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Estimation of Phylogeny of Nineteen Sedoideae Species Cultivated in Korea Inferred from Chloroplast DNA Analysis

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The genetic diversity and relationships among plants belonging to the subfamily Sedoideae (Crassulaceae), some of which are indigenous to Korea or introduced from other countries, were determined using chloroplast (cp) nucleotide sequence analysis. To analyze genetic diversity and variation among 19 plants including species belonging to *Sedum*, *Hylotelephium*, and *Phedimus*, the tRNA-Leucine gene (*trn*L [UAA]) and adjoining spacer in chloroplast DNA (cpDNA) were sequenced and compared across species. Species were divided into two main groups based on the cpDNA sequence comparison. The generated phylogeny indicated that many native *Sedum* species had diverged from *S. album*. Members of the *Phedimus* and *Hylotelephium* species, and several *Sedum* species analyzed here, clustered distinctively in different groups. Using cpDNA sequence analysis, we successfully discriminated Sedoideae plants cultivated in Korea from each other, even at the intraspecific level, and the results were reflective of the morphological and biogeographical characteristics. These findings could be useful for classifying samples for proper naming, choosing breeding materials for new cultivars, or identifying species for conservation of horticultural crop resources.

Key Words: classification, Crassulaceae, *Hylotelephium*, *Phedimus*, *Sedum*.

Introduction

The Crassulaceae family includes 35 genera in six subfamilies that are divided into two lineages, a *Crassula* lineage and a *Sedum* lineage. The latter lineage is composed of three subfamilies (Echeverioideae, Sedoideae, and Sempervivoideae) (Berger et al., 1930). Despite the general consensus that a substantial part of it is highly artificial, Berger's classification for Crassulaceae has been widely used because of its comprehensiveness and great practical value (Van Ham and 'T Hart, 1998). *Sedum*, the largest genus of Crassulaceae, is a polyphyletic genus that encompasses much of the morphological diversity present in the family. The *Sedum* genus is, however, still poorly characterized and requires further investigation (Mort et al., 2001). Plants of the genus *Sedum* occur across the Northern Hemisphere, including in the Mediterranean countries, Mexico, and Far Eastern countries like China, Japan, and Korea (Stephenson, 1994). *Sedum* is known to contain about 470 species (Mayuzumi and Ohba, 2004), 22 of which are indigenous to Korea (http://www.nature.go.kr). The taxonomy of the subfamilies in Crassulaceae is based on the number and arrangement of floral parts, the degree of sympetaly, and the phyllotaxis, with these factors featuring in each of the larger genera (Van Ham and 'T Hart, 1998). Plants in the genus *Sedum* have succulent leaves, and the flowers usually have five petals, and only occasionally four or six. These features, along with their chromosome counts, define the genus *Sedum* (Ohba, 1977). While the basic genome chromosome number in *Sedum* species is eight (x = 8), chromosome doubling has occurred repeatedly, and some *Sedum* species contain x = 16, 32, and 64. Polyploidy appears to be common throughout the *Sedum* genus, and some members also show euploidy with chromosome counts of x = 7 or 31 (Stephenson, 1994). Especially in Korea, *Sedum* taxonomy is mainly based on morphological characteristics, and there is currently no accurate method of classifying *Sedum* species by genetic variation and divergence from the closest relative. *Hylotelephium* and *Phedimus* species have been previously classified as *Sedum* species (e.g., *Sedum telephium*) (Ohba, 1977). However,

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there is still confusion in the common or public naming system in Korea. *Sedum* species, such as *S. sarmentosum*, which is known as 'Dolnamul' in Korean, have been used traditionally as food. Recently, *Sedum* species were recognized as useful plant resources for landscape architecture or materials for ground cover in Korea. Despite these uses, a classification system for *Sedum* and related species based on genetic identification has not been properly established in Korea. To avoid naming confusion in the commercial market and improper identification, we aimed to identify indigenous *Sedum* and related species and those previously imported and now cultivated in Korea using molecular genetics data from chloroplast DNA (cpDNA) sequences to facilitate the conservation of horticultural resources and breeding materials.

Since DNA sequence data has led to reconstructions of classifications that often conflict with traditional taxonomy, cpDNA sequences have proven to be a valuable tool for phylogeny construction in plants because of their small size, high copy number, and maternally inherited nature (Chase and Palmer, 1989; Dong et al., 2013; Wu et al., 2010). The simultaneous alignment of many nucleotide sequences is an essential tool in molecular biology to detect homology among species, and the use of cpDNA in multiple sequence alignment to examine relationships and evolutionary changes is advantageous. To resolve phylogenetic relationships at several taxonomic levels, the maturase K (*mat*K) gene of cpDNA was used (Soltis et al., 1996). Recently, the phylogeny of the *Dendrobium* species in Thailand was reconstructed using data from a partial *mat*K gene and rDNA internal transcribed spacer (ITS) sequences (Srikulnath et al., 2015). Using sequences in a noncoding region in cpDNA, Crassulaceae species have been analyzed by several research groups for their molecular evolution or discrimination within the genus (Van Ham and 'T Hart, 1998; Van Ham et al., 1994). In particular, Mayuzumi and Ohba (2004) evaluated the phylogenetic positions of Eastern Asian Sedoideae plants using *trn*L-F (spacers between tRNA-Leucine and Phenylalanine) data. Recently, *trn*L-F regions were applied to estimate the relationships and track the origins among 31 hostas (Lee and Maki, 2015). Random amplified polymorphic DNA (RAPD) has also been used previously to set conservation priorities and design management strategies for taxa in *Sedum* species (Olfelt et al., 2001). Korean *Sedum* plants, in particular, are thought to have diverse phenotypes at both the species and interspecies levels (Kwon and Jeong, 1999). When investigating the relationships between 31 ecotypes of *S. sarmentosum* distributed in Korea, researchers found that the results from RAPD markers significantly correlated with morphological characteristics (Kim et al., 2008). Specifically, our study included 19 newly generated cpDNA sequences of 11 *Sedum*, 3 *Hylotelephium*, and 5 *Phedimus* species, and used molecular markers to examine the relationships between them. Sedoideae species used in this study either grow naturally or have been recently introduced to Korea and are cultivated. To discriminate native species from imported species and to develop hybrid cultivars showing useful new traits in breeding programs, the genetic characteristics and relationships need to be assessed. Therefore, we aimed to estimate the genetic relationships between Sedoideae plants from various origins by examining cpDNA sequences. The information obtained in this study could be useful for discriminating the native plants from each other for identification during resource conservation programs. The evaluation of molecular cpDNA markers would allow the development of proper discrimination methods among Sedoideae plants cultivated in Korea and their application for various practical uses, including in breeding programs.

Materials and Methods

Plant materials and genomic DNA extraction

Nineteen sample plants were obtained from the National Institute of Horticultural and Herbal Science located in Gyeonggi province, Korea, in May, 2014. Samples comprised eleven *Sedum* species, five *Phedimus* species, and three *Hylotelephium* species (Table 1). The closely related genera *Aeonium* and *Greenovia* were used as outgroups for the analysis. The species used and their overall characteristics are shown in Table 1. As the imported Sedoideae plants have been cultivated in Korea over several years, they were used in addition to the indigenous plants. Genomic DNA was extracted using an DNeasy Plant Mini Kit (Qiagen, USA). For each sample, 100 mg FW of plant leaves was used. The DNA quantity and quality were measured by absorbance at 260 nm with an ND 2000 spectrophotometer (Nanodrop Technologies, USA), and isolated DNA was checked by visualization on a 1% agarose gel with ethidium bromide (EtBr).

Amplification of cpDNA and sequencing

To compare cpDNA sequence variation, regions consisting of a tRNA-UAA gene (*trn*L) and *trn*L-*trn*F intergenic spacer (*trn*L-F) located in the large single-copy region of cpDNA were amplified. Samples were analyzed using primers encompassing *trn*L and flanking two intergenic spacers, *trn*L-F and *trn*T-L, as previously described by Taberlet et al. (1991). PCR amplification reactions contained 10–20 ng of genomic DNA, 20 pM of each primer (Taberlet et al., 1991), 10 mM Tris-HCl at pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.25 mM each of dATP, dGTP, dCTP, and dTTP, one unit of *Taq* DNA polymerase (Takara, Japan), and sterilized water in a total volume of 20 μL. PCR amplifications were performed at 94°C for 3 min, followed by 35 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, and finally incubated at 72°C for 5 min. PCR fragments were electrophoresed on a 1% agarose gel in 0.5×TAE

Table 1. Morphological, phenological, and biogeographical characteristics of Sedoideae species used in this study.

Scientific name	Abbreviation (in this study)	Phyllotaxis	Leaf					Flowering	Leaf phenology	Distribution	Synonym
			Apices	Margin	Cross-section	Shape	Petiole				
Phedimus kamtschaticus	Pkam	Alternate	Obtuse	Dentate	Flat	Linear-spatulate	Petiolate	Jun.–Aug.	Deciduous	Russia, N[z], C[y], E[x] China, Japan, Korea	*Sedum kamtschaticum*
Phedimus middendorffianus	Pmid	Alternate	Obtuse	Dentate	Flat	Linear-spatulate	Petiolate	Jun.–Aug.	Deciduous	Russia, China, Japan, N Korea	*Sedum middendorffianum*
Phedimus takesimense	Ptak	Alternate	Obtuse	Dentate	Flat	Linear-obovate	Petiolate	Jul.	Evergreen	Korea	*Sedum takesimense*
Phedimus spurius	Pspu	Opposite	Obtuse	Crenate	Flat	Obovate	Petiolate	May–Jun.	Evergreen	Georgia, Iran, Turkey	*Sedum spurium*
Phedimus spurius 'Tricolor'	PspuT	Opposite	Obtuse	Crenate	Flat	Spatulate to obovate	Petiolate	Apr.–May.	Semievergreen	Georgia, Iran, Turkey	*Sedum spurium* 'Tricolor'
Sedum sarmentosum	Ssar	Whorls	Subacute	Entire	Flat	Oblanceolate to oblong	Sessile	May–Jun.	Deciduous	China, Korea, Japan	—
Sedum lineare	Slin	Whorls	Obtuse	Entire	Flat	Linear to broadly oblanceolate	Sessile	May–Jun.	Semievergreen	SE[w] China, Japan, Korea	—
Sedum makinoi	Smak	Opposite	Acute	Entire	Flat	Spatulate to obovate	Sessile	May–Jun.	Evergreen	China, Japan, Korea	—
Sedum polystichoides	Spol	Alternate	Acute	Entire	Flat	Linear-lanceolate to oblong	Sessile	Aug.–Sep.	Evergreen	E China to Korea, Russian border, Japan	—
Sedum oryzifolium	Sorg	Alternate	Obtuse	Entire	Semiterete	Linear-elliptic to linear-obovate	Sessile	May–Jul.	Evergreen	Korea, Japan	*Sedum uniflