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Molecular phylogenetic study of *Cardamine amaraeformis* Nakai using nuclear and chloroplast DNA markers

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ABSTRACT. The internal transcribed spacer regions of nuclear ribosomal DNA, *trn*L and *trn*L-F chloroplast genes of *Cardamine amaraeformis* Nakai (Brassicaceae) were sequenced and analyzed with the sequences of related *Cardamine* sp available in GenBank to detect the pattern of *C. amaraeformis* evolutionary differentiation. *C. amaraeformis*, which is endemic to South Korea, formed a clade with *Cardamine pedata* (69 bootstrap support) in all trees resulting from combined sequence data analyses of internal transcribed spacer, *trn*L and *trn*L-F genes. The phylogenetic analysis also clearly revealed that *C. amaraeformis* is distinct from the morphologically similar *Cardamine scutata*.

Key words: Molecular systematic; *Cardamine amaraeformis*; ITS; *trn*L; *trn*L-F

INTRODUCTION

The genus *Cardamine* L. (family Brassicaceae) comprises approximately 200 species and has indigenous taxa on all continents except Antarctica (Al-Shehbaz, 1988; Lihova and Marhold, 2006). In Korea, the genus *Cardamine* is represented by 16 species, i.e., *C. parviflora*, *C. impatiens*, *C. fallax*, *C. amaraeformis*, *C. flexuosa*, *C. scutata*, *C. komarovi*, *C. bellidifolia*, *C. changbaiana*, *C. pratensis*, *C. glechomifolia*, *C. leucantha*, *C. koreana*, *C. prorepens*, *C. yezoensis*, and *C. lyrata*. *C. amaraeformis* is known in Korean as Kkot-hwang-sae-naeng-i. *C. amaraeformis* is endemic to Korea, and was taxonomically described by Nakai in 1912 (see Park, 2007). This species has been morphologically characterized as follows: cauline leaves usually with two pairs of leaflets; leaflets oblong or oval, nearly equal in size and shape, margins undulate or sinuate; petals obovate, white or pink, distinctly netted veined, 7-12 mm long. A detailed perusal of the literature on the genus *Cardamine* reveals that the species status of *C. amaraeformis* is questionable, and the relationships within the genus have not yet been established (Ali MA, personal observation). Morphologically, *C. amaraeformis* is very similar to *C. scutata.* Hence, this study was undertaken to compare the internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA and chloroplast *trn*L and *trn*L-F in *C. amaraeformis*, *C. scutata* and other related species of the genus *Cardamine* to detect patterns of evolutionary differentiation.

MATERIAL AND METHODS

Fresh leaf material of *C. amaraeformis* was collected from the wild during plant exploration in South Korea (Voucher Changyoung KRIB0023213). Total DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN, Amsterdam, The Netherlands). ITS sequences of nuclear ribosomal DNA, *trn*L and *trn*L-F genes were amplified via the polymerase chain reaction (PCR) using AccuPower HF PCR PreMix (Bioneer, Daejeon, South Korea). One round of amplification consisted of denaturation at 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 49°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR products were purified with a SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) before sequencing. The purified fragments were directly sequenced using dye terminator chemistry following the manufacturer protocol. Cycle sequencing was conducted with the primers used for amplification, BigDye version 3 reagents and an ABI PRISM 3730XL DNA Analyzer (Perkin-Elmer, Applied Biosystems, USA) following manufacturer instructions. Cycling conditions included initial denaturing at 94°C for 5 min, followed by 30 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Each sample was sequenced in the sense and antisense direction. The sequences were analyzed with ABI Sequence Analysis and the ABI Sequence Navigator software (Perkin-Elmer/Applied Biosystems). Nucleotide sequences of both DNA strands were obtained and compared to ensure accuracy. For the phylogenetic analysis of ITS, *trn*L and *trn*L-F sequences of *C. amaraeformis*, 38 related species of *Cardamine* and 2 outgroup sequences of *Barbarea vulgaris* and *Rorippa divaricata* were retrieved from the National Center for Biotechnology Information GenBank database (www.ncbi.nlm.nih.gov; Table 1).

Table 1. Taxon and GenBank accession numbers used for the molecular systematic study of *Cardamine amaraeformis*.

Taxon	*trn*L	*trn*L-F	ITS
Cardamine alpine	FJ464509	FJ464529	AM905716
Cardamine amaraeformis	HM237352	HM237353	HM237351
Cardamine bonariensis	EU819200	EU819241	EU819314
Cardamine blaisdellii	EU819152	EU819303	EU819313
Cardamine carnosa	FJ384269	FJ384333	FJ384181
Cardamine constancei	EU819205	EU819244	EU819322
Cardamine debilis	DQ268059	DQ268226	DQ268392
Cardamine douglassii	EU819209	EU819247	EU819332
Cardamine enneaphyllos	FJ464515	FJ464537	EF136405
Cardamine fallax	DQ268123	DQ268288	DQ268464
Cardamine fialae	FJ384279	FJ384341	FJ384230
Cardamine glauca	FJ384262	FJ384327	FJ384178
Cardamine graeca	EU819164	FJ384356	FJ384197
Cardamine impatiens	DQ268171	DQ268339	AM905720
Cardamine longifructus	DQ268155	DQ268322	DQ268498
Cardamine maritima	FJ384295	FJ384355	FJ384224
Cardamine microphylla	EU819173	FJ464532	EU819347
Cardamine microzyga	EU819221	EU819266	EU819348
Cardamine monteluccii	FJ384253	FJ384319	FJ384208
Cardamine niigatensis	DQ268165	DQ268332	DQ268493
Cardamine nuttallii	FJ464523	EU819267	EU819350
Cardamine ovata	EU819225	EU819270	EU819353
Cardamine pancicii	FJ384263	FJ384328	FJ384179
Cardamine rupestris	FJ384283	FJ384343	FJ384200
Cardamine parviflora	DQ268070	DQ268237	DQ209133
Cardamine pedata	EU819176	EU819277	EU819356
Cardamine pectinata	DQ268175	DQ268338	DQ268502
Cardamine pensylvanica	DQ268136	DQ268304	DQ268469
Cardamine paucijuga	AY047640	DQ268294	DQ268455
Cardamine resedifolia	FJ464510	FJ464530	EU819364
Cardamine raphanifolia	AF079335	EF067933	AY260612
Cardamine rupicola	EU819232	EU819278	EU819368
Cardamine scutata	EU819227	EU819279	EU819372
Cardamine serbica	FJ384274	FJ384337	FJ384212
Cardamine tangutorum	EU819234	EU819282	EU819376
Cardamine tanakae	EU819233	EU819281	EU819375
Cardamine trifolia	FJ464526	FJ464548	DQ209114
Cardamine umbellata	EU819191	EU819297	EU819380
Cardamine victoris	EU819195	EU819300	EU819383
Rorippa divaricata	AF361900	AY030247	AF100693
Barbarea vulgaris	DQ479855	DQ518352	AJ232915

ITS = internal transcribed spacer.

Sequence alignments were performed using ClustalX version 1.81 (Thompson et al., 1997). Sequence alignments were subsequently adjusted manually using BioEdit (Hall, 1999). Gaps were treated as missing data in phylogenetic analyses. All sequences generated in the present study were deposited in GenBank, and the GenBank accession numbers are included in Table 1. Molecular evolutionary analyses were conducted using the maximum parsimony method implemented in Molecular Evolutionary Genetics Analysis (MEGA) version 4 (Eck and Dayhoff, 1966; Nei and Gojobori, 1986; Kumar and Gadagkar, 2001; Tamura et al., 2004, 2007). The maximum parsimony tree was obtained using the close-neighbor-interchange algorithm (Nei and Kumar, 2000) with search level three (Nei and Kumar, 2000; Tamura et al., 2007), in which the initial trees were obtained with the random addition of sequences (10 replicates). The codon positions included were 1st+2nd+3rd+noncoding. All positions containing

gaps and missing data were eliminated from the dataset (Complete Deletion option). A total of 1098 positions were included in the final dataset, of which 122 were parsimony informative.

RESULTS AND DISCUSSION

The amplified regions of ITS (ITS1-5.8S-ITS2), *trn*L, and *trn*L-F were 619 (GC content 54%), 401 (GC content 34%), and 288 bp (GC content 25%) long, respectively. The phylogenetic trees resulting from combined sequence data analyses of the ITS, *trn*L, and *trn*L-F genes clearly reveal that *C. amaraeformis* forms a strong clade with *C. pedata* (69 bootstraps) and is distinct or separate from the clade in which the morphologically similar *C. scutata* is nested. The bootstrap strict consensus tree of 14 maximally parsimonious trees of *Cardamine* is shown in Figure 1.

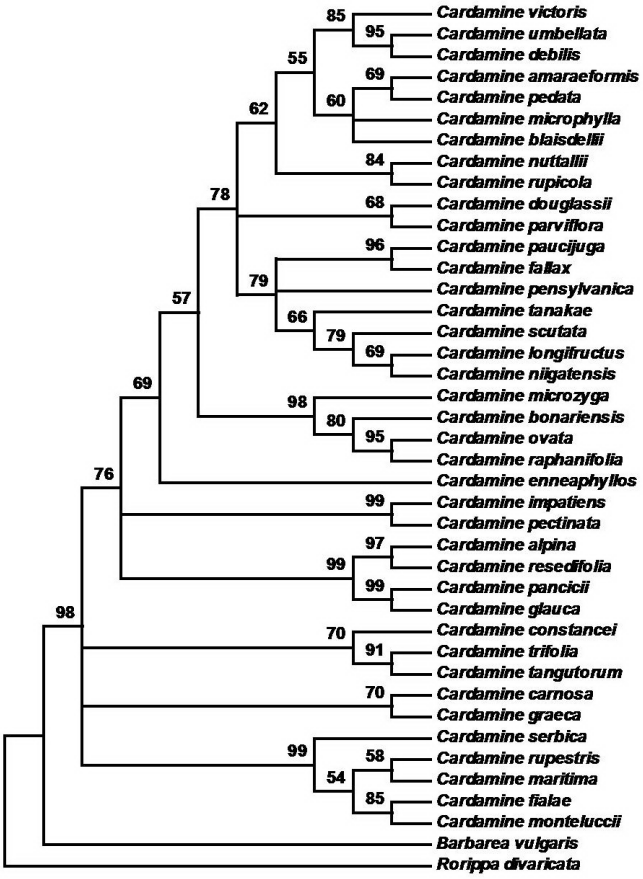

Figure 1. The bootstrap strict consensus of 14 maximally parsimonious trees of *Cardamine* inferred from the combined sequence data analysis of the internal transcribed spacer region of nuclear ribosomal DNA, *trn*L and *trn*L-F chloroplast genes. Bootstrap values greater than 50% in 100 replicates are shown above lines. Branches corresponding to partitions reproduced in less than 50% trees are collapsed. The consistency index is 0.5767, the retention index is 0.6892, and the composite index is 0.5115.

The genus *Cardamine* shows great morphological and karyological diversity and a complex evolutionary history strongly affected by both historical and more recent reticulation events (Lihova and Marhold, 2006) that provide an opportunity to study mechanisms of plant diversification. Major centers of diversity, assessed by species richness and endemism, occur in the Far East and the Himalayas, with approximately 70 *Cardamine* taxa reported (Al-Shehbaz, 1988). Several species have been reported to have spread as weeds beyond their natural ranges after introduction to distant areas and even different continents. Apart from those of the European species, most of which have been thoroughly investigated taxonomically (Lihova and Marhold, 2006), the taxonomies of species from other continents - South America (Sjostedt, 1975), Australia and New Zealand (Hewson, 1982; Webb et al., 1988), and eastern Asia (Ohwi, 1984; Zhou et al., 2001) - are extraordinarily complex and remain in many cases, controversial and unresolved. The phylogenetic analysis also clearly revealed that *C. amaraeformis* is distinct from the morphologically similar *Cardamine scutata*. This report is the first to recognize *C. amaraeformis* as a distinct species based on cladistic analysis of the ITS region of nuclear ribosomal DNA, *trn*L and *trn*L-F chloroplast gene sequences.

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REFERENCES

Al-Shehbaz IA (1988). The genera of Arabideae (Cruciferae, Brassicaceae) in the Southeastern United States. *J. Arnold Arbor.* 69: 85-166.

Eck RV and Dayhoff MO (1966). Atlas of Protein Sequence and Structure. National Biomedical Research Foundation, Silver Springs.

Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41: 95-98.

Hewson HR (1982). *Cardamine*. In: Flora of Australia (George AS, ed.). Australian Government Publishing Service, Canberra, 311-318.

Kumar S and Gadagkar SR (2001). Disparity index: A simple statistic to measure and test the homogeneity of substitution patterns between molecular sequences. *Genetics* 158: 1321-1327.

Lihova J and Marhold K (2006). Phylogenetic and Diversity Patterns in *Cardamine* (Brassicaceae) - A Genus with Conspicuous Polyploid and Reticulate Evolution. In: Plant Genome: Biodiversity and Evolution (Sharma AK and Sharma A, eds.). Science Publishers, Enfield, 149-186.

Nei M and Gojobori T (1986). Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Mol. Biol. Evol.* 3: 418-426.

Nei M and Kumar S (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Ohwi J (1984). Flora of Japan. Smithsonian Institution, Washington.

Park C (2007). The Genera of Vascular Plants of Korea. Academy Publishing Co., Seoul.

Sjostedt B (1975). Revision of the genus *Cardamine* L. (Cruciferae) in South and Central America. *Bot. Notiser* 128: 8-19.

Tamura K, Nei M and Kumar S (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. U. S. A.* 101: 11030-11035.

Tamura K, Dudley J, Nei M and Kumar S (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* 24: 1596-1599.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, et al. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25: 4876-4882.

Webb CJ, Sykes WR and Garnock-Jones PJ (1988). Flora of New Zealand. Vol. 4. Botany Division, D.S.I.R., Christchurch.

Zhou TY, Lu LL, Yang G and Al-Shehbaz IA (2001). Brassicaceae. In: Flora of China (Wu ZY and Raven PH, eds.). Science Press, Beijing, Missouri Botanical Garden Press, St. Louis, Missouri, 1-193.

