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Phylogenetic Relationships among *Allium* subg. *Rhizirideum* Species Based on the Molecular Variation of 5S rRNA Genes

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This study has demonstrated the molecular variation of 5S rRNA genes in 15 *Allium* subgenus *Rhizirideum* and 1 *Allium* subg. *Allium*. For cloning of the 5S rRNA genes, PCR products were obtained from amplification with oligonucleotide primers which were derived from the conserved coding region of 5S rRNA genes. These amplified PCR products were cloned and identified by FISH and sequence analysis. The 5S rRNA loci were primarily located on chromosomes 5 and/or 7 in diploid species and various chromosomes in alloploid species. The size of the coding region of 5S rRNA genes was 120 bp in all the species and the sequences were highly conserved within *Allium* species. The sizes of nontranscribed spacer (NTS) region were varied from 194 bp (*A. deltoide-fistulosum*, 2*n*=16) to 483 bp (*A. sativum*). Two kinds of NTS regions were observed in *A. victorialis* var. *platyphyllum* a diploid, *A. wakegi* an amphihaploid, *A. sacculiferum*, *A. grayi*, *A. deltoide-fistulosum* and *A. senescens* all allotetraploids, while most diploid species showed only one NTS region. The species containing two components of NTS region were grouped with different diploid species in a phylogenetic tree analysis using the sequences of 5S rRNA genes and adjacent non-coding regions.

The edible *Allium* species are of major economic and dietary importance. *Allium* is a large genus which includes more than 700 species (Mes et al., 1999) and contains several major agricultural crops such as the bulb onion (*A. cepa*), Japanese bunching or Welsh onion (*A. fistulosum*), Chinese chive (*A. tuberosum*) and garlic (*A. sativum*). The current classification (Hanelt et al., 1992) into six subgenera *Allium*, *Amerallium* Traub, *Bromatorrhiza* Ekb., *Caloscordum* (Herb.) R.M. Fritsch, *Melanocrommyum* (Webb et Berth.) Rouy, and *Rhizirideum* (G. Don ex Koch) Wendelbo is mainly based on characters from morphology, karyology, anatomy, seed coat structure, and isozyme data. Neverthless, the phylogenetic relationships in some variable groups and between cultivated and wild species are not yet clear (Pich et al., 1996).

Many studies aimed at elucidation of the evolutionary relationships of *Allium* species. Groups within the genus have been delimited with various sorts of data, for example investigations of genomic relationships of diploid (2*n*=16) and polyploid (2*n*=32) *Allium* species have been based on karyotype analyses (Ved Brat, 1965), numerical taxonomy (El-Gadi and Elkington, 1977), and restriction enzyme analysis of the chloroplast (Havey, 1991). Isozymes have measured the genetic variability of between- and within-population variation (Rouamba et al., 1993). Recently, taxonomic studies carried out with RFLP markers revealed high differentiation between species, especially for species belonging to the section *Cepa* (Bradeen and Havey, 1995). Mes et al. (1999) reported the evolution of the chloroplast genome and polymorphic ITS regions in *Allium* subg. *Melanocrommyum*. Commonly, domesticated *Allium* species in Korea have a basic chromosome number of 8 (McCollum, 1976) and most of them are bulbous plants (Hanelt, 1990). Although there are some auto- and allopolyploids in *Allium* species, unequivocal confirmation of the genomic origins in these species has not been possible by C-banding or conventional karyotypes due to the similarity in size and morphology of somatic chromosome components (Seo and Kim, 1989; Seo et al., 1989; Kim et al., 1990).

The subgenus *Rhizirideum* (G. Don ex Koch) Wendelbo is highly heterogeneous as reported by morphological (Hanelt et al., 1992), karyological (Friesen, 1988), and molecular data (Linne von Berg et al., 1996). All the taxa from rhizomes, although sometimes they are reduced to disc-like corms, similar to that in the common onion *A. cepa*. Sectional subdivision needs further research.

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The 5S ribosomal RNA (rRNA) genes of higher plants are organized into clusters of tandem repeats with thousands of copies at one or more positions in the genome (Sastri et al., 1992; Baum and Johnson, 1994). Each repeat consists of a highly conserved 5S rRNA coding region of approximately 120 base pairs (bp) in length and of NTS regions that vary in size between 100 and 700 bp (Sastri et al., 1992). Most repeats appear to be uniform in a species. On the basis of the high degree of stability during the course of evolution, comparative studies of the nucleotide sequences of rRNA genes provide a means for analyzing phylogenetic relationships over a wide range of taxonomic levels (Medlin et al., 1988). For the 5S rRNA genes, some studies that include the NTS regions as well as the number of 5S rRNA loci, have been reported in many species (Gerlach and Dyer, 1980; Lapitan, 1992). Recent studies have demonstrated that the variation in sizes and sequences of the NTS of the 5S rRNA gene was found to be useful for the phylogenetic reconstruction of species (Appels and Baum, 1991; Baum and Johnson, 1998), and to discover differences between cultivars in barley and wheat, and between lines in maize (Kolchinsky et al., 1991).

Assuming that sequences of NTS of an allopolyploid contain multiple/both variants of its original parent lines, with subsequent sequence divergence during evolution after natural or artificial hybridization between parental lines, comparative analysis of size differences and sequence variation of NTS is likely to permit a more accurate identification of the parental origins of parts of the genome of allopolyploids. The purpose of this study is to analyze the variation between 5S rRNA genes consisting of coding and NTS regions in the 15 *Allium* subg. *Rhizirideum* (G. Don ex Koch) Wendelbo and 1 *Allium* subg. *Allium* distributed in Korea is a subject of interest, to determine whether the NTS could be used as a taxanomic marker, and to investigate its utility for phylogenetic studies of the genus *Allium*.

Materials and Methods

Plant material and DNA isolation

Sixteen wild and cultivated species of *Allium* including ten diploids ($2n$=16), one amphihaploid ($2n$=16), four allotetraploids ($2n$=32), and one autotetraploid ($2n$=32) have been examined (Table 1). The plant material was collected from 1986 to 1989, and has been maintained in the greenhouse at Kyungpook National University. Genomic DNA of each species was extracted from fresh leaves according to the method of Rogers and Bendich (1988).

Amplification and cloning of 5S rRNA genes

Polymerase chain reaction (PCR) was used to amplify the 5S rRNA genes including coding and NTS region in each species. To amplify the complete coding region and NTS region of 5S rRNA genes in each species, we designed primer sequences of the forward (position 30 to 47) and reverse (position 11 to 29) in the conserved coding region of tandemly repeated 5S rRNA genes (Fig. 3). Total genomic DNA of each species was used as template DNA for the amplification of the 5S rRNA gene. The reaction mixture (25 μl) contained 10 ng of template DNA, 5 pM each of the forward (5′-GGATCCCATCAGAACTCC-3′) and the reverse (5′-

Table 1. Summary of the characteristics of *Allium* species and 5S rRNA gene repeat sequences

Species	Abbreviation	Clone	Size of repeat (bp)	EMBL/GenBank /DDBJ accession number
		Allium subgenus *Allium*		
A. sativum	SAT	pSA617	603	AF101249
		Allium subgenus *Rhizirideum* (G. Don ex Koch) Wendelbo		
A. cepa	CEP	pCE605	352	AF101244
A. cyaneum var. *deltoides*	CVD	pCD623	345	AF101246
A. cyaneum R.	CYA	pCY620	358	AF101245
A. detoide-fistulosum	DF2	pDF2611	314	AF101247
A. fistulosum	FIS	pFI599	344	AF101248
A. splendens	SPL	pSP635	355	AF101251
A. senescens var. *minor*	SVM	pSM626	433	AF101250
A. thunbergii	THU	pTH632	355	AF101252
A. victorialis var. *platyphyllum*	VIC-1	pVI161	394	AF101253
A. victorialis var. *platyphyllum*	VIC-2	pVI162	407	AF101254
[a]*A. wakegi*	WAK-1	pWA609	339	AF101255
[a]*A. wakegi*	WAK-2	pWA402	349	AF101256
[b]*A. deltoide-fistulosum*	DF4-1	pDF4652	339	AF144401
[b]*A. deltoide-fistulosum*	DF4-2	pDF4614	358	AF101257
[b]*A. grayi*	GRA-1	pGR639	355	AF101258
[b]*A. grayi*	GRA-2	pGR678	397	AF144400
[b]*A. sacculiferum*	SAC-1	pCC407	351	AF101259
[b]*A. sacculiferum*	SAC-2	pCC415	358	AF101260
[b]*A. senescence*	SEN-1	pSE629	434	AF101261
[b]*A. senescence*	SEN-2	pSE654	436	AF144402
[c]*A. tuberosum*	TUB	pTU602	541	AF101262

[a]Amphihaploid ($2n$=16). [b]Allotetraploid ($2n$=32). [c]Autotetraploid ($2n$=32). All other entries are diploid ($2n$=16).

GGTGCTTTAGTGCTGGTAT-3′) primer, 200 μM each of dATP, dGTP, dCTP, and dTTP, and 2.5 U *Ex Taq* Polymerase (Takara Shuzo) in 1X Ex *Taq* buffer (Takara Shuzo) with 2 mM magnesium chloride. The PCR reaction was performed in a Perkin Elmer Cetus System 9600 thermal cycler. Amplification involved a preliminary 2 min denaturation at 94℃, 30 cycles for 1 min at 94℃ (denaturation), 1 min for 55℃ (annealing) and 2 min for 72℃ (extension), and a final extension at 72℃ for 10 min followed by slow cooling to room temperature. PCR products were visualized by electrophoresis in 1.2% agarose gels. The prominent band corresponding to the 5S rRNA genes was isolated using a Geneclean II kit (Bio 101), ligated into pT7Blue(R) T-vector, and introduced into DH5d by transformation (Hanahan, 1983). Each clone that contained a 5S rRNA gene insert was labeled with digoxigenin-11-dUTP, and was subsequently identified by fluorescence by *in situ* hybridization (FISH) on metaphase chromosomes according to methods reported by Mukai et al. (1990).

Sequencing and sequence analysis

The size of the insert was estimated by electrophoresis after digestion with *Eco*RI and *Pst*I. The 5S rRNA gene was sequenced using a PAGE gel with an ABI Prism 377 sequencer (Perkin-Elmer) as an automated fluorescent sequencing system. Sequencing of the 5S rRNA gene was carried out at least ten clones per species. CLUSTAL W 1.7 (Thompson et al., 1994), a multiple alignment program, was used to locate homologies of the rRNA gene sequences of *Allium*. The sets of sequences were converted to the format of PHYLIP package (version 3.5) (Felsenstein, 1993). We used bootstrap analysis with 100 bootstrap samples. The DNAPARS program was employed to find the most parsimonious trees. Finally, the trees were reduced to a single tree. The ambiguously aligned regions were excluded from the analysis (Fig. 3). The BLASTIN program (Altschul et al., 1990) was used to search the homologous region to rDNA sequences through data bases such as GenBank, EMBL, and DDBJ.

Since most of the other species are from subgroup, *Rhizirideum*, it was used for the 5S repeat of *A. sativum* which belongs to subg. *Allium* as an outgroup.

Results

Amplification and identification of 5S rRNA genes

The amplified 5S rRNA fragments mostly consisted of a single band of variable size with the exception of *A. victorialis* var. *platyphyllum* (Fig. 1). The size of the *Allium* 5S rRNA repeat ranged from 300 to 600 bp sizes on electrophoresis. These amplified PCR products were cloned and identified using electrophoresis after *Eco*RI and *Pst*I double digestion (data not shown). For the identification of chromosomal localization, FISH was conducted using the corresponding recombinant

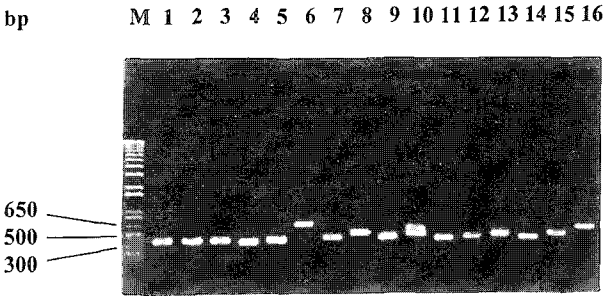


Fig. 1. Agarose electrophoresis of 5S rRNA genes and NTS of 16 *Allium* species. Lane 1, *A. cepa*; Lane 2, *A. cyaneum*; Lane 3, *A. cyaneum* var. *deltoides*; Lane 4, *A. deltoide-fistulosum* (2*n*=16); Lane 5, *A. fistulosum*; Lane 6, *A. sativum*; Lane 7, *A. splendens*; Lane 8, *A. senescens* var. *minor*; Lane 9, *A. thunbergii*; Lane 10, *A. victorialis* var. *platyphyllum*; Lane 11, *A. wakegi*; Lane 12, *A. deltoide-fistulosum* (2*n*= 32); Lane 13, *A. grayi*; Lane 14, *A. sacculiferum*; Lane 15, *A. senescens*; Lane 16, *A. tuberosum*. M indicates size marker of 1 kb Plus DNA ladder.

plasmid of 5S rRNA genes as probes. From the detailed signals on the chromosomal locations, we confirmed that the inserted fragment is the 5S rRNA gene (Fig. 2). A pair of 5S rRNA gene signals on the short arm of chromosome 5 and two pairs of signals on both arms of chromosome 7 were detected in *A. cyaneum* (Fig. 2b), *A. cyaneum* var. *deltoides* (Fig. 2c), *A. deltoide-fistulosum* (2*n*=16) (Fig. 2d), *A. splendens* (Fig. 2g) and *A. thunbergii* (Fig. 2i). *A. senescens* var. *minor* and *A. fistulosum* showed one pair of signals on the short arm of chromosome 7 (Fig. 2h and 2e), *A. cepa* two pairs on the short arm of chromosome 7 (Fig. 2a), *A. sativum* two pairs on both arms of chromosome 7 (Fig. 2f) and *A. victorialis* var. *platyphyllum* three pairs on the short arm of chromosome 7 (Fig. 2j). The signals in *A. wakegi*, an amphihaploid, were detected in the intercalary region of the short arm of chromosome 15 and in two regions of the short arm of chromosome 9 (Fig. 2k). In allotetraploid species, *A. deltoide-fistulosum* showed four pairs of signals on chromosomes 10, 13, 14, and 15 (Fig. 2l), *A. senescens* three pairs on chromosomes 8, 14 and 15 (Fig. 2o), *A. grayi* two pairs on chromosomes 9 and 10 (Fig. 2m) and *A. sacculiferum* six pairs on chromosomes 7, 8, 9, 11, 13 and 14 (Fig. 2n), respectively. *A. tuberosum*, an autotetraploid, had two sets of chromosomes showing 5S rRNA loci on the proximal position of the short arm of chromosome 3 and the intercalary region on the long arm of chromosome 6 (Fig. 2p).

