J. Plant Biol. 39(1) : 1~8 (1996)

Comparative Analysis of ITS Sequences from *Acer* Species (Aceraceae) in Korea

Suh, Youngbae*, Hye-Jeong Cho[1], Sangtae Kim[1] and Chong-Wook Park[1]

Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea;
and [1]Department of Biology, College of Natural Sciences, Seoul National University, Seoul 151-742, Korea

Sequences from the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA were determined to assess their potential as a phylogenetic tool for Korean *Acer* species, including *A. okamotoanum* and *A. takesimense* which are endemic on Ullung Island of volcanic origin. Although the genus *Acer* has been studied by various authors, different infrageneric dispositions have been suggested, and the phylogeny of the genus has been in dispute. The variation of ITS sequences from seven species of *Acer* was very low among species within the same section, but comparative analyses of the molecular data obtained suggest that ITS sequences may provide enough phylogenetic resolution for sectional relationships in the genus.

Keywords: *Acer*, Aceraceae, ITS, ribosomal DNA, molecular phylogeny

The genus *Acer*, consisting of about 150 species with many infraspecific taxa, is primarily distributed in temperate regions of the Northern Hemisphere and some tropical regions including southeast Asia (Murray, 1970; Delendick, 1981). The genus is taxonomically very difficult and highly variable; a large number of microspecies and inconsequential varieties and formae were generated due to the complicated patterns of morphological variation (Delendick, 1981; Park *et al.*, 1993). Since the genus *Acer* was established by Linnaeus in 1753 (Linnaeus, 1753), close to 6,000 scientific names have been reported, indicating that botanical nomenclature has been well provided for the genus.

However, species delimitation is still in dispute for many instances (Delendick, 1981; Chang, 1991, 1994; Park *et al.*, 1993, 1994), and infrageneric dispositions and phylogenetic schemes for the genus produced by different workers do not approach closely one another (Monotani, 1962; Fang, 1966; Ogata, 1967; Murray, 1970; de Jong, 1976; Delendick, 1981). For example, Monotani's (1962) and Fang's (1966) treatments are very different from those by more recent workers (Murray, 1970; de Jong, 1976; Delendick, 1981). Murray (1970) proposed seven subgenera with 23 sections, but de Jong (1976) divided the genus into 14 sections, providing a quite different treatment from Murray's (1970). Delendick (1981) recognized 20 sections, and his sectional treatment is more or less similar to that of de Jong's (1976). In addition, Delendick (1981) disposed 20 sections he recognized into five groups in consideration of morphology, palynology, and flavonoid chemistry, but he did not assign formal taxonomic ranks to his groups (Delendick, 1981).

Eighteen species are found in Korea (Lee, 1979), and two of them, *A. okamotoanum* Nakai and *A. takesimense* Nakai, are endemic on Ullung Island which is of volcanic origin (Lee and Yang, 1981). Ullung Island lies about 150 km east from the Korean Peninsula, and has never been connected with the continent since it was formed by volcanic activity between the late Tertiary and the early Quaternary (Kim, 1971; Park and Park, 1981). Although *A. okamotoanum* and *A. takesimense* are endemic on the island, these two species are not phylogenetically related with each other, and they were probably given rise to by independent evolutionary events.

Molecular phylogenetic studies have demonstrated that the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (nrDNA) are very useful to reconstruct phylogenies at infrageneric or infrafamiliar levels, because their rates of divergence

*Corresponding author: Fax +82-2-740-8906

are considerably higher in comparison to protein or rRNA coding genes such as *rbcL* and 18S/26S ribosomal DNA (Baldwin, 1992; Suh *et al.*, 1993; Kim and Jansen, 1994). In addition, recent studies of endemic genera of the Asteraceae on the Juan Fernandez Islands proved that comparative analyses of the ITS sequences are also useful for addressing phylogenetic questions on recent evolutionary events (Sang *et al.*, 1994, 1995).

In this study, as a part of comprehensive phylogenetic study of the genus *Acer*, we analyzed the ITS sequences of nrDNA from seven species to examine their potential as a phylogenetic tool for *Acer* species in Korea and to address phylogenetic questions on the differentiation of two endemic species of Ullung Island, *A. okamotoanum* and *A. takesimense*. We also examined restriction fragment length polymorphism (RFLP) of the intergenic spacer (IGS) region of nrDNA for *A. takesimense* and its close relatives.

MATERIALS AND METHODS

Plant materials and DNA extraction

Leaves of seven species of *Acer* including two endemic species on Ullung Island were collected in the field and transported to the laboratory on the ice (Table 1). Leaves were powdered in liquid nitrogen and kept in −70°C deep freezer until DNA extraction. Total DNA was extracted by the 2× CTAB method (Doyle and Doyle, 1989), and further purified with Geneclean II Kit (Bio 101, CA, USA) for PCR.

Table 1. Species of *Acer* used for sources of DNA. Vouchers are at SNU

Species	Voucher[a]	Locality
Sect. *Platanoidea*		
A. mono Maxim.	KJH 301	Kangwon Prov.: Mt. Sorak
A. okamotoanum Nakai	KTJ 101	Kyungbuk Prov.: Ullung Island
A. platanoides L.	KTJ 9512	Kyunggi Prov.: Kwangreung Arboretum
A. truncatum Bunge	KJH 9501	Kyunggi Prov.: Kwangreung Arboretum
Sect. *Palmata*		
A. japonicum Thunb.	CHO 572	Chungnam Prov.: Chullipo Arboretum
A. pseudosieboldianum (Pax) kom.	CHO 204	Kyunggi Prov.: Kwangreung Arboretum
A. takesimense Nakai	CHO 108	Kyungbuk Prov.: Ullung Island

[a]KTJ stands for collection by T. J. Kim, KJH by J.-H. Kim, and CHO by H.-J. Cho.

PCR and sequencing

PCR was carried out in 100 μL final volume containing 0.5 ng template DNA, 2.5 units of Taq polymerase (Perkin-Elmer Cetus, USA), 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 μM of each dNTP, and 0.5 μM of each primer. PCR primers were 'ITS1' and 'ITS4' designed by White *et al.* (1990). Amplification was performed in a Perkin Elmer Cetus DNA Thermal Cycler programmed for 30 cycles of denaturation for 1 minute at 95°C, annealing for 1 minute at 55°C, and extension for 45 seconds at 72°C. Primer extension time was gradually increased by 3 seconds with each cycle. A pre-denaturation step for 3 minutes at 95°C preceded the first cycle, and a final extension step for 7 minutes at 72°C followed the completion of 30 thermal cycles. Double stranded PCR product was directly sequenced by using Sequenase PCR Product Sequencing Kit (USB, OH, USA). The excess dNTPs, primers, and extraneous single-stranded DNAs produced by the PCR were removed by Shrimp Alkaline Phosphatase and Exonuclease I. In addition to PCR primers, 'ITS2' and 'ITS3' of White *et al.* (1990) were used as internal primers for sequencing in both directions. Electrophoresis was performed with denaturing formamide gel, and glycerol tolerant buffer containing taurine was used instead of TBE buffer (protocols for Sequenase PCR Product Sequencing Kit, 2nd ed., USB).

RFLP of IGS

The IGS region was amplified by PCR technique using 'NS1R' (reverse primer of 'NS1' of White *et al.*, 1990) and 'NLL' (5'-GACGGGGTATTGTAAGTG-3'; designed by H.-J. Cho). Amplification was performed in a Perkin Elmer Cetus DNA Thermal Cycler programmed for 30 cycles of denaturation for 1 minute at 95°C, annealing for 1 minute at 46°C, and extension for 1 minute at 72°C. Primer extension time was gradually increased by 3 seconds with each cycle. A pre-denaturation step for 3

minutes at 95°C preceded the first cycle, and a final extension step for 7 minutes at 72°C followed the completion of 30 thermal cycles. PCR was carried out in 100 μL final volume containing 0.5 ng template DNA, 0.5 μM of each primer, 4.5 mM $MgCl_2$, 250 μM of each dNTP, 2.5 units of Taq polymerase, and 1× PCR buffer supplied by the vendor (Takara LA PCR kit; Takara Shuzo Co., Japan). PCR product band was identified on the 1.5% agarose gel and sliced from the gel. DNA was eluted by squeezing the frozen agarose block, and purified by 70% ethanol precipitation. DNA was treated with restriction enzymes, and restriction fragments were separated by electrophoresis in 1.5% agarose gel.

Sequence alignment and phylogenetic analyses

The sequence boundaries of ITS 1, 5.8S coding region, and ITS 2 were determined by the comparison with those from previous studies (Yokota *et al.*, 1989; Baldwin, 1992; Suh *et al.*, 1993; Kim and Jansen, 1994). Sequences were aligned by using Clustal V program (provided by D. Higgins), and then finally adjusted by eyes. Gaps were excluded in sequence divergence calculation and phylogenetic analysis. Phylogeny was reconstructed with Wagner parsimony by PAUP (ver. 3.1.1; Swofford, 1993). The branch and bound searches with collapse of zero-length branches were conducted, and character state changes were equally weighted in the analysis. The tree was rooted at the longest branch separating clades of sects. *Platanoidea* Pax and *Palmata* Pax (Lundberg, 1972). Sequence divergence values were calculated using the Kimura's two-parameter method (Kimura, 1980). Kimura's distances and the neighbor-joining tree were obtained using DNADIST program with default settings (transition vs. transversion =2:1) and NEIGHBOR program in PHYLIP (ver. 3.5; Felsenstein, 1992), respectively.

RESULTS

The size of ITS 1 for seven *Acer* species examined ranged from 234 to 236 bp, and that of ITS 2 ranged from 235 to 239 bp. The size of the 5.8S coding region was 164 bp for all species examined. The G+C content in ITS 1 was 62.4–64.0%, ITS 2 60.9–62.3%, and 5.8S 55.4% (Table 2).

Sequence alignment required three independent indels for ITS 1 and five indels for ITS 2. Out of eight indels, the largest gap was a 4-base indel. The 2-base indels occurred in five instances, and 1-base indels in two instances (Fig. 1). The range of sequence divergence (Kimura's K×100; Kimura, 1980) of ITS 1 and ITS 2 was 0–0.86% and 0–1.29%, repectively, among species of sect. *Platanoidea*. Sequences from three species of sect. *Palmata, A. japonicum* Thunb., *A. pseudosieboldianum* (Pax) Kom., and *A. takesimense*, were identical throughout ITS 1, ITS 2, and 5.8S. However, PCR yielded different products for IGS from these three species. Amplified PCR product of IGS from *A. takesimense* was shorter than those from *A. japonicum* and *A. pseudosieboldianum* by about 40 bases (Fig. 2). Between sects. *Platanoidea* and *Palmata*, sequence divergence ranged from 9.62 to 10.10% for ITS 1 and from 11.11 to 11.65% for ITS 2. In the combination of ITS 1 and ITS 2, it was 0–1.05% among species of sect. *Platanoidea* and 10.36–10.87% between sects. *Platanoidea* and *Palmata* (Table 3).

Parsimony analysis generated two shortest trees. The only difference between the two trees is the collapse of branch at the basal node, where *A. oka-*

Table 2. Size and base composition of ITS 1, ITS 2, and 5.8S coding region of nuclear ribosomal DNA from seven *Acer* species included in the study

Species	ITS 1		ITS 2		5.8S	
	Length (bp)	G+C (%)	Length (bp)	G+C (%)	Length (bp)	G+C (%)
Sect. *Platanoidea*						
A. mono	234	62.4	235	60.9	164	55.4
A. okamotoanum	234	62.8	236	61.4	164	55.4
A. platanoides	236	62.7	236	62.3	164	55.4
A. truncatum	234	62.4	235	60.9	164	55.4
Sect. *Palmata*						
A. japonicum	236	64.0	239	61.9	164	55.4
A. pseudosieboldianum	236	64.0	239	61.9	164	55.4
A. takesimense	236	64.0	239	61.9	164	55.4

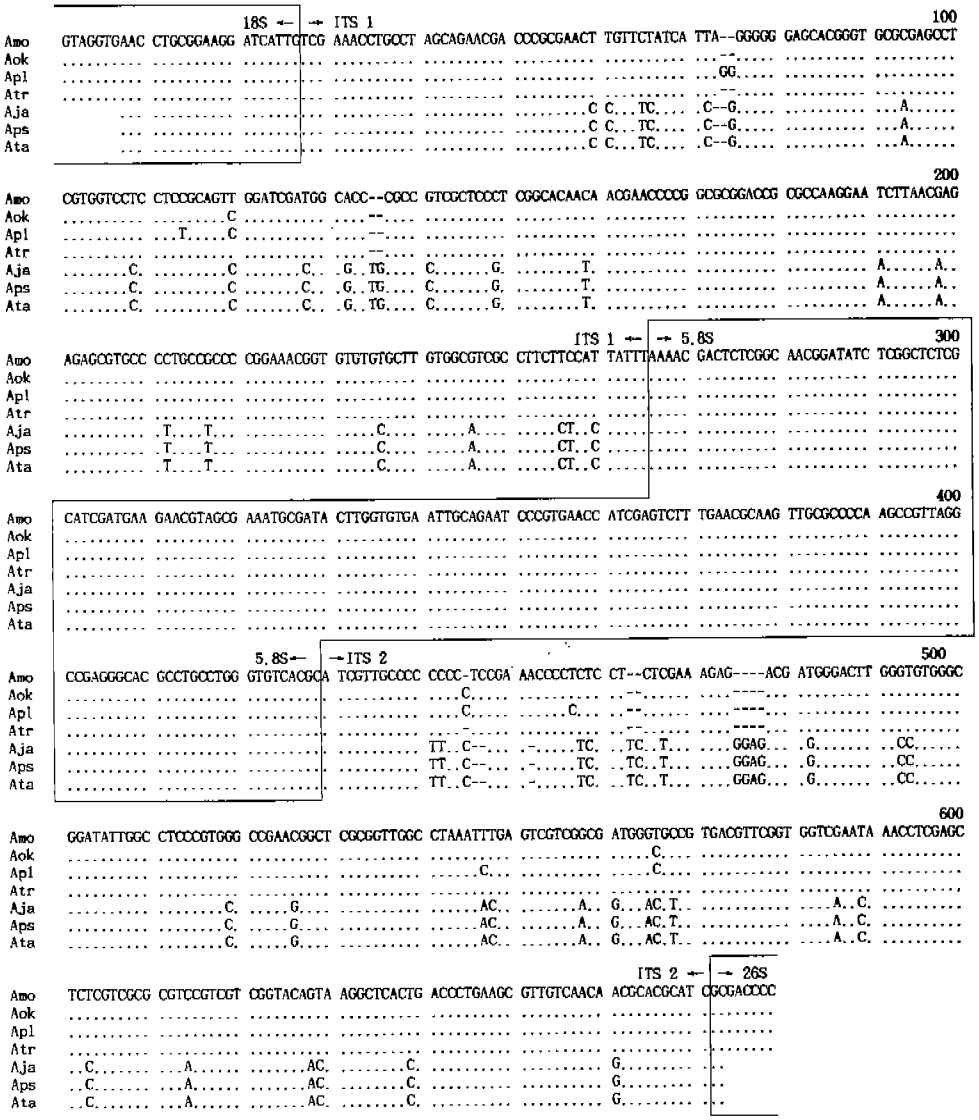

Fig. 1. Aligned sequences of ITS from seven species of *Acer*. Dots in lines indicate same nucleotides as the first taxon (Amo), and dashes represent gaps required for alignment. Sequences have been deposited in the GenBank database with accession numbers of u57772 – u57778.

motoanum is separated from the clade of *A. mono* Maxim. and *A. truncatum* Bunge (Fig. 3). The neighbor-joining tree (Saitou and Nei, 1987) constructed from Kimura's distance matrix (Kimura, 1980) shows an identical topology with one of the two most parsimonious trees (Fig. 3). Out of 50 steps on the tree, 44 changes occur at the branch separating sects. *Platanoidea* and *Palmata*, and changes among species within each section are very low. In addition, *A. okamotoanum*, an endemic species of Ullung Island, appears to have been differentiated before *A. mono* and *A. truncatum* were differentiated (Fig. 3).

DISCUSSION

The sizes of ITS 1 and ITS 2 lie within the range of those reported previously for other angiosperms (ITS 1, 194 – 268 bp; ITS 2, 188 – 237 bp), and the size of the 5.8S coding region is also similar to other plants (Takaiwa *et al.*, 1985; Kavanagh and Timmis, 1988; Kiss *et al.*, 1988; Rathgeber and Capesius, 1989; Schiebel and Hemleben, 1989; Yokota *et al.*, 1989; Unfried and Gruendler, 1990; Venkateswarlu and Nazar, 1991; Baldwin, 1992; Suh *et al.*, 1993; Kim and Jansen, 1994; Sang *et al.*, 1994,

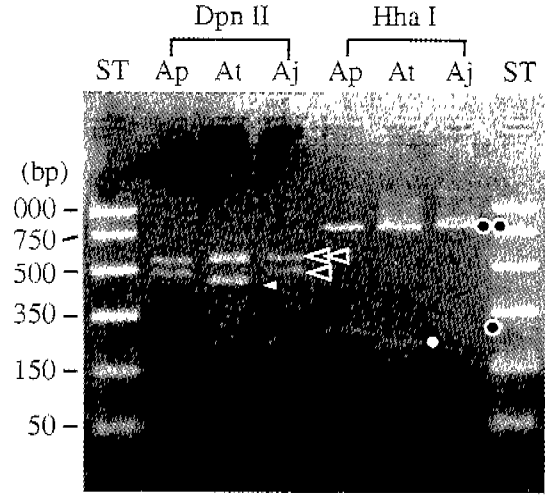

Fig. 2. Deletion of 40 bp in IGS of *Acer takesimense* as compared to those of *A. pseudosieboldianum* and *A. japonicum*. The PCR products were digested with *Dpn* II and *Hha* I, and fragments were separated by electrophoresis in 1.5% agarose gel. The *Dpn* II fragments were 600 bp (◀◀) and 490 bp (◀) for *A. pseudosieboldianum* and *A. japonicum*, but 600 bp and 450 bp (◁) for *A. takesimense*. The Hha I fragments were 820 bp (● ●) and 270 bp (●) for *A. pseudosieboldianum* and *A. japonicum*, but 820 bp and 230 bp (○) for *A. takesimense*. The 40 bp deletion in IGS of *A. takesimense* is demonstrated by the small fragments (◁, ○) of both restriction enzyme digests. ST= molecular size marker, Ap=*A. pseudosieboldianum*, At= *A. takesimense*, Aj=*A. japonicum*.

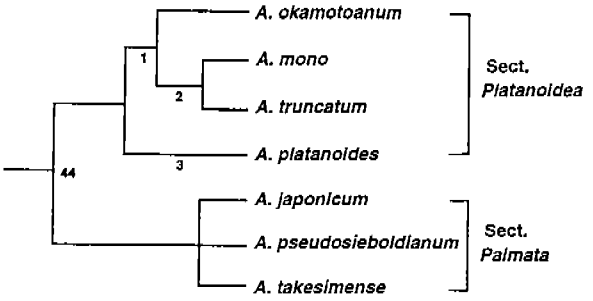

Fig. 3. One of two most parsimonious trees which is preferred by the neighbor-joining method. The tree is rooted arbitarily to separate the clade of sect. *Platanoidea* from sect. *Palmata*. Numbers below branches indicate the number of nucleotide changes.

1995). In *Acer* species, ITS 1 is slightly longer than ITS 2 in size. The G+C content in the ITS regions also lies within the range of those reported by previous studies listed above.

The ITS regions from *Acer* shows a striking feature in sequence divergence. Between sects. *Platanoidea* and *Palmata* in the genus *Acer*, sequence divergence is comparable to other angiosperms. In the Winteraceae, the range of nucleotide divergence is 0−11.8% for ITS 1 and 0−12.9% for ITS 2 between pairs of genera (Suh *et al.*, 1993). In the Brassicaceae, sequence divergence between *Sinapis alba* and *Arabidopsis thaliana* is 24.3% for ITS 1 and 18.9% for ITS 2 (Rathgeber and Capesius, 1989; Yokota *et al.*, 1989). In the Asteraceae, sequence divergence within the subtribe Madiinae ranges from 0 to 12.9%. Within the tribe Heliantheae of the Asteraceae, intertribal divergence between Madiinae and two outgroup taxa ranges from 0.8 to 22.9% in ITS 1 and from 7.2 to 19.6% in ITS 2 (Baldwin, 1992). Interspecific divergence in *Krigia* of the Asteraceae ranges from 1.61 to 15.19% and from 0 to 6.64% in ITS 1 and ITS 2, respectively. Intergeneric divergence among genera of the tribe Lactuceae ranges from 15.6 to 44.5% in ITS 1 and from 8.0 to 28.6% in ITS 2 (Kim and Jansen, 1994). On the other hand, sequence divergence between pairs of species within the same section of *Acer* appears to be very low. Species in sect. *Platanoidea* show sequence divergence value ranging from 0 to 0.86% for ITS 1 and from 0 to 1.29% for ITS 2 (Table 3). Moreover, three species of sect. *Palmata*, *A. japonicum*, *A. pseudosieboldianum*, and *A. takesimense*, show no differences in nucleotide sequences in both ITS 1 and ITS 2. Morphologically, *A. takesimense* is very similar to *A. pseudosieboldianum* and *A. japonicum*, and its taxonomic identity has been controversial (Chang, 1991, 1994; Park *et al.*, 1993, 1994). However, examination of the IGS region showed that *A. takesimense* is distinguished from the latter two species by a deletion of about 40 bp (Fig. 2). This result, in conjunction with morphological evidence (Park *et al.*, 1993) strongly suggests that the former is taxonomically distinct from the latter two. In general, the IGS region is known to evolve much faster than the ITS regions, and further investigation on the restriction fragment length polymorphism of the IGS region from closely related species in *Acer*, including *A. takesimense* and *A. pseudosieboldianum*, is currently under way to examine the patterns and the levels of differentiation.

As mentioned previously, the divergence of ITS sequences is very low among species within the same section, but considerably high between sections in *Acer*. If we assume a local molecular clock which ticks in constant rhythm within a certain lineage, this drastic difference in sequence divergence between intersectional and interspecific levels

Table 3. Sequence divergence of ITS region in *Acer* species. Number of substitutions per 100 sites (Kimura's K× 100; Kimura, 1980) is shown above diagonal and observed number of nucleotide differences in pairwise comparison below diagonal

	Amo	Aok	Apl	Atr	Aja	Aps	Ata
ITS 1							
A. mono	–	0.43	0.86	0.00	10.10	10.10	10.10
A. okamotoanum	1	–	0.43	0.43	9.62	9.62	9.62
A. platanoides	3	2	–	0.86	10.05	10.05	10.05
A. truncatum	0	1	3	–	10.10	10.10	10.10
A. japonicum	25	24	25	25	–	0.00	0.00
A. pseudosieboldianum	25	24	25	25	0	–	0.00
A. takesimense	25	24	25	25	0	0	–
ITS 2							
A. mono	–	0.43	1.29	0.00	11.65	11.65	11.65
A. okamotoanum	1	–	0.85	0.43	11.11	11.11	11.11
A. platanoides	2	1	–	1.29	11.60	11.60	11.60
A. truncatum	0	1	2	–	11.65	11.65	11.65
A. japonicum	22	21	22	22	–	0.00	0.00
A. pseudosieboldianum	22	21	22	22	0	–	0.00
A. takesimense	22	21	22	22	0	0	–
ITS 1+ITS 2 (combined)							
A. mono	–	0.43	1.07	0.00	10.87	10.87	10.87
A. okamotoanum	2	–	0.64	0.43	10.36	10.36	10.36
A. platanoides	5	3	–	1.07	10.82	10.82	10.82
A. truncatum	0	2	5	–	10.87	10.87	10.87
A. japonicum	47	45	47	47	–	0.00	0.00
A. pseudosieboldianum	47	45	47	47	0	–	0.00
A. takesimense	47	45	47	47	0	0	–

may indicate an interval of long period between two stages of differentiation. The genus *Acer* may have differentiated into major lineages leading to extant sections or major groups long ago, and then quite later, each of these lineages underwent recent speciation in regional floras, giving rise to many hardly distinguishable microspecies or varieties. Although ITS sequences do not provide enough phylogenetic resolution among species within the same section, the nucleotide divergence of the ITS regions between sects. *Platanoidea* and *Palmata* indicates that they would be useful for elucidating phylogenetic relationships among sections of *Acer*.

ACKNOWLEDGMENTS

This research was supported by a grant from the Ministry of Education, Korea (BSRI – 94 – 4416). We are grateful to Dr. J.-H. Kim and T. J. Kim for helping us collect plant materials.

LITERATURE CITED

Baldwin, B.G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. *Molec. Phylogenet. Evol.* **1**: 3-16.

Chang, C.-S. 1991. A morphometric analyses of genus Acer L., section *Palmata* Pax, series *Palmata. Korean J. Pl. Taxon.* **21**: 165-186.

Chang, C.-S. 1994. A critique of "Reexamination of vascular plants in Ullung Island, Korea II: Taxonomic identity of *Acer takesimense* Nakai (Aceraceae)." *Korean J. Pl. Taxon.* **24**: 279-284.

De Jong, P.C. 1976. Flowering and sex expression in *Acer* L. A biosystematic study. *Meded. Landbouwhogeschool* **76**: 1-201.

Delendick, T.J. 1981. A systematic review of the Aceraceae. Ph. D. dissertation, The City University of New York, New York.

Doyle, J.J. and J.L. Doyle. 1989. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull. Bot. Soc. Amer.* **19**: 11-15.

Fang, W.-P. 1966. Revisio Taxorum Aceracearum Sin-

icarum. *Acta Phytotax. Sin.* **11**: 139-189.

Felsenstein, J. 1992. PHYLIP (Phylogeny Inference Package) ver. 3.5. Department of Genetics, The University of Washington, Seattle.

Kavanagh, T.A. and J.N. Timmis. 1988. Structure of melon rDNA and nucleotide sequence of the 17 – 25S spacer region. *Theor. Appl. Genet.* **76**: 673-680.

Kim, K.-J. and R.K. Jansen. 1994. Comparisons of phylogenetic hypotheses among different data sets in dwarf dandelions (*Krigia*): Additional information from internal transcribed spacer sequences of nuclear ribosomal DNA. *Pl. Syst. Evol.* **190**: 157-185.

Kim, T.J. 1971. Natural geography of Ullung Island (in Korean). *In* A Report on the Scientific Survey of Ullung-do. Bureau of Cultural Property, Ministry of Culture and Information, Republic of Korea, pp. 15-26.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Molec. Evol.* **16**: 111-120.

Kiss, T., M. Kiss, S. Abel and F. Solymosy. 1988. Nucleotide sequence of the 17S – 25S spacer region from tomato rDNA. *Nucl. Acids Res.* **16**: 7179.

Lee, T.B. 1979. Illustrated Flora of Korea. Hyangmunsa, Seoul.

Lee, W.T. and T.S. Yang. 1981. The flora of Uleung Island and Dogdo Island (in Korean). *Report KACN* **19**: 61-95.

Linnaeus, C. 1753. Species Plantarum. 1st Ed., Stockholm.

Lundberg, J.G. 1972. Wagner networks and ancestor. *Syst. Zool.* **21**: 398-413.

Monotani, Y. 1962. Taxonomic study of the genus *Acer* with special reference to the seed proteins. III. System of Aceraceae. *Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, Biol.* **29**: 177-189.

Murray, E. 1970. A monograph of the Aceraceae. Ph. D. dissertation, Pennsylvania State University.

Ogata, K. 1967. A systematic study of the genus *Acer*. *Misc. Inform. Tokyo Univ. Forests* **63**: 89-206.

Park, C.-W., S.-H. Oh and H. Shin. 1993. Reexamination of vascular plants in Ullung Island, Korea II: Taxonomic identity of *Acer takesimense* Nakai (Aceraceae). *Korean J. Pl. Taxon.* **23**: 217-231.

Park, C.-W., S.-H. Oh and H. Shin. 1994. Character analysis and taxonomic identity of *Acer takesimense* Nakai. *Korean J. Pl. Taxon.* **24**: 285-294.

Park, D.W. and S.P. Park. 1981. Landforms of Ullung and Dogdo Islands (in Korean). *Report KACN* **19**: 37-59.

Rathgeber, J. and I. Capesius. 1989. Nucleotide sequence of the 18S – 25S spacer region from mustard DNA. *Nucl. Acids Res.* **17**: 7522.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molec. Biol. Evol.* **4**: 406-425.

Sang, T., D.J. Crawford, S.-C. Kim and T.F. Stuessy. 1994. Radiation of the endemic genus *Dendroseris* (Asteraceae) on the Juan Fernandez Islands. *Amer. J. Bot.* **81**: 1494-1501.

Sang, T., D.J. Crawford, T.F. Stuessy and M. Silva O. 1995. ITS sequence and the phylogeny of the genus *Robinsonia* (Asteraceae). *Syst. Bot.* **20**: 55-64.

Schiebel, K. and V. Hemleben. 1989. Nucleotide sequence of the 18S – 25S spacer region from rDNA of mung bean. *Nucl. Acids Res.* **17**: 2852.

Suh, Y., L.B. Thien, H.E. Reeve and E.A. Zimmer. 1993. Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. *Amer. J. Bot.* **80**: 1042-1055.

Swofford, D.L. 1993. PAUP (Phylogenetic analysis using parsimony) ver. 3.1.1. Illinois Natural History Survey, Champaign.

Takaiwa, F., K. Oono and M. Sugiura. 1985. Nucleotide sequence of the 17S – 25S spacer region from rice rDNA. *Pl. Molec. Biol.* **4**: 355-364.

Unfried, I. and P. Gruendler. 1990. Nucleotide sequence of the 5.8S and 25S rRNA genes and of the internal transcribed spacers from *Arabidopsis thaliana*. *Nucl. Acids Res.* **18**: 4011.

Venkateswarlu, K. and R. Nazar. 1991. A conserved core structure in the 18 – 25S rRNA intergenic region from tobacco, *Nicotiana rustica*. *Pl. Molec. Biol.* **17**: 189-194.

White, T.J., T. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols: A guide to methods and application. M. Innis, D. Gelfand, J. Sninsky and T. White (eds.). Academic Press, San Diego, pp. 315-322.

Yokota, Y., T. Kawata, Y. Iida, A. Kato and S. Tanifuji. 1989. Nucleotide sequences of the 5.8S rRNA gene and internal transcribed spacer regions in carrot and broad bean ribosomal DNA. *J. Molec. Evol.* **29**: 294-301.

(Received February 13, 1996)

韓國産 단풍나무屬 植物의 系統 硏究를 위한 ITS 鹽基序列 分析

徐 榮 倍*·趙 惠 晶[1]·金 相 泰[1]·朴 鍾 郁[1]

서울大學敎 天然物科學硏究所, [1]서울大學敎 自然科學大學 生物學科

적 요

단풍나무속 식물의 계통을 연구하기 위하여 일차적으로 울릉도의 고유종인 우산고로쇠(*Acer okamotoanum*)와 섬단풍나무(*A. takesimense*)를 포함한 한국산 단풍나무속 7종에 대하여 핵 리보솜 DNA 중 5.8S 리보솜 RNA 유전자 및 ITS 구간의 염기 서열을 결정, 비교하였다. 단풍나무속은 많은 학자들에 의하여 분류학적 연구가 이루어져 왔으나, 속내 분류군의 분류학적 한계 및 근연관계에 대하여 매우 다양한 견해가 제시되어 왔다. 그 결과, 현재에 이르기까지 단풍나무속 식물의 전체적인 계통 유연관계에 대하여는 일치된 견해에 도달하지 못하고 있다. 조사한 단풍나무속 7종의 경우, ITS 염기 서열의 변이는 같은 절 내의 종 간에는 다른 분류군에 비하여 매우 적었으나, 절 간에는 상당한 변이가 나타남으로써 ITS 염기 서열의 분석은 단풍나무속의 전체적인 계통 유연관계를 규명하기 위한 도구로 매우 유용하게 사용될 수 있는 것으로 판명되었다.

주요어: 단풍나무속, 단풍나무과, ITS, ribsomal DNA, 분자계통

*교신저자: Fax (02) 740-8906