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RESEARCH ARTICLE

Organization of the 5S rRNA gene units in Korean *Lilium* species

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Abstract

The 5S rRNA gene unit was characterized from *Lilium* species distributed in Korea. Sequence analyses revealed that the 5S rDNA, harboring a highly conserved coding region (120 bp) and a divergent non transcribed spacer (NTS), ranged from 470 bp to 640 bp. The NTS regions showed length heterogeneity (350 bp-520 bp) due to the presence of several indels, and extensive sequence divergence among the species. Despite heterogeneity in length and nucleotide composition, the NTS regions possess some common features across the species, which include a T stretch region CTTTT and an identical motif of 11 bp (CAATGTATGAC) at the 3′ and 5′ flanking sequence of the coding region, respectively. These may play a role in the initiation and termination of transcription. The chromosomal distribution of the 5S rRNA gene was mapped on the long arm of chromosome 3 by fluorescence *in situ* hybridization (FISH). The phylogenetic analysis based on the NTS sequence broadly divided the *Lilium* species into two major groups which were referred to as the section *Sinomartagon* and *Martagon* by Comber's classification of the genus *Lilium*. The present study showed that the 5S rDNA sequence was very useful to unravel the genetic relationships among *Lilium* species.

Keywords *Lilium*; 5S rRNA gene; NTS; genetic variation; FISH mapping

Introduction

The genus *Lilium*, a bulbous group of the family Liliaceae, consists of approximately 100 species, the majority of which are widely distributed in Eastern Asia. Of these, eleven species are known to be present in the Korean peninsula (Kim, 1996; Rhee et al., 2005) and some of these have been used as genetic resources for lily commercial breeding.

Based on morphological and physiological characters (Comber, 1949), *Lilium* plants in Korea have been assigned to the sections *Sinomartagon* and *Martagon*. Comber's classification, however, has been revised consequently (Lighty, 1968; De Jong, 1974; Asano, 1986; Haw, 1986; Baranova, 1988). Due to similar morphological characters, especially conserved flower shapes in distantly related species (Mitchell, 1998), species identification in the genus *Lilium* by just external morphology has been very difficult. Moreover, in Korean species, *L. lancifolium* is morphologically more similar to *L. maximowiczii* (Noda, 1986; Song and Seo, 1988). Recently, molecular genetic approaches have become popular to overcome the influence of morphological similarity between species.

RAPD and PCR-RFLP analyses have been conducted in various *Lilium* species and their hybrids (Lee et al., 1993; Yamagishi, 1995; Haruki et al., 1997), and the intergenic spacers and coding regions of the chloroplast genome have also been examined (Hayashi and Kawano, 2000; Nishikawa et al., 2002). Using the ITS sequence, sixteen *Lilium* species and one variety endemic to or naturalized in Japan were analyzed to evaluate the Comber's classification system of *Lilium*, and the sequence data also provided molecular evidence for the transfer of *L. dauricum* to section *Sinomartagon* (Dubouzet and Shinoda, 1999a). Nishikawa et al. (1999, 2001) investigated ITS sequences to formulate more appropriate hypotheses about the relationships among the diverse clades within the genus and among the species in the section *Sinomartagon*. Furthermore, ITS sequences were also found to be useful in verifying the pedigree of a putative hybrid *L. maculatum* between *L. concolor* and *L. dauricum* (Dubouzet and Shinoda, 1999b). In the *L. carniolicum* group which consists of several taxonomically dubious taxa, ITS sequences were used to unravel the phylogenetic relationships and section delimitations among taxa comprising the group and to provide the phylogenetic position of the group within the genus (Rešetnik et al., 2007). In the genus *Lilium*, however, the ITS region has been studied extensively, while the organization of 5S rRNA gene

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has not been studied yet. Hence, the potential of the NTS sequence remains unutilized in elucidating the relationships among species.

The 5S rRNA gene occurs in high copy numbers (1000-50000 copies) to form a multigene family (Long and Dawid, 1980; Goldsbrough et al., 1982) and is arranged in clusters of tandemly repeated units at specific locations in the genome (Lapitan, 1992; Sastri et al., 1992). Each 5S rDNA unit consists of a 120 bp coding region, and a NTS region which is believed to play a role in the initiation and termination of transcription (Scoles et al., 1988). Although the coding region is highly conserved in higher eukaryotes, the NTS region tends to be diverged in length between 95 bp to 730 bp (Udovicic et al., 1995; Prado et al., 1996;) and is variable with regards to nucleotide composition among closely related species or even within a species (Gottlob-Mchugh et al., 1990; Trontim et al., 1999; Baker et al., 2000). Variations in the NTS gave rise to heterogeneity in the 5S rDNA unit within and between species and these have been used for inferring phylogenetic relationships at lower taxonomic ranks (Persson, 2000; Becerra 2003; Lee et al., 2010).

In this report, the 5S rRNA gene was analyzed for the first time to demonstrate its structural organization in *Lilium*, and to elucidate the inter-specific relationships among their closely related species.

Materials and Methods

Plant materials

Lilium species, *L. lancifolium* Thunberg diploid, *L. lancifolium* Thunberg triploid, *L. maximowiczii* Regel, *L. amabile* Palibin, *L. callosum* Siebold et Zuccarini, *L. cernuum* Komarov, *L. concolor* Salisbury var. *partheneion* Baker, *L. concolor* var. *buschianum* Baker, *L. dauricum* Ker-Gawl, *L. distichum* Nakai ex Kamibayashi, *L. hansonii* Leichtlin and *L. tsingtauense* Gilg were obtained from the Lily Experimental Station (Taean, Chungnam prov.), Hantaek Botanical Garden (Kyunggi prov.), Breeding Institute of Korean native plants (Wonju, Kangwon prov.), Yeongwol (Kangwon prov.) and Ockcheon (Chungbuk prov.) of Korea and transplanted to the plant growth facility in Chungnam National University, Korea.

Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from young leaves using the DNeasy® Plant Mini Kit (Qiagen, USA) according to the manufacturer's instructions. The 5S rDNA from each species was PCR-amplified using the primer set (5'-GGATCCCATC-AGAACTCC-3' and 5'-GGTGCTTTAGTGCTGGTATG-3') (Fukui et al., 1994). The PCR reaction mixture of a total volume of 25 μℓ was comprised of 5 pM of each primer set, 10 ng of template DNA, 2.5 units of Ex *Taq* polymerase (Takara, Japan) and 0.2 mM dNTPs. The PCR amplification was carried out in a thermal cycler (Takara, Japan) and the reaction conditions included an initial denaturation at 94℃ for 5 min, followed by 30 cycles of amplification (denaturation at 94℃ for 30 Sec, annealing at 59℃ for 30 sec and extension at 72℃ for 2 min) with a final extension at 72℃ for 7 min. Amplified rDNA was fractionated in a 1.5 % agarose gel by electrophoresis. The 5S rDNA fragments were then isolated from the agarose gel using a gel extraction kit (Cosmo Genetech, Korea).

Cloning and sequencing of 5S rDNA

The amplified 5S rDNA fragments were cloned into pGEM-T-Easy vector (Promega, USA), and transformed into *E. coli* DH5α cells. More than five clones of each species were sequenced to avoid mutation introduced by *Taq* polymerase. DNA sequence data were analyzed using the BLAST algorithm, provided by the National Centre for Biotechnology Information (NCBI, USA). The 5S rRNA gene sequences of all species were multiply aligned using Clustal W ver. 1.8 (Thompson et al. 1994).

Phylogenetic analysis

Phylogenetic analysis was done by the neighbor joining tree method based on NTS sequences using MEGA software version 4.1 (http://www.megasoftware.net). Published data of *Allium senescens* (Lee et al., 1999) was used as an outgroup species for phylogenetic reconstruction.

Chromosome preparation and FISH

Chromosome preparation, probe labeling and FISH of the *Lilium* plants were performed according to previous study (Sultana et al., 2010). In brief, root tips were macerated using an enzyme cocktail (2% cellulase, 1.5% macerozyme and 1% pectolyase in 1 mM EDTA, pH 4.2) at 37℃ for 1 hr. Chromosomal DNA on the slide was denatured in 70% formamide at 65℃ for 90 sec followed by dehydration in a ethanol gradient (70, 95 and 100%) at -20℃ for 3 min each. The probe mixture containing 50% formamide (v/v), 10% dextran sulfate (w/v), 5 ng/μl salmon sperm DNA and 500 ng/mℓ of each probe was denatured at 99℃ for 15 min and immediately chilled on ice for 5 min. 25 μℓ of the probe mixture was applied to the denatured chromosomal DNA and covered with a glass cover slip. Slides were then placed in a humid chamber at 37℃ for 18 hr. Each probe was detected with avidin-FITC (Vector Lab, USA) and anti-digoxigenin rhodamine (Roche, Germany). The preparations were counterstained with Vectashield (Vector Lab, USA) containing 1.5 ng/μℓ DAPI. All images were acquired using a Leica epi-fluorescence microscope equipped with a FITC-DAPI two-way or FITC-rhodamine-DAPI three-way filter set (Leica, Germany) and processed with a cooled CCD camera (CoolSNAP, Photometrics, USA) and the Meta Imaging Series ™ 4.6 software.

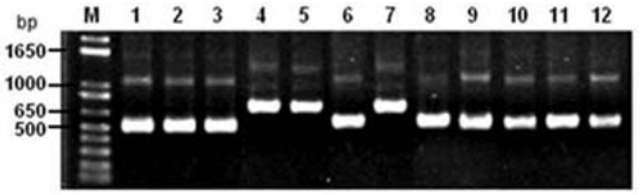

Figure 1. Agarose gel electrophoresis of PCR products for the 5S rDNA units: *L. lancifolium* 2X (1), *L. lancifolium* 3X (2), *L. maximowiczii* (3), *L. amabile* (4), *L. callosum* (5), *L. cernuum* (6), *L. concolor* var. *partheneion* (7), *L. concolor* var. *buschianum* (8), *L. dauricum* (9), *L. distichum* (10), *L. hansonii* (11), *L. tsingtauense* (12). M, DNA size marker.

Results and Discussion

The 5S rRNA gene units of each *Lilium* species were amplified by PCR (Fig. 1). In each species one major band was identified with considerable length variation of approximately 450 bp to 650 bp in addition of repeating units. Three species, *L. concolor* var. *partheneion*, *L. callosum and L. amabile* exhibited similar sized bands of 650 bp, which were significantly larger compared to other species. In addition to the prominent band, these three species posses 850 bp and 1200 bp repeating units. Most species showed a prominent band corresponded to approximately 500 bp and a repeating unit of about 1050 bp. The PCR products were directly cloned into the vector and the clones with an approximate insert size of about 500 bp to 650 bp were selected for sequence analysis.

To assess the heterogeneity in NTS regions, PCR-cloned 5S rRNA gene units from *Lilium* species were sequenced. For each species, at least more than five clones were picked and sequenced separately. The various aligned sequences within each species for NTS regions were found to be similar in length and constitution, and thus the consensus sequences from the clones of each species were obtained. Sequence analyses revealed that the 5S rRNA gene, including the full length of NTS region of considerable size (350 bp-520 bp), was flanked by 91 and 29 bp of coding sequence at 3′ and 5′ ends, respectively (Fig. 2, Table 1). These sequences are deposited in the GenBank nucleotide sequence databases with the accession numbers HQ724828-HQ724839. The coding region of the 5S rRNA gene was highly conserved (120 bp), while greater variation was identified in the NTS regions, which were species-specific. Length variation (350 bp-520 bp) in the NTS regions gave rise to the heterogeneity of 5S rDNA units, ranging from 470 bp (*L. dauricum*) to 640 bp (*L. amabile*) (Fig. 2, Table 1). These values are in accordance with the amplified 5S rDNA data obtained by PCR (Fig. 1). The variations in the NTS region have been described in various plant species such as Poaceae (Röser et al., 2001), *Populus* (Negi et al., 2002), *Vitis* (Falistocco, 2007) and *Vigna* (Saini and Jawali, 2009).

Indels or duplications located in the spacer are major sources of repeating unit variation in the 5S rDNA (Baum and Bailey, 1997; Negi et al., 2002; Baum et al., 2004). In *Lilium* species, heterogeneity in the NTS region occurred due to the presence of four species-specific indel regions. Indels 1, 2 (variable length) and indel 3 (8 bp) are AT rich regions and present in 3′ and 5′ flanking sequences of the coding region, respectively (Fig. 2). These were responsible for the smaller size of the 5S rRNA gene unit in *L. lancifolium* (479 bp) and *L. dauricum* (470 bp). Indel 4 (137 bp), which was placed in sequences of *L. concolor* var. *partheneion*, *L. callosum* and *L. amabile*, was highly conserved and species-specific, and contributed to the larger size of the 5S rRNA gene unit (615 bp-640 bp) compared to other species.

The NTS sequence is divided into three regions, which are the 3′ downstream (ds), mid spacer (ms) and 5′ upstream (us) regions (Sastri et al., 1992). The 3′ downstream region is the portion of the spacer that follows the 3′ end of the coding region corresponding to approximately 133 bp and is characterized by 4-8 tracts of a T stretch region (CTTTT) down-

Table 1. Analysis of 5S rDNA non transcribed spacers (NTS) in *Lilium* species along with their GenBank accession numbers.

Species	GenBank accession number	5S rDNA gene unit (bp)	Coding region (bp)	NTS length (bp)	Variation in NTS region								
					Nucleotide composition (%)				GC %	No. of variable sites			
					T	A	G	C		T	A	G	C
L. lancifolium 2X	HQ724836	479	120	359	43.0	24.8	18.2	14.0	32.2	4	11	12	7
L. lancifolium 3X	HQ724837	479	120	359	43.0	24.0	19.0	14.0	33.0	4	11	15	7
L. maximowiczii	HQ724838	521	120	401	38.8	25.2	19.3	16.7	36.0	3	13	10	11
L. amabile	HQ724828	640	120	520	42.8	23.5	20.0	13.7	33.7	38	23	26	16
L. callosum	HQ724829	615	120	495	43.7	24.9	19.0	12.4	31.4	40	28	26	15
L. cernuum	HQ724830	521	120	401	41.9	23.8	18.3	16.0	34.3	0	5	3	9
L. concolor var. *partheneion*	HQ724831	615	120	495	44.1	24.9	18.8	12.2	31.0	41	29	25	15
L. concolor var. *buschianum*	HQ724832	521	120	401	39.0	25.5	18.2	17.3	35.5	1	12	7	14
L. dauricum	HQ724834	470	120	350	41.8	24.7	19.2	14.3	33.5	4	10	12	9
L. distichum	HQ724833	511	120	391	41.1	25.5	17.2	16.2	33.4	3	8	8	8
L. hansonii	HQ724835	511	120	391	40.8	25.5	17.2	16.5	33.7	6	8	8	11
L. tsingtauense	HQ724839	511	120	391	41.8	24.7	17.3	16.2	33.5	4	6	8	9

stream from the coding region across the species. This could act as a terminator signal for RNA polymerase III in the termination of transcription (Korn 1982; Scoles, 1988; Röser et al., 2001; Falistocco et al., 2007). The termination signal is followed by a GC-rich region of different lengths. The 76 bp ms region separates the 3′ ds and 5′ us spacer (Sastri et al., 1992), and is characterized by different lengths of poly-T and poly-A tracts. The 5′ us region corresponds to approximately 190 bp upstream of the 5′ end of the coding region, where a highly conserved AT rich motif with the consensus sequence TAATTA was identified. This AT rich sequence has been reported as the 'TATA box' in many plant species (Röser et al., 2001; Negi et al., 2002; Saini and Jawali, 2009). The 5′ us region ended with a conserved motif of 11 bp (CAATGTATGAC) among the *Lilium* species examined. Detailed analysis of 5S rRNA genes of many species has revealed that the spacer regions flanking upstream of the gene are conserved and may have a role in the initiation of transcription (Cloix et al., 2000; Falistocco et al., 2007). The NTS regions usually end with the final base G, A or C in many plant species (Gottlob-McHugh et al., 1990; Cronn et al., 1996; Röser et al., 2001).

In *Lilium*, the coding sequence started with GGG and ended with CCC to make the 120 bp coding region. The beginning

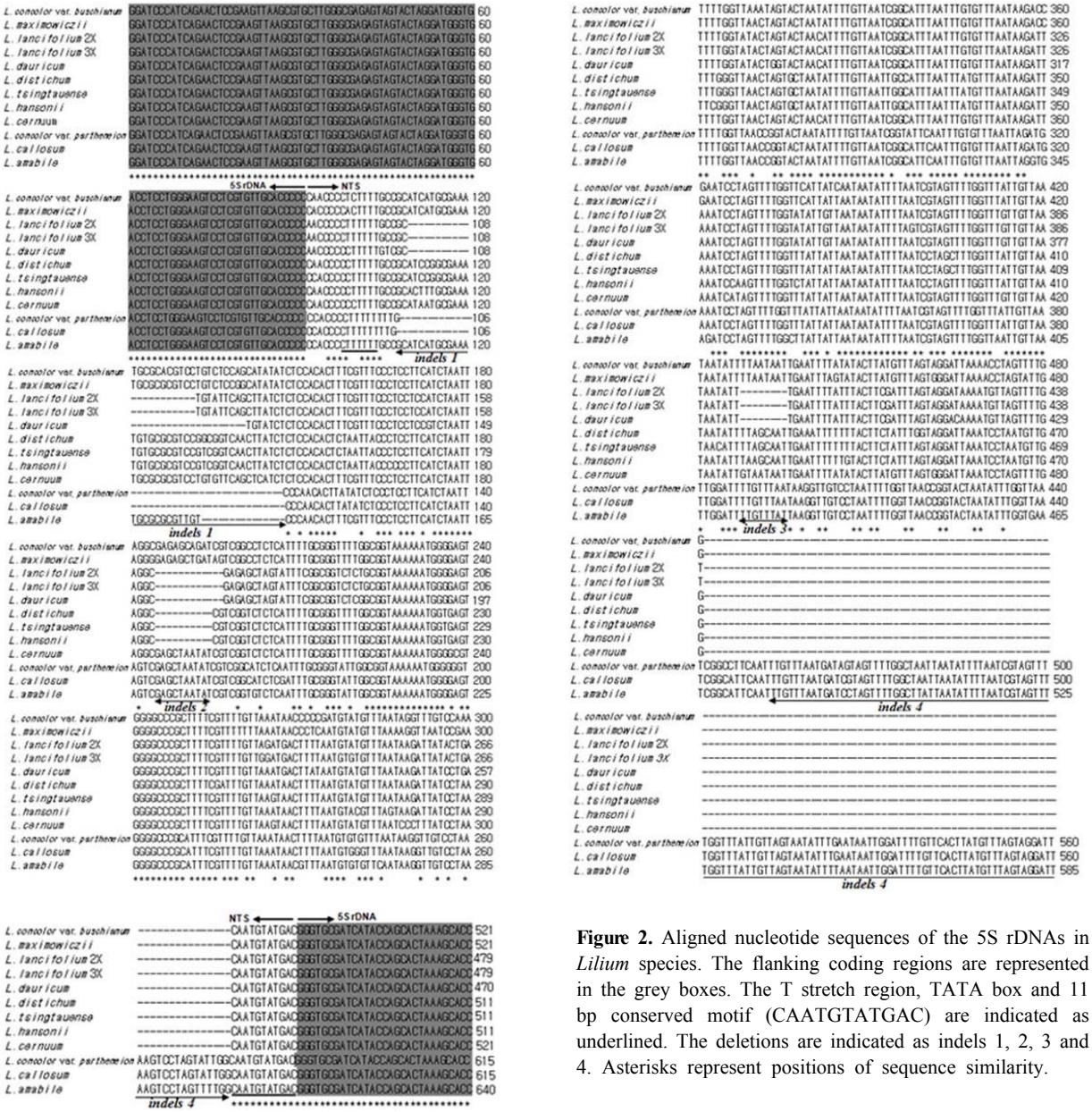

Figure 2. Aligned nucleotide sequences of the 5S rDNAs in *Lilium* species. The flanking coding regions are represented in the grey boxes. The T stretch region, TATA box and 11 bp conserved motif (CAATGTATGAC) are indicated as underlined. The deletions are indicated as indels 1, 2, 3 and 4. Asterisks represent positions of sequence similarity.

Table 2. Comparison of ITS1, ITS2 of 45S rRNA gene and NTS of 5S rRNA gene unit in the *Lilium* species originated from Korea and Japan.

Genomic region analyzed	Country of origin	Length variation (bp)	Polymorphic sites	Constant sites	Parsimony informative sites	Reference
ITS1	Japan	228-231	170	65	108	Nishikawa et al.(1999)
	Korea	229	47	182	21	Data not Shown
ITS2	Japan	232-233	163	79	100	Nishikawa et al.(1999)
	Korea	233	55	178	28	Data not shown
NTS	Korea	350-520	186	351	131	Present study

of the coding region generally shows some family specificity (Volkov et al., 2001), such as GGA (or TGA) in *Graminae* (Baum and Johnson, 1996), GGG in *Gossypium* (Cronn et al., 1996), AGG in *Legume* (Gottolob-McHugh et al., 1990) and GGA in *Solanum* (Zanke et al., 1995). In other species such as *Hordeum* and *Vigna*, the coding region ended with GTG (or TCC) and CCT, respectively (Baum and Johnson, 1996; Saini and Jawali, 2009).

The GC content of the NTS region revealed wide variation ranging from 31.0% (*L. concolor* var. *partheneion*) to 36.0% (*L. maximowiczii*) (Table 1). To examine the NTS sequence heterogeneity, the homology percentage was compared based on the sequence alignment (Supplemented Table 2). The sequence similarity in NTS regions ranged from 64% (between *L. hansonii* and *L. concolor* var. *partheneion)* to 99% (between diploid and triploid *L. lancifolium*) which indicates a high degree of interspecific nucleotide diversity (up to 36%). The NTS regions showed significant nucleotide sequence heterogeneity (Table 1). *L. concolor* var. *partheneion* showed highest number of variable sites in the NTS region followed by *L. callosum* and *L. amabile* among the species. In spite of high sequence variation, the NTS regions were characterized by the conserved order of (T>A>G>C) in nucleotide abundance among the *Lilium* species (Table 1). In *Vigna,* the NTS regions showed (T>C>A>G) in nucleotide abundance (Saini and Jawali, 2009)

In the genus *Lilium*, the 5S rDNAs have not been cloned yet and therefore sequence information is not available. This is the first attempt in the genus *Lilium* characterizing the NTS region of 5S rRNA gene unit, while the ITS region in this genus has been studied extensively from different origin. The 5S rDNA NTS region was evaluated for the phylogenetic informativeness and compared to ITS regions from the related species originated from other countries. The ITS region was also used for understanding species relationships among *Lilium* species (Dubouzet and Shinoda, 1999; Nishikawa et al., 1999). The ITS data from related species originated from Japan, China and Korea showed that the ITS region exhibits lesser polymorphism than the NTS region in this genus (Supplemented Table 1 and 2). Sequences of the ITS regions were around 230 bp (228 to 231 bp in ITS1 and 231-246 bp in ITS2) among the species from different origin, whereas the NTS regions were found more variable in length, ranging from 350-520 bp (Supplemented Table 1). The NTS was comparable to both ITS regions in size and number of variable sites, as it had substantially more polymorphic and parsimony informative sites (Table 2). As it is known that different sequences have different rates of evolution (Saini and Jawali, 2009), the analysis of the NTS region could give additional insights into evolution of a group of species.

In our previous study, FISH mapping using 45S and 5S rDNA probes in Korean *Lilium* species have demonstrated that the location of 5S rRNA gene was detected only on the long arm of chromosome 3 in all *Lilium* plants examined, whereas 45S rDNA loci were distributed in most of the chromosomes except for chromosomes 8, 9 and 12 (Sultana et al., 2010). Fig. 3 shows only one chromosome complement with FISH signals of 5S and 45S rRNA genes from representative *L. lancifolium*.

Based on the NTS sequences, a neighbor joining tree was constructed to elucidate the phylogenetic relationships among *Lilium* species (Fig. 4). The species were divided into two major groups (Cluster I and II). Cluster I was represented by most species which belonged to the section *Sinomartagon,* whereas Cluster II included only three species *L. hansonii, L. tsingtauense* and *L. distichum* belonging to the section *Martagon* (Comber, 1949). Sequence data was also consistent

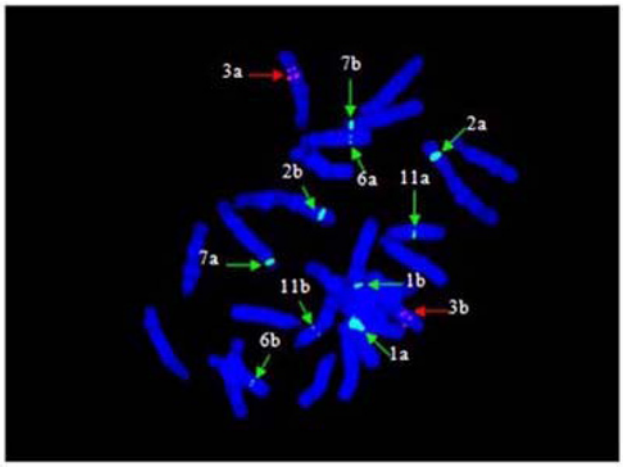

Figure 3. FISH mapping of digoxigenin-labeled 5S rDNA (red signals) and biotin-labeled 45S rDNA (green signals) in diploid *L. lancifolium*.

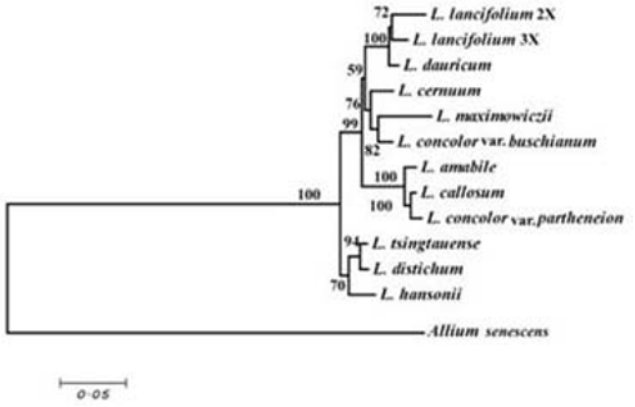

Figure 4. Phylogenetic relationships of the Korean wild *Lilium* species based on NTS sequences inferred from the neighbor joining method. *Allium senescens* used as an outgroup species. The numbers at the nodes represent bootstrap values (%) for a 1000 replicate analysis.

with previous cytogenetic FISH results (Sultana et al., 2010). Cluster I was further divided into three distinct clades. According to Kim and Lee (1990), the Korean *Lilium* species belonging to the section *Sinomartagon* could be divided into three groups. In the first clade, diploid and triploid *L. lancifolium* have a closer phylogenetic relationship (99% sequence identity) and *L. dauricum* was a sister group to this clade (Fig. 4 and Supplemened Table 2). The second clade revealed that *L. cernuum* had very high sequence similarity (92%) with *L. maximowiczii* and *L. concolor* var. *buschianum*. *L. amabile.* In the third clade, it showed 96% sequence similarity with *L. concolor* var. *partheneion* and *L. callosum,* while *L. callosum* showed a closer relationship with *L. concolor* var. *partheneion* by 98% sequence homology (Supplemened Table 2).

In conclusion, analysis of the 5S rRNA gene unit organization may provide new insights to clarify the inter-specific relationships among the closely related *Lilium* species. The phylogenetic results based on NTS sequences were in agreement with previous morphology-based classification and cytogenetic data in the genus.

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References

Asano Y (1986) A numerical taxonomic study of the genus *Lilium* in Japan. J. Fac. Agr. Hokkaido Univ. 62: 333-341.

Baker WJ, Hedderson TA and Dransfield J (2000) Molecular phylogenetics of *Calamus* (Palmae) and related rattan genera based on 5S nrDNA spacer sequence data. Mol. Phylogenet. Evol. 14: 218–221.

Baum BR, Bailey LG, Belyayev A, Raskina O and Nevo E (2004) The utility of the nontranscribed spacers of 5S rDNA units grouped into unit classes assigned to haplomes - a test on cultivated wheat and wheat progenitors. Genome 47: 590-599.

Baum BR and Bailey LG (1997) The molecular diversity of the 5S rRNA in *Kengyilia alatavica* (Drobov) J.L.Yang, Yen & Baum (Poaceae: Triticeae): potential genomic assignment of different rDNA units. Genome 40: 215-228.

Baum BR and Johnson DA (1996) The 5S rRNA gene units in ancestral two-rowed barley (*Hordeum spontaneum* C. Koch) and bulbous barley (*H. bulbosum* L.): sequence analyses and phylogenetic relationships with 5S rDNA units of cultivated barley (*H. vulgare* L.). Genome 39: 140-149.

Baranova MV (1988) A synopsis of the system of the genus *Lilium* (*Liliaceae*). Bot. Zhur. 73: 1319-1329.

Becerra JX (2003) Evolution of Mexican Bursera (Burseraceae) inferred from ITS, ETS, and 5S nuclear ribosomal DNA sequences. Mol. Phylogenet. Evol. 26:300–309.

Cloix C, Tutois S, Mathieu O, Cuvillier C, Espagnol MC, Picard G and Tourmente S (2000) Analysis of 5S rDNA arrays in *Arabidopsis thaliana*: physical mapping and chromosome specific polymorphism. Genome Res. 10: 679-690.

Comber HF (1949) A new classification of the genus *Lilium*. Lily Yearbook. Royal Hort. Soc. London.13: 86–105.

Cronn RC, Zhou X, Paterson AH and Wendel JF (1996) Polymorphism and concerted evolution in a tandem repeated family: 5S ribosomal DNA in diploid and allopolyploid cottons. J. Mol. Evol. 42: 685-705.

De Jong PC (1974) Some notes on the evolution of lilies. Yearbook North American Lily Soc. 27: 23-28.

Dubouzet JG and Shinoda K (1999a) Phylogenetic analysis of the internal transcribed spacer region of Japanese *Lilium* species. Theor. Appl. Genet. 98: 954-960.

Dubouzet JG and Shinoda K (1999b) ITS DNA sequence relationships between *Lilium concolor* Salisb., *L. dauricum* Ker-Gawl. and their putative hybrid, *L. maculatum* Thunb. Theor. Appl.Genet. 98: 213 –218.

Felistocco E, Passeri V and Marconi G (2007) Investigations of 5S rDNA of *Vitis vinifera* L.: sequence analysis and physical mapping. Genome 50: 927-938.

Fukui K, Ohmido N and Khush GS (1994) Variability in rDNA loci in the genus *Oryza* detected through fluorescence *in situ* hybridization. Theor. Appl. Genet. 87: 893−899.

Goldsbrough RB, Ellis THN and Lomonosoff GD (1982) Sequence variation and methylation of the flax 5S RNA genes. Nucleic Acids Res. 10: 450-454.

Gottlob-McHugh SG, Levesque M, MacKenzie K, Oslon M, Yarosh O and Johnson DA (1990) Organization of the 5S rRNA genes in the soybean *Glycine max* (L.) Merrill and conservation of the 5S rDNA repeat structure in higher plant. Genome 33:486–494.

Haruki K, Hosoki T, Nako Y and Ohta K (1997) Possibility of classification in some species of *Lilium* by PCR-RFLP of the ribulose-1, 5-bisphosphate carboxylase large subunit (rbcL) gene and ribosomal RNA gene. J. Japan Soc. Hort. Sci. 66: 189-192.

Haw SG (1986) The lilies of China. BT Batsford Ltd, London.

Hayashi K and Kawano S (2000) Molecular systematics of *Lilium* and allied genera (Liliaceae): phylogenetic relationships among *Lilium* and related genera based on the *rbc*L and *mat*K gene se-

quence data. Plant Species Biol. 15: 73–93.
Kim Y (1996) Lily industry and research, and native *Lilium* species in Korea. Acta Hort. (ISHS). 414: 69-80.
Kim YS and Lee WB (1990) A study of morphological characters on the genus *Lilium* L. in Korea. Kor. J. Plant Tax. 20: 165-178.
Korn LJ (1982) Transcription of *Xenopus* 5S ribosomal RNA genes. Nature 295: 101-105.
Lapitan NLV (1992) Organization and evolution of higher plant genomes. Genome 35: 171−181.
Lee SH, Choi HW, Sung JS and Bang JW (2010) Inter-genomic relationships among three medicinal herbs: *Cnidium officinale*, *Ligusticum chuanxiong* and *Angelica polymorpha*. Genes Genom. 32: 95-101.
Lee SH, Do GS and Seo BB (1999) Chromosomal localization of 5S rRNA gene loci and the implications for relationships within the *Allium* complex. Chromosome Res. 7: 89-93.
Lee WB, Choi SY and Kim YS (1993) An application of random amplified polymorphic DNA (RAPD) to systematics of some species of *Lilium* in Korea. Kor. J. Plant Tax. 23: 43–56.
Lighty RW (1968) Evolutionary trends in lilies. Lily Yearbook. Royal Hort. Soc. London. 31: 40-44.
Long EO and Dawid IB (1980) Repeated gene in eukaryotes. Ann. Rev. Biochem. 43: 727- 764.
Mitchell RM (1998) Species DNA research report. NALS Q Bull. 52: 8–9.
Negi MS, Rajagopal J, Chauhan N, Cronn and Lakshmikumaran (2002) Length and sequence heterogeneity in 5S rDNA of *Populus deltoids*. Genome 45:1181-1188.
Nishikawa T, Okazaki K and Nagamine T (2002) Phylogenetic relationships among *Lilium auratum* Lindley, *L. auratum* var. *platyphyllum* Baker and *L. rubellum* Baker based on three spacer regions in chloroplast DNA. Breed. Sci. 52: 207–213.
Nishikawa T, Okazaki K, Arakawa K and Nagamine T (2001) Phylogenetic analysis of section *Sinomartagon* in genus *Lilium* using sequences of the internal transcribed spacer region in nuclear ribosomal DNA. Breed. Sci. 51: 39–46.
Nishikawa T, Okazaki K, Uchino T, Arakawa K and Nagamine T (1999) A molecular phylogeny of *Lilium* in the internal transcribed spacer region of nuclear ribosomal DNA. J. Mol. Evol. 49: 238–249.
Noda, S (1986) Cytogenetic behavior, chromosomal differences, and geographic distribution in *Lilium lancifolium* (*Liliaceae*). Plant Species Biol. 1: 69-78.
Persson C (2000) Phylogeny of *Neotropical Alibertia* group (Rubiaceae), with emphasis on the genus *Alibertia*, inferred from ITS and 5S ribosomal DNA sequences. Amer. J. Bot. 87:1018–1028.
Prado EA, Faivre-Rampant P, Schneider C and Darmency MA (1996) Detection of a variable number of ribosomal DNA loci by fluorescent *in situ* hybridization in *Populus* species. Genome 39: 1020 –1026.
Rešetnik I, Liber Z, Satovic Z, Cigić P and Nikolić T (2007) Molecular phylogeny and systematics of the *Lilium carniolicum* group (Liliaceae) based on nuclear ITS sequences. Plant Syst. Evol. 265: 45-58.
Rhee HK, Lim JH and Kim YJ (2005) Improvement of breeding efficiency for interspecific hybridization of lilies in Korea. Acta Hort. ISHS. 673: 107-112.
Röser M, Winterfeld G, Grebenstein B and Hemleben V (2001) Molecular diversity and physical mapping of 5S rDNA in wild and cultivated oat grasses (Poaceae: Aveneae). Mol. Phylogenet. Evol. 21(2): 198–217.
Sastri DC, Hilu K, Appels R, Lagudah ES, Playford J and Baum BR (1992) An overview of evolution in plant 5S rDNA. Plant Syst. Evol. 183: 169−181.
Saini A and Jawali N (2009) Molecular evolution of 5S rDNA region in *Vigna* subgenus *Ceratotropis* and its phylogenetic implications. Plant Syst. Evol. 280: 187-206.
Scoles GJ, Gill BX, Xin ZY, Clarke BC, McIntyre CL, Chapman C and Appels R (1988) Frequent duplications and deletion events in the 5S RNA genes and the associated spacer regions in the *Triticeae*. Plant Syst. Evol. 160:105–122.
Song NH and Seo BB (1988) Cytogenetics study of *Lilium maximowiczii* Regel using differential Giemsa stain. Res. Rev. Kyungpook Natl. Univ. 45: 63-68.
Sultana S, Lee SH, Bang JW and Choi HW (2010) Physical mapping of rRNA gene loci and inter-specific relationships in wild *Lilium* distributed in Korea. J. Plant Biol. 53: 433-443.
Thompson JD, Higgings DG and Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl. Acids Res. 22: 4673-4680.
Trontin JF, Grandemange C and Favre JM (1999) Two highly divergent 5S rDNA unit size classes occur in composite tandem array in European larch (*Larix decidua* Mill.) and Japanese larch (*L. kaempferi* Lamb. Carr.). Genome 42: 837–848.
Udovicic R, McFadden GI and Ladiges PY (1995) Phylogeny of *Eucalyptus* and *Angophora* based on 5S rDNA spacer sequence data. Mol. Phylogenet. Evol. 4: 247–256.
Volkov RA, Zanke C, Panchuk II and Hemleben V (2001) Molecular evolution of 5S rDNA of *Solanum* species (sect. Petota): application for molecular phylogeny and breeding. Theor. Appl. Genet. 103: 1273–1282.
Yamagishi M (1995) Detection of section-specific random amplified polymorphic DNA (RAPD) markers in *Lilium*. Theor. Appl. Genet. 91: 830–835.
Zanke C, Borisjuk N, Rouss B, Schilde-Rentschler L, Ninnemann H and Hemleben V (1995) A species specific oligonucleotide of the 5S rDNA spacer and species-specific elements identify symmetric somatic hybrids between *Solanum tuberosum* and *S. pinnatisectum*. Theor. Appl. Genet. 90: 720-72.