To determine whether the 5S rRNA genes vary in base substitutions and size per clones, we sequenced at least ten clones per species. Most species contained identical or nearly identical sequences showing less than one percent divergence, and as a consequence no significant variation in base composition and size existed in these clones. In the present study, two kinds of NTS sequences were obtained from in *A. victorialis* var. *platyphyllum* (2*n*=16), *A. wakegi* (2*n*=16), *A. sacculiferum* (2*n*=32), *A. senescens* (2*n*=32), *A. grayi* (2*n*=32) and *A. deltoide-fistulosum* (2*n*=32). We desig-

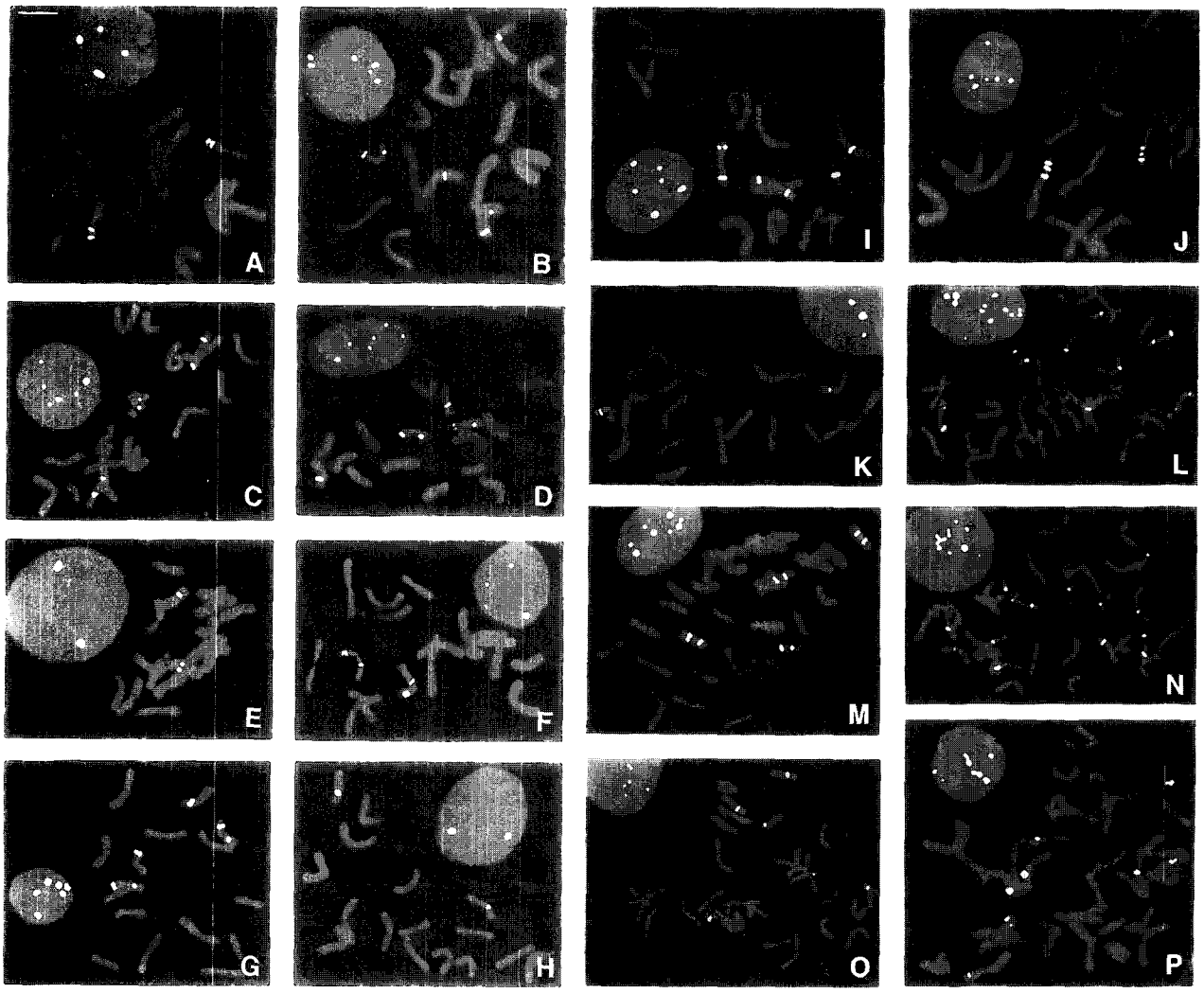
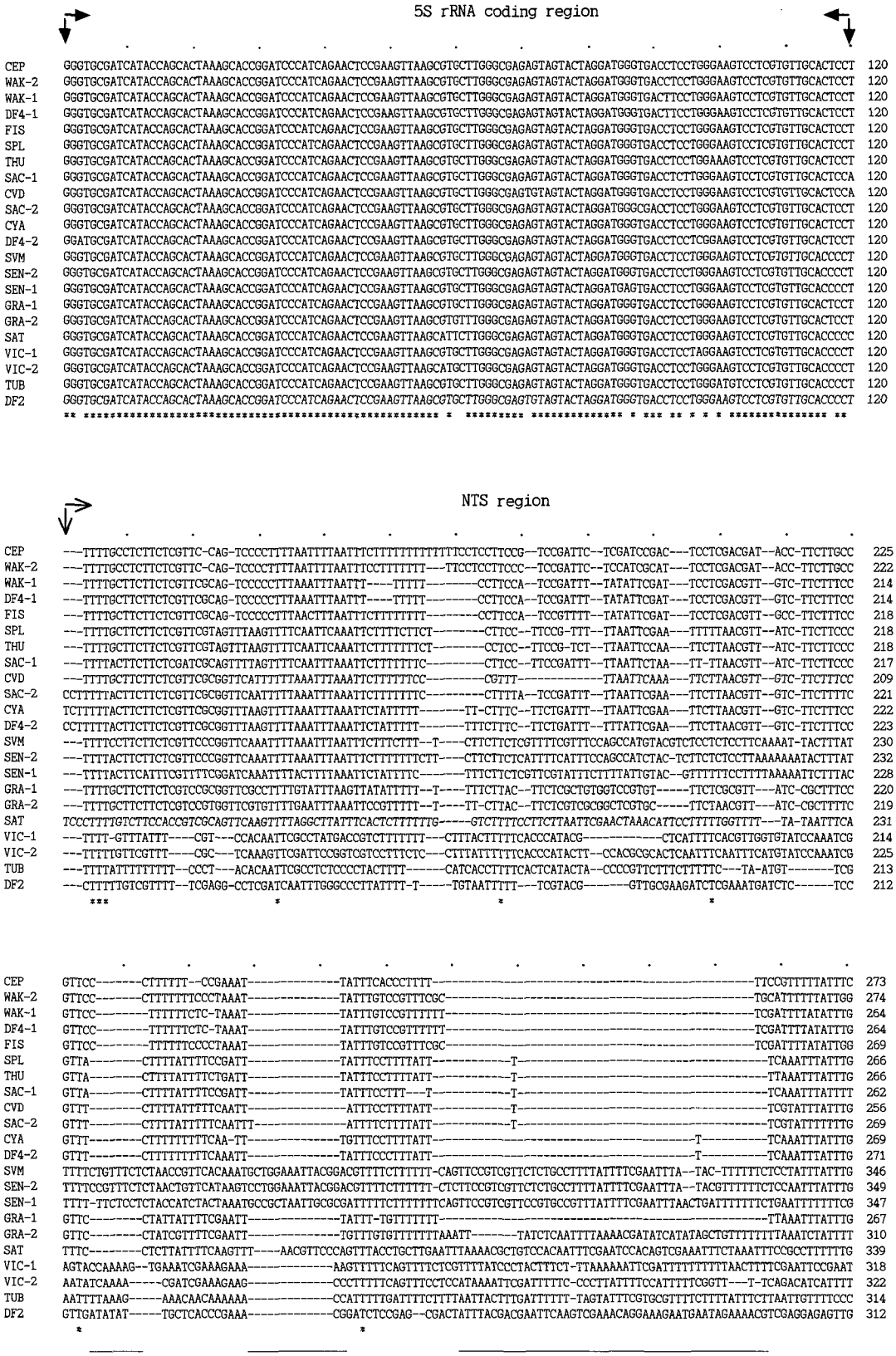


Fig. 2. FISH patterns of 5S rRNA genes in 16 *Allium* species. A, *A. cepa*; B, *A. cyaneum*; C, *A. cyaneum* var. *deltoides*; D, *A. deltoide-fistulosum* (2*n*=16); E, *A. fistulosum*; F, *A. sativum*; G, *A. splendens*; H, *A. senescens* var. *minor*; I, *A. thunbergii*; J, *A. victorialis* var. *platyphyllum*; K, *A. wakegi*; L, *A. deltoide-fistulosum* (2*n*=32); M, *A. grayi*; N, *A. sacculiferum*; O, *A. senescens*; P, *A. tuberosum*. The digoxigenin-labeled 5S rRNA genes was detected by antidigoxigenin-rhodamine conjugate (red color) on DAPI counterstained metaphase chromosomes (blue color). Scale Bar=10 μm.

nated these 22 recombinant plasmids as pCE605, pCD623, pCY620, pDF2611, pFI599, pSA617, pSP635, pSM626, pTH632, pVI161, pVI162, pWA609, pWA402, pDF4652, pDF4614, pGR639, pGR678 pCC407, pCC415, pSE629, pSE654, and pTU602 according to initials and clone number in *Allium* species. Table 1 summarizes the clone number, size of NTS, and EMBL/GenBank/DDBJ accession number. The DNA homology search of the NTS region using the EMBL nucleic acid data bank revealed no similar DNA sequences registered in the data bases. The size of NTS region was varied from 194 to 483 bp in length (Table 1) and the sequence alignment of the 5S rRNA genes including coding and NTS region of all examined species are given in Fig. 3.

Clones of all diploids except for *A. victorialis* var. *platyphyllum* showed only one sequence variant. In *A. victorialis* var. *platyphyllum*, variations of 5S rRNA genes were observed in two kinds of NTS with different sizes of 274 bp (VIC-1) and 287 bp (VIC-2). In *A. wakegi* which is known as an amphihaploid, and *A. sacculiferum, A. senescens, A. grayi* and *A. deltoide-fistulosum* that are allotetraploids, two 5S rRNA repeat variants were cloned. Because the two types showed distinct variations in sequence and size (Fig. 3), we can argue that the interspecific hybrid species originated from different genomic species must have shared with its different 5S rRNA gene sequence. After cloning of the 5S rRNA fragment, the probe of 5S rRNA genes was amplified, and labeled with digoxigenin-11-dUTP from each recombinant plasmid as template.


5S rRNA coding region
NTS region

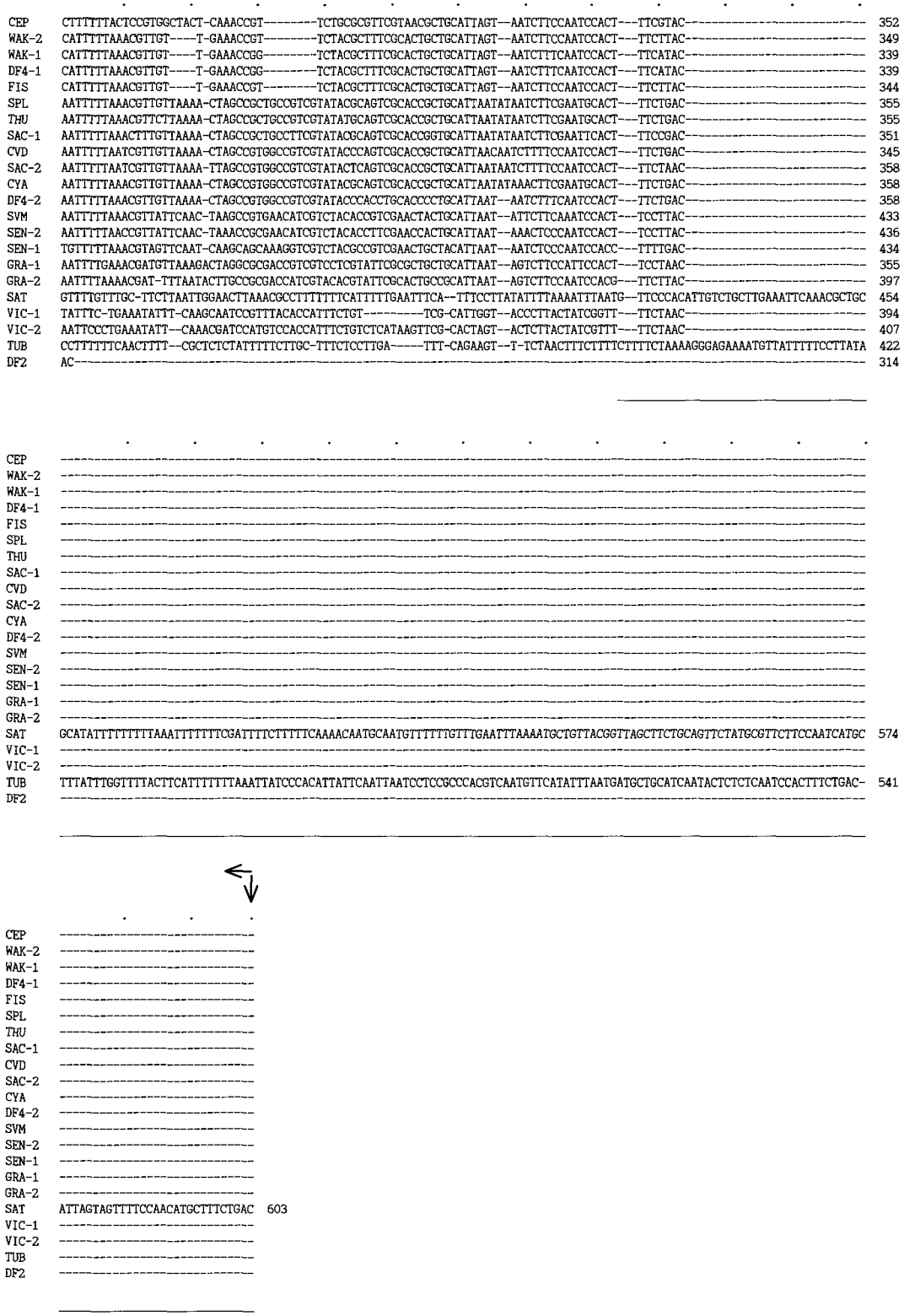


Fig. 3. Multiple alignment of 22 *Allium* 5S rRNA genes and NTS repeat sequences. The symbols of taxa are indicated by the three letter codes (see Table 1). The first nucleotide of 5S rRNA gene is position 1 and the last nucleotide is position 120. The boundaries of the 5S rRNA coding region and NTS region are indicated by arrows, respectively. The asterisks below the aligned sequences indicate constant nucleotides across all the taxa, and ambiguously aligned sequence positions (excluded from the analysis) are underlined. The dots refer to the position of every 10 bases relative to the starting point of the sequence. The hyphens indicate the gaps assumed.

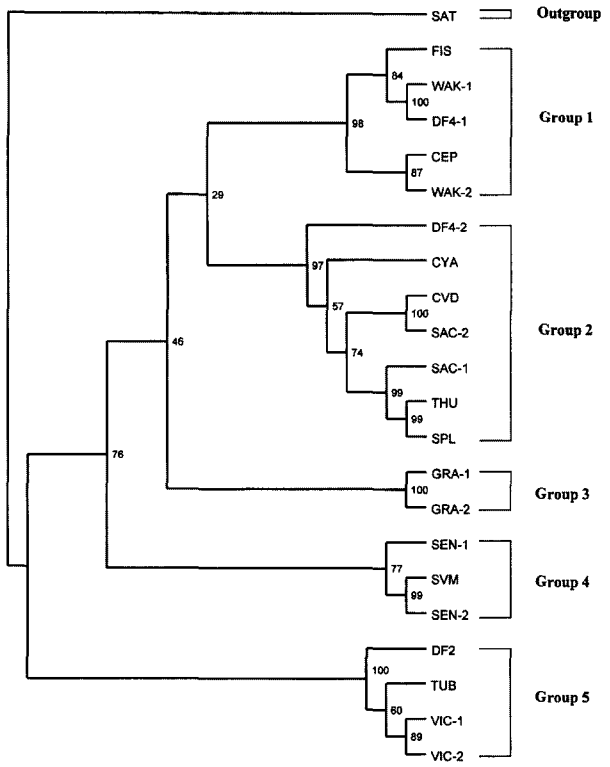


Fig. 4. The most parsimonious tree using DNAPARS in PHYLIP package. The numbers among the branches are confidence levels of consensus tree from 100 bootstrap replicates. The tree was constructed from the alignment shown in Fig. 3.

Sequence and phylogenetic analysis

The size of the coding region of 5S rRNA was 120 bp in all the species. While no variation of sequence size of 120 bp of 5S coding region was found within a species. For the most part, the sequences of coding regions of 5S rRNA genes are highly conserved within *Allium* species. The coding regions of 120 bp sequence size show high homology to other plant 5S rRNA gene sequences using the EMBL nucleic acid data bank. By comparison to other 5S genes, we infer that the coding region of the 5S rRNA gene starts with GGGTGC for the *Allium* species used in this study. The 3′ end of the coding region is likely located within a stretch of CCT, CCA or CCC residues between position 118 and position 120. The 5′ end of the NTS region contains blocks of T-rich sequences implicated in transcription termination (Korn, 1982), and terminates with an AC sequence in all species (Fig. 3).

Because only few variation in the coding region of the 5S rRNA was found, the function of coding region is deduced to be functional. However, the size and sequence in the NTS region during evolutionary pathway from putative ancient species would not be so critical that deletion or addition of a few base pair within NTS regions would not be affected by species differentiation. Our result corroborates with the same aspects in the view of coding region and NTS region. As shown in Fig. 3, NTS regions showed little homology among *Allium* species and outside of regulartory signals, do not code for any known function. *A. sativum* showed an extraordinary large NTS region about two times longer than the smallest NTS sequence variant.

Fig. 4 showed the parsimonious tree using the entire unambiguously aligned NTS region shown in Fig. 3. The cladogram showed little agreement with grouping of *Allium* diploid species based on chromosomal *in situ* hybridization patterns of 5S rDNA reported by Lee et al. (1999). In Fig. 4, sixteen *Allium* species are grouped into five clades from the phylogenetic analysis. *A. sativum* which belongs to subg. *Allium* formed separate clades on the parsimonious tree. In the phylogenetic Group 1, two components of NTS sequences of *A. wakegi* (WAK-1 and WAK-2) were grouped with those of *A. cepa* (CEP) and *A. fistulosum* (FIS). One component of *A. deltoide-fistulosum* (2*n*=32) (DF4-1) showed high affinity to the Group 1. Group 2 comprises *A. cyaneum* var. *deltoide* (CVD), *A. splendens* (SPL), *A. cyaneum* (CYA), *A. thunbergii* (THU), one component of *A. deltoide-fistulosum* (2*n*=32) (DF4-2), and two components of *A. sacculiferum* (SAC-1 and SAC-2). One 5S repeat variant of *A. sacculiferum* (SAC-2) was closely related to *A. cyaneum* var. *deltoide*, while another variant (SAC-1) was related to *A. splendens*, and *A. thunbergii*. Therefore, the origin of *A. sacculiferum* could be inferred from genomic hybrid of these related species. Group 3 comprises only two variants of NTS regions of *A. grayi* (GRA-1 and GRA-2). Group 4 comprises two kinds of NTS of *A. senescens* (SEN-1 and SEN-2) and that of *A. senescens* var. *minor* (SVM). One 5S repeat variant of *A. senescens* (SEN-2) was closely related to *A. senescens* var. *minor* (SVM). Group 5 comprises *A. deltoide-fistulosum* (2*n*=16) (DF2), *A. tuberosum* (TUB), and two components of NTS sequences of *A. victorialis* var. *platyphyllum* (VIC-1 and VIC-2). Group 5 was closely placed to the outgroup, *A. sativum*.

Discussion

This study has demonstrated the molecular variation of 5S rRNA genes in 15 *Allium* subg. *Rhizirideum* and 1 *Allium* subg. *Allium*. Examination of the nucleotide sequences in 5S rRNA genes revealed that sequences of the coding regions were highly conserved with a few base substitutions in the 16 *Allium* species studied. However, the minor base changes would not cause any problems in functioning as a 5S subunit. The size of the 5S NTS region normally range from 194 bp to 483 bp and contained conserved sequence motifs which have been postulated to have a regulatory function (Fig. 3). An AT-rich element which is similar to the sequence required for the transcription initiation of 5S rRNA genes in *Neurospora* (Selker et al., 1986) and found upstream of many other plant 5S rRNA genes (Rafalski et al., 1982; Venkateswarlu et al., 1991;

Schmidt et al., 1994) is localized between -29 and -21 upstream of the *Allium* 5S rRNA genes. The C residue at -1 supports the proposed cytosine conservation at this position (Venkateswarlu et al., 1991).

Khvyrleva et al. (1988) reported that two types of 5S rRNA gene sequences were present in barley, one class with a short, approximately 180 bp long NTS, and a class with a larger NTS according to the variable number of internal TAG repeats. Kanazin et al. (1993) found that the 5S rRNA genes in barley exist as two classes of repeat, a long and short, and the repeat with the larger NTS located on chromosome 3 and smaller NTS on chromosome 2, respectively. Mukai et al. (1990) reported that two units of 5S rRNA loci, long and short, were localized on separate chromosomes of homologous groups 1 and 5 of Chinese Spring wheat and that signal intensity was different. In this study, however, two kinds of 5S rRNA genes are primarily located on the intercalary region of chromosomes 5 and/or 7 in diploid species and various chromosomes in alloploid species. Moreover, regardless of location, these genes show no difference in signal strength.

Phylogenetic relationships among the *Allium* species were not well understood, and taxonomic classifications have been based on relatively few morphological characters. Bard and Elkington (1978) recommended that morphological, cytological, and chemical data should be used in combination to a more informative classification of *Allium* and for the origin and evolution of polyploid species. But all the earlier studies failed to identify the genome donors to allotetraploid species of *Allium*. In this study, we applied the knowledge of the molecular variation of 5S rRNA genes to reveal the genomic origin of allotetraploids and the genetic relationships among 15 *Allium* subg. *Rhizirideum* and 1 *Allium* subg. *Allium*. Because of their universal occurrence, the rRNA genes have been a frequency and source of data for phylogenetic analysis, and for probing specific sequences to aid species identification. Hence, the data obtained from sequence homology of the NTS region flanking the 5S rRNA coding region gave a better understanding of the evolution of the *Allium* species as well as the delineation of groups of *Allium* species within which interspecific hybrids potentially might have been generated. As shown in Fig. 4, *A. cyaneum* (CYA), *A. cyaneum* var. *deltoides* (CVD) and *A. thunbergi* (THU) belong to the same clade of Group 2. This result agrees with the result of Giemsa C-banging pattern by Seo et al. (1989) and of the FISH pattern of 5S rDNA by Lee et al. (1999). However, additional *Allium* species in this study do not correlate with grouping of *Allium* species based on chromosomal localization by FISH pattern of 5S rDNA reported by Lee et al. (1999).

Although it is dangerous that phylogenetic similarity was discussed as a clue for grouping based on sequence variation of 5S rRNA genes, these results could assist in determinig the phylogenetic pathway of the alloploid species to some extent. The evolutionary tree estimated by the maximally parsimonious trees was essentially similar in its topology to the maximum likelihood method (data not shown). When interpreting the phylogeny, however, the taxa used in this analysis can be clustered into 5 groups. The results obtained from 5S rRNA genes sequence in diploid and alloploid species (Fig. 3) could assist in determining the phylogenetic pathway of the alloploid species. Diploid species showed only one sequence in all species with the exception of *A. victorialis* var. *platyphyllum*. The allotetraploid and amphihaploid species showed two kinds of sequences. In one of these species, i.e. *A. wakegi*, two 5S rRNA variants gene sequence (Group 1) in Fig. 4 led to the conclusion that its genomic constitution is from two different genomes present in different species. Because *A. deltoide-fistulosum* ($2n$= 32) has shown to have a very different C-banding patterns and external morphology than those from *A. wakegi* (Seo and Kim, 1989), it was apparent that two 5S repeat variants of *A. wakegi*, WAK-1 and WAK-2, were closely related to *A. fistulosum* and *A. cepa*, respectively. These results could bring up a possible insight for genomic origin of the allotetraploid. *A. wakegi* was known to be a hybrid between *A. cepa* and *A. fistulosum* from the genome analysis of *A. wakegi* on the basis of chromosome pairing (Tashiro, 1984), genomic *in situ* hybridization (GISH) using both total DNA (Hizume, 1994), and FISH using 5S and 18S-26S rRNA genes (Lee and Seo, 1997). Likewise, we could infer the possibility of the evolutionary pathway of allotetraploids of *A. sacculiferum*, *A. senescens*, *A. deltoide-fistulosum*, and *A. grayi*. On the basis of the phylogenetic analysis in Fig. 4, it is likely that *A. grayi is* more primative than other *Allium* species according to 5S rRNA gene sequences, which requires more extensive examinations to confirm its exact taxonomic position.

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