evaluation, random amplified polymorphic DNA, karyotype, floral morphology, taxonomy

Introduction

The genus *Lycoris* consists of about 20 species distributed in warm temperate and subtropical zones from southwestern China, southern Korea and Japan with a few in the areas of northern Indochina and Nepal. To date, more than 29 taxa of *Lycoris* has been described, although some of these were classified as infraspecific rank, hybrids, or questionable for their existence in nature (Hsu, et al., 1994). Many *Lycoris* species, except *L. radiata*, commonly known as Red Spider Lily, and *L. squamigera*, known as Surprise Lily, but incorrectly in commerce as *Amaryllis Halli*, are not well known to gardeners in western countries (Waddick, 2000, 2001).

Due to natural hybridization among many fertile species, hybrids of diverse morphological features occur in nature and in cultivation. It has been difficult to identify unknown and newly acquired *Lycoris* accessions. Considering the long history of ornamental horticulture in China, some plants treated as wild

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and natural species could be actually the descendent of garden plants that were subjected to natural hybridization. Further, it is possible that botanical descriptions could have been obtained from plants of garden origin, but not from the wild population (Cooke, 2001, personal communication). Classification of the genus *Lycoris* is based traditionally on the morphology and the color of flowers (Hsu, et al., 1994). Cytological karyotype and palynological studies documented by Hsu, et al., (1994), Kurita (1987), and Lee and Kim (1987) indicate a revision of the work by Traub and Moldenke (1949) may be necessary.

Random amplified polymorphic DNA (RAPD) (Welsh and McClelland, 1990; Williams et. al., 1990) as compared to amplified fragment length polymorphism has several advantages to study the genetic diversity, plant taxonomy, population genetics, systematics, and relatedness in plant populations (Weising, et al., 1995). The RAPD technique does not require genetic or sequence information. It is easily applied to most of the species, and produces numerous polymorphic markers for analysis. Molecular markers generated by RAPD are independent from environmental influences and DNA for amplification can be obtained during any growth and development stage of plants. Because of these advantages, RAPD has been widely used to identify unknown hybrids, to discriminate cultivars, and to examine intraspecific variations in *Picea glauca* (Moench) Voss (Khasa and Dancik, 1996), *Campanula takesimana* Nakai (Kim, et al., 1996), *Olea europeana* L. (Sanz-Cortés, et. al., 2001), and *Acer griseum* (Franch) Pax (Joung, et al., 2001a).

Phylogenetic relationships in *Lycoris* have been based on morphological and cytological characteristics. Molecular markers and isozyme analysis were used to characterize a few Korean *Lycoris* (Tae and Ko, 1997; Lee, et al., 2001). However, molecular fingerprinting techniques to identify and characterize *Lycoris* taxa collected from China, Japan, and Korea were not performed. The objective of this research was to use molecular markers generated by RAPD to study phylogenetic relationships in taxa of *Lycoris* and to use this information to characterize unidentified accessions collected from Korea.

Materials and Methods

Accessions of Lycoris. Samples of 80 *Lycoris*, some of them as bulbs or leaves, were collected from various sources between 1984 and 1999 (Table 1). The origin of collection sites of some accessions from the horticultural trade were unknown or not verified. Accessions were tentatively assigned to a species (Table 2) following the description provided by the collaborators. Samples acquired from Chiba University, Japan, were verified cytologically which is consistent with the herbarium collections (Hsu, et al., 1994) and those acquired from James Waddick (Kansas City, Missouri, USA) were verified morphologically at flowering. These were treated as authentic taxa, unless noted in the text following identification.

Preliminary analysis. Twenty three primers from random primer kits A, B, and C (Operon Technologies, Alameda, CA) were screened to select informative primers using DNA from *Lycoris* samples of *L. sprengeri* (S1), *L. anhuiensis* (S5), *L. chinensis* var. *sinuolata* (S8), *L. aurea* (S11), *L.* × *albiflora* (S13), and *L. chejuensis* (S17), and 15 primers (OPA1, OPA7, OPA9, OPA11, OPB13, OPB17, OPB18, OPB19, OPC1, OPC5, OPC9, OPC10, OPC13, OPC15, OPC19) that produced a high ratio of polymorphic bands were selected. Due to the large number of samples that cannot be loaded into one gel with 30 lanes for electrophoresis, they were divided into three groups based on the descriptions obtained from the collaborators. Six primers (OPA1, OPA9, OPA11, OPB13, OPB17, and OPB18) were used for preliminary analysis; group A of 27 accessions of *L.* × *albiflora, L. aurea, L. chejuensis, L. flavescens, L. anhuiensis, L. chinensis* var. *chinensis, L. chinensis* var. *sinuolata, L. elsiae, L. flavescens* var. *flavescens*, and two unknowns, group B of 27 accessions of *L.* × *haywardii*, *L.* × *houdyshelii*, *L. incarnata*, *L. longituba* var. *longituba*, *L. radiata* var. *radiata*, *L. radiata* var. *pumila*, and *L. sanguinea* var. *koreana*, and group C of 28 accessions of *L.* × *rosea*, *L. sanguinea* var. *sanguinea*, *L. sanguinea* var. *kiusiana*, *L. sanguinea* var. *koreana*, *L. shaanxiensis*, *L. sprengeri*, *L. squamigera*, *L. straminea* and *L. traubii*. All samples of each group were loaded onto a gel. For final analysis, 15 primers were used for selected accessions based on the results from the preliminary analyses.

Characterization and identification. Following preliminary identification and characterization of all accessions that were divided into three groups excluding those accessions that could be misidentified or mis-labeled in the trade, 26 taxa were selected for a final classification based on the preliminary results. They were *L.* × *albiflora* (O4 and S13), *L. anhuiensis* (S5), *L. aurea* (HM1), *L. caldwellii* (JW10), *L. chejuensis* (K11), *L. chinensis* var. *chinensis* (S7); *L. chinensis* var. *sinuolata* (S8), *L. elsiae* (JW16), *L. flavescens* var. *flavescens* (S16), *L.* × *haywardii* (B5), *L.* × *houdyshelii* (D3), *L. incarnata* (HM6), *L. sanguinea* var. *koreana* (K3), *L. longituba* var. *longituba* (S6), *L. radiata* var. *radiata* (K1), *L. radiata* var. *pumila* (D8), *L.* × *rosea* (B1), *L. sanguinea* var. *sanguinea* (S2), *L. sanguinea* var. *kiusiana* (S3), *L. sanguinea* var. *koreana, L. shaanxiensis* (JW3), *L. sprengeri* (B3), *L. squamigera* (H6), *L. straminea* (JW18), *L. traubii* (L1 and S12), and two unidentified accessions (K2 and K5). With these samples, amplification was repeated twice. Most of the major polymorphic bands were reproduced when bands were compared with those from the preliminary and final RAPD am-plifications.

DNA extraction, PCR amplification, and data analysis. DNA was extracted from the tissue of young leaves or of the base of leaves near the neck of the bulb using the cetyitrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). Polymerase chain reaction (PCR) was carried out in 25 μL reaction mix containing 5 pmol 10-mer random primer (Operon Technologies, Inc., Alameda, CA.), 20 ng genomic DNA and PCR mix (1.5 unit Taq DNA polymerase, 10 mM Tris-HCl pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 200 mM of each dNTP). PCR amplification was 94°C for 3 min followed by 35 cycles of 95°C for 5 sec, 37°C for 30 sec, and 72°C for

Table 1. Sources of *Lycoris* accessions.

Sample identification[z]	Species	Sample identification[z]	Species
B1	*L.* × *rosea*	JW17	*L.* × *rosea*
B2	*L. chinensis* var. *chinensis*	JW18	*L. straminea*
B3	*L. sprengeri*	JW19	*L. sanguinea* var. *kiusiana*
B4	*L. aurea*	JW20	*L. traubii*
B5	*L.* × *haywardii*	K1	*L. radiata* var. *radiata* Sooncheon
B6	*L. longituba* var. *longituba*	K2	*L.* species (*L. incarnata*), Cheju Is.
B7	*L. radiata* var. *radiata* (Jiangsu Prov.)	K3	*L. sanguinea* var. *koreana*, Iksan,
B8	*L. radiata* var. *radiata* (Shaanxi Prov.)	K4	*L. squamigera*, Cheju Is.
D1	*L. radiata* var. *pumila*	K5	*L. species,* Cheju Is.
D2	*L.* × *albiflora*	K6	*L. chejuensis*, Cheju Is.
D3	*L.* × *houdyshelii*	K7	*L.* species, Iksan
D4	*L.* × *houdyshelii* (different from D3)	K8	*L. chejuensis*, Cheju Is.
D5	*L. radiata* var. *radiata,* China	K9	*L. squamigera* (?), Cheju Is.
D8	*L. radiata* var. *pumila*	K10	*L. squamigera* (?) (*L. radiata* var. *radiata*), Cheju Is.
D10	*L.* × *houdyshelii* (different from from D3)	K11	*L. chejuensis* (White-flower), Cheju Is.
H1	*L. aurea*, 10419	L1	*L. traubii*
H2	*L. sanguinea*, 10421	O4	*L.* × *albiflora*
H3	*L.* × *albiflora*, 10418	O5	*L. sanguinea* var. *sanguinea*
H4	*L. radiata* var. *radiata*, 10420	O6	*L. sanguinea* var. *kiusiana*
H5	*L. sprengeri*, 110603	O8	*L. aurea* var. *traubii*
H6	*L. squamigera*	O9	*L. radiata* var. *radiata*
HM1	*L. aurea*	S1	*L. sprengeri*, Hangzhou, China
HM2	*L. sprengeri*	S2	*L. sanguinea* var. *sanguinea*, Chiba, Japan
HM3	*L.* × *albiflora*	S3	*L. sanguinea* var. *kiusiana*, Nagasaki, Japan
HM4	*L. radiata* var. *pumila* (type II)	S4	*L. sanguinea* var. *koreana*, Naejang Mt., Korea
HM5	*L. radiata* var. *radiata* (type I)	S5	*L. anhuiensis*, Langya Mt., Anhui, China
HM6	*L. incarnata*	S6	*L. longituba* var. *longituba*, Tang Mt., Nanjing, China
JY1	*L. sprengeri*	S7	*L. chinensis* var. *chinensis*, Mogan Mt., Zhejiang, China
JY2	*L. squamigera*	S8	*L. chinensis*, var. *sinuolata,* Naejang Mt., Korea
JW1	*L. radiata* var. *radiata*	S9	*L. aurea* var. *latifolia*, Guangdong, China
JW3	*L. shaanxiensis* (?)	S10	*L. aurea* var. *aurea*, Hongkong, China
JW4	*L. sprengeri*	S11	*L. aurea* var. *purpurea*, Hunan, China
JW5	*L. incarnata*	S12	*L. traubii*, Kagoshima, Kyushu, Japan
JW6	*L.* × *houdyshelii*	S13	*L.* × *albiflora*, Kagoshima, Japan
JW7	*L. squamigera*	S14	*L. radiata* var. *pumila*, Mogan Mt., Zhejiang, China
JW8	*L. sanguinea* (?)	S15	*L. radiata* var. *radiata,* Chiba, Japan
JW9	*L. chinensis* var. *chinensis*	S16	*L. flavescens* var. *flavescens*, Naejang Mt., Korea
JW10	*L. caldwellii*	S17	*L. chejuensis*, Cheju Is., Korea
JW11	*L. longituba*	Y1	*L. radiata* var. *radiata*
JW16	*L. elsiae*	Y2	*L. aurea*

[z]B: Zhao, China, bulb; D: Bobbie Lively-Diebold, USA, leaf; H: van Bourgondien & Sons, bulb; HM: M. Hamada, Japan, bulb; JY: M. Hiromitsu, Japan, bulb; JW: James W. Waddick, USA, leaf; K: Jong Suk Lee, Jong-Suk Lee, Yoon Jeom Park, Jeung Keun Suh, Jeong Seob Song, Korea, leaf or bulb; L: Louisiana Nursery, USA, bulb; O: Eikou Ooyabu and Mr. Oono, Japan, bulb.

60 sec and a final extension at 72°C for 3 min. The RAPD fragments were separated by electrophoresis on 1.2% agarose gel and visualized with ethidium bromide. Amplification products were scored and polymorphic bands were clustered using an unweighted pair-group method with arithmetic average (UPGMA) or neighbor-joining analysis of genetic distances (Saitou and Mei, 1987).

Results and Discussion

Preliminary analysis for identification and classification of known and unknown accessions. Some accessions in a given species were not clustered together according to the dendrogram composed by the polymorphic RAPD bands. In group A, *L.* × *albiflora* (HM3) was not clustered with other *L.* × *albiflora* (O4 and H3), yet all were collected from Japan (Fig. 1). Two accessions (S13 and D2) of *L.* × *albiflora* are similar even though they were collected from the United States (D2) and Japan (S13). *Lycoris elsiae* from Australia (JW16) and the un-

Table 2. Karyotype, phenology, and native site of the genus *Lycoris*.

Species	Sub genus	Karyotype[z]	Phenology (leaf/inflorescence[y])	Native to
L. × *albiflora* Koidzumi	*Lycoris*	2n=5M+1T+11A=17, 2n=5M+1T+11A+1m=18	A/Sept.-Oct.	Japan
L. anhuiensis Xu & Fan	*Symmanthus*	2n=6M+10T=16	S/Aug.	China
L. argentea Worseley	*Symmanthus*	no report		Burma
L. aurea Herbert	*Lycoris*	2n=8M+6T=14 2n=7M+8T=15 2n=7M+1A+7T=15, 2n=6M+10T=16	leaf: at or before inflorescence: Aug.-Sept.	China
L. caldwellii Traub	*Lycoris*	2n=6M+10T+11A=27	S/Aug.-Sept.	China
L. chejuensis K.H. Tae & S.C. Ko	*Symmanthus*	2n=3M+4T+1sm+22A=30	S/Aug	Korea
L. chinensis var. *chinensis* Traub	*Lycoris*	2n=6M+10T=16	S/July-Aug.	China
L. chinensis Traub var. *sinuolata* K.H. Tae & S.C. Ko	*Lycoris*	2n=6M+8T+2sm=16	S/July	Korea
L. elsiae Traub	*Lycoris*	2n=17	A/Aug.-Sept.	Japan
L. flavescens var. *flavescens* M. Kim & S. Lee	*Symmanthus*	2n=3M+4T+1sm+11A=19	S/Aug.	Korea
L. guangxiensis Xu & Fan	*Lycoris*	no report	S/July-Aug.	China
L. × *haywardii* Traub	*Symmanthus*	2n=22A=22	A/July-Aug.	China
L. × *houdyshelii* Traub	*Lycoris*	2n=3M+6T+21A=30	A/July-Aug.	China
L. incarnata Comes ex C. Spreng	*Symmanthus*	2n=3M+1M′+3T+22A+1m=30	S/Sept.	China, Japan
L. longituba var. *longituba* Y. Hsu & G.J. Fan	*Symmanthus*	2n=6M+10T=16	S/July-Aug.	China
L. radiata (L'Hérit) Herb. var. *radiata*	*Lycoris*	2n=33A, 2n=31A+1M=32	A/Sept.-Oct.	China, Japan Korea, Nepal
L. radiata (L'Hérit) Herb. var. *pumila* Grey	*Lycoris*	2n=22A=22	A/Sept.-Oct.	China
L. × *rosea* Traub & Moldenke	*Lycoris*	2n=22A=22	A/Sept.	China
L. sanguinea Maxim. var. *sanguinea*	*Symmanthus*	2n=22A=22	S/July-Aug.	Japan
L. sanguinea Maxim. var. *kiusiana* (Makino)	*Symmanthus*	2n=22A=22	S/July-Aug.	Japan
L. sanguinea var. *koreana* Nakai	*Symmanthus*	2n=22A=22	S/Aug.	Korea
L. shaanxiensis Xu & Hu.	*Lycoris*	no report	S/Aug.-Sept.	China
L. sprengeri Comes ex Baker	*Symmanthus*	2n=22A=22	S/Aug.-Sept.	China
L. squamigera Maxim.	*Symmanthus*	2n=6M+10T+11A=27	S/Aug.	China, Japan Korea
L. straminea Lindley	*Lycoris*	2n=3M+5T+11A=19	S/Aug.	China
L. traubii Hayward	*Lycoris*	2n=10M+2T=12 2n=9M+4T=13 2n=8M+6T=14	A/Sept.-Oct.	Japan, Taiwan

[z]Refer Hsu, et al., (1994) for a complete list of karyotype.
[y]Season of leaf emergence/month of flowering.

identified *Lycoris* from Korea (K5 and K7) were clustered with all *L.* × *albiflora* accessions. *Lycoris elsiae*, native to Japan, resembles *L.* × *houdyshelii* or *L.* × *albiflora*, and is considered a hybrid involving *L. radiata* (Cooke and Phan, 1999) or a hybrid between *L. traubii* and *L. sanguinea* var. *kiusiana* (Hsu, et al., 1994). Two unidentified accessions, K5 and K7, obtained as white-flower *Lycoris* from Cheju Island, were clustered together with *L.* × *albiflora* (HM 3). This suggested that they could be related to *L.* × *albiflora*. *Lycoris* × *albiflora* that was reported as native to Korea (Park, 1994; Tae, et al., 1987) should be characterized as *L. chejuensis* (Kim and Lee, 1991), based on karyotype and morphological descriptions. However, K5 and K7 were not clustered with either *L. chejuensis* (K6, K8, K11, S17) or *L. flavescens* var. *flavescens* (S16) and *L. chinensis* var. *sinuolata* (S8).

Three *L. aurea* accessions (S9, S10, and S11) were not clustered with other *L. aurea* accessions (H1, B4, and HM1) (Fig. 1). *Lycoris aurea* S9 and Y2 were clustered together, but clustered separately from other *L. aurea* accessions, particularly from S10 and S11. It is not certain whether this diversity of *L. aurea* could be attributed to the difference of geographical origins or chromosome numbers. Floral morphology, but not leaf morphology, of three *L. aurea* (S10, S11, and S12) was similar. Some *L. aurea* accessions could be *L. traubii*, another yellow flowered species that occurs only in Taiwan and southern Japan (Hsu, et al., 1994). These two species are frequently confused in the bulb trade.

Three accessions of *L. chejuensis* (K6, K11 and S17) collected from Cheju Island were grouped together and these accessions were clustered with *L. chinensis* var. *sinuolata* (S8) (Fig. 1). *Lycoris chejuensis* (K8) collected from Cheju Island

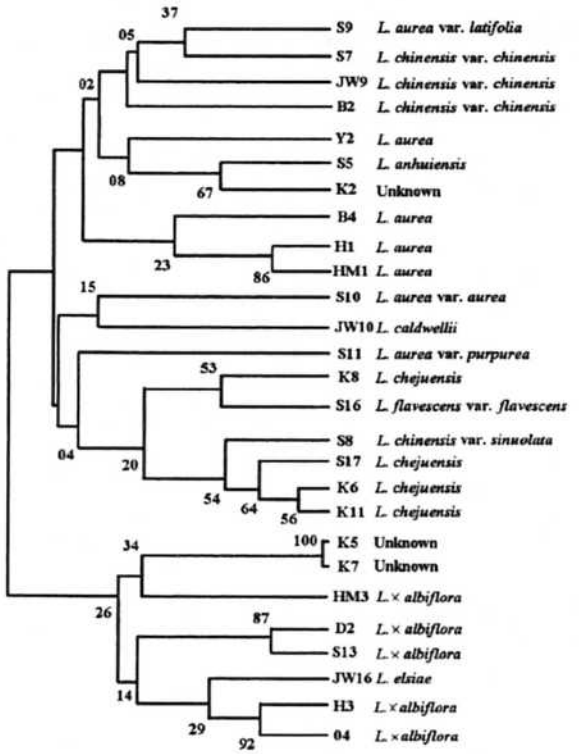

Fig. 1. Clustering of Group A *Lycoris* by unweighted pair group method using arithmetic average.

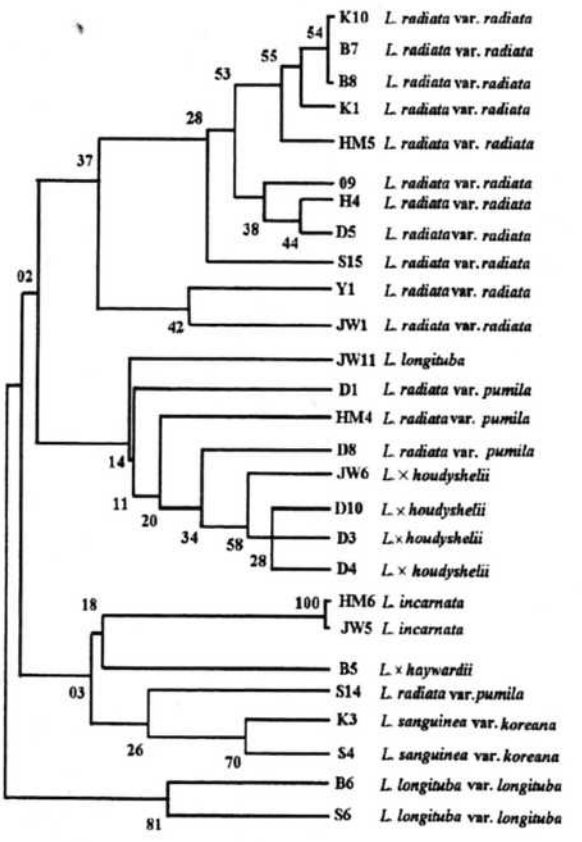

Fig. 2. Clustering of Group B *Lycoris* by unweighted pair group method using arithmetic averages.

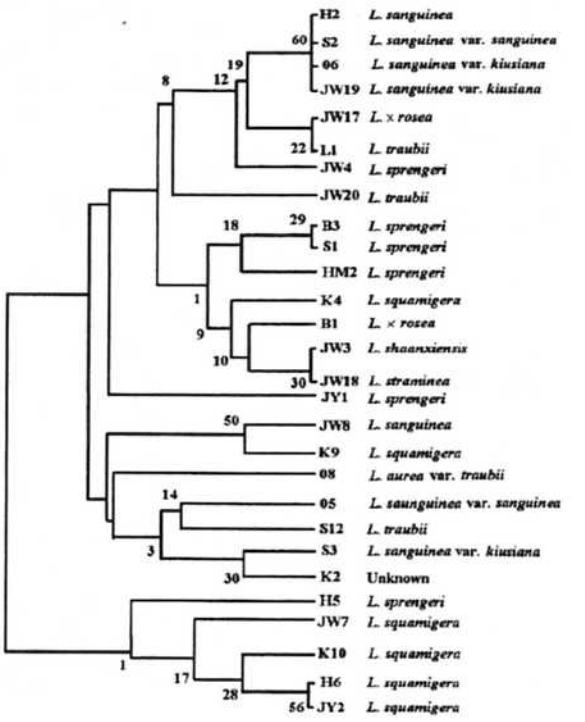

Fig. 3. Clustering of Group C *Lycoris* by unweighted pair gr method using arithmetic average.

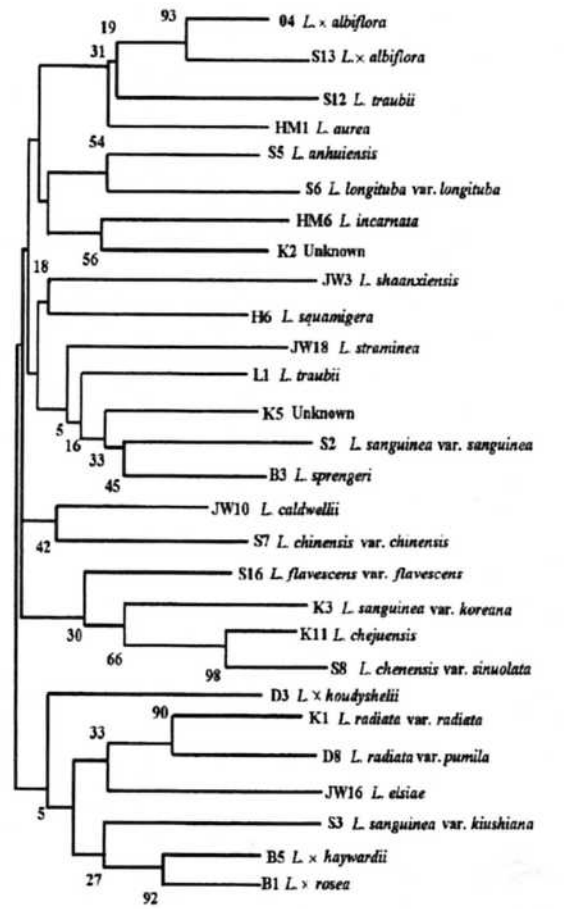

Fig. 4. Clustering of selected *Lycoris* taxa by neighbor-joining an ysis.

was clustered with *L. flavescens* var. *flavescens* (S16) which was collected from Mt. Naejang in the Korean peninsula. This suggests that *L. chejuensis*, *L. flavescens*, and *L. flavescens* var. *uydoensis* (Kim, 1996) are closely related. Three accessions of *L. chinensis* var. *chinensis* (B2, S7, and JW9) from China that were clustered together were distantly related to Korean accession (S8) of *L. chinensis*. This suggested that *L. chinensis* native to Korea is different from *L. chinensis* native to China. Unidentified accession K2 collected from Cheju Island was clustered with *L. anhuiensis* (S5) from China, although the floral morphology and flower color between these two are different. From group A, accessions of HM1, JW10, JW16, K2, K11, O4, S5, S7, S8, S13, and S16 (Table 1, 2) were selected for a final analysis, excluding accessions which were mis-identified in the trade.

In group B, *L. longituba* (JW 11) and *L. radiata* var. *pumila* (S14) that were not clustered with other *L. longituba* var. *longituba* accessions (B6 and S6) or *L. radiata* var. *pumila* (HM4, and D8), respectively, may have been mislabeled or misidentified (Fig. 2). *L. radiata* (D1) should be identified as *L. radiata* var. *pumila*. Each of two accessions of *L. incarnata* (HM6 and JW 5), *L. longituba* (B6 and H6), and *L. sanguinea* var. *koreana* (K3, S4) were clustered together. *Lycoris radiata* which was obtained as Type II *L. radiata* (HM 4) (Table 1) should be recorded as *L. radiata* var. *pumila*, while Type I *L. radiata* (HM5) as *L. radiata* var. *radiata* which was clustered together with other accessions of *L. radiata* var. *radiata*. *Lycoris radiata* var. *radiata* could further be sub-divided into three groups, but it is considered as monophyletic in spite of the multiple origins at different distribution areas. Four accessions of *L.* × *houdyshelii* were grouped together and also with *L. radiata* var. *pumila* (D1, D8, and HM4). *Lycoris* × *houdyshelii* is considered a triploid hybrid (2n=3M+6T+21A=30) between *L. longituba* var. *longituba* that produced the gamete having 3M (metacentric chromosomes) + 5T (telocentric chromosomes), and one of following with an unreduced 22A (acrocentric chromosomes): *L. radiata* var. *pumila*, *L.* × *rosea*, or *L. sprengeri* (Kurita, 1987). From group B, accessions of B5, D3, D8, HM6, K1, S6, and S12 were selected for a final analysis (Table 1, 2).

Lycoris × *rosea, L. sanguinea, L. sprengeri, L. squamigera* and *L. traubii* in group C were quite variable among accessions of a given species (Fig. 3). This variability could be attributed to the genotypic variations resulting from their wide distribution and sexual reproduction. Two accessions (K4 and K9) of *Lycoris squamigera* not clustered with four other accessions (JW7, H6, JY2, and K10) could have been misidentified. Three *L. sprengeri* accessions (S1, B3, and HM2) were clustered, while three other accessions of *L. sprengeri* (H5, JY1, and JW4) were very distantly related. Both *L. traubii* (L1 and S12) and *L.* × *rosea* (JW14 and B1) were also clustered distantly. *Lycoris. traubii* (L1) could be a hybrid or similar to *L. rubroaurantiaca* (2n=17) which is considered a hybrid between *L. traubii* and *L. sanguinea*. *Lycoris traubii* (S12) and *L. aurea* var. *traubii* (O8) were clustered together. When unidentified *Lycoris* accession K2 was included in group C, it was clustered together with *L. sanguinea* var. *kiusiana* (S3). From group C, accessions of B1, B3, H6, JW3, JW18, K2, L. S2, S3, and S12 were selected for a final analysis (Table 1, 2).

According to the results from the initial identification, 28 acces sions including two accessions (K2 and K5) were selected for final classification and possible identification of two unidentifie accessions, K2 and K5. When accessions of the same taxon wer not clustered closely, for example, *L.* × *rosea*, selection was base on the taxonomical treatments (Hsu, et al., 1994), thus selectin accession B1 for *L.* × *rosea*. Further, questionable accessions suc as *L. shaanxiensis* (JW3) and *L. traubii* (L1) were included. The were *L.* × *albiflora* (O4 and S13), *L. anhuiensis* (S5), *L. aure* (HM1), *L. caldwellii* (JW10), *L. chejuensis* (K11), *L. chinensi* var. *chinensis* (S7); *L. chinensis* var. *sinuolata* (S8), *L. elsia* (JW16), *L. flavescens* var. *flavescens* (S16), *L.* × *haywardii* (B5 *L.* × *houdyshelii* (D3), *L. incarnata* (HM6), *L. longituba* var. *lon gituba* (S6), *L. radiata* var. *radiata* (K1), *L. radiata* var. *pumil* (D8), *L.* × *rosea* (B1); *L. sanguinea* var. *sanguinea* (S2), *L. san guinea* var. *kiusiana* (S3), *L. sanguinea* var. *koreana* (K3), *L shaanxiensis* (JW3), *L. sprengeri* (B3), *L. squamigera* (H6), *L straminea* (JW18), *L. traubii* (L1 and S12), and two unidentifie accessions (K2 and K5).

Characterization and identification of Lycoris. All *Lycori* accessions could be clustered into 4 or 5 broad groups (Fig. 4). The first group consisted of *L.* × *albiflora* with a white flowe (Fig. 5-A), *L. traubii* from Japan with a yellow flower (Fig. 5-CC), *L. aurea* with a yellow flower (Fig. 5-C, 5-D, 5-E), *L anhuiensis* with a yellow flower (Fig. 5-B), *L. longituba* var *longituba* with a white flower (Fig. 5-R), *L. incarnata* with a white to light rose pink with a reddish band in tepals (Fig. 5-Q), and unidentified K2 with a flower color the same as *L. incarnata* (Fig. 5-T). *Lycoris* × *albiflora, L. traubii*, and *L. aurea* could be grouped separately from *L. anhuiensis, L. longituba var. longituba, L. incarnata*, and unidentified K2.

The highly sterile *L.* × *albiflora* (2n=5M+1T+11A=17 and 2n=5M+1T+11A+1m=18) is considered a hybrid between *L. traubii* and *L. radiata* according to cytological and morphological data (Kurita, 1987). The present results by RAPD analysis may not support this hypothesis. *L. traubii* (S12), which is clustered closely with *L. aurea* (HM1) and *L.* × *albiflora*, but clustered distantly to *L. traubii* (L1) suggests that *L. traubii* (L1) avail- able on the market in the United States could be of hybrid origin, that multiple species are present, or misidentified. To identify the nature of *L. traubii* (L1), other methods such as isozyme analysis (Lee, et al., 2001) and karyotype examination should be performed. Although it was suspected that *L. traubii,* now in commercial, could be similar to *L. aurea* (Cooke and Phan, 1999; J. Waddick, Personal communication, 2000), *L. traubii* (L1) was clustered with *L. straminea* and *L. sanguinea* var. *sanguinea* and also *L. sprengeri* (Fig. 4) and with *L.* × *rosea* (Fig. 3). The stigma tip in *L. traubii* was pink and the style was curved upward. *Lycoris traubii* (Fig. 5-DD) that was introduced from the Netherlands to Korea (N. B. Park, personal communication, 2001) has a yellow tip and a straight stigma. *Lycoris traubii* seems closely related to *L. aurea*, its flower is smaller and hardier to cold (Waddick, 2000). Both *L. traubii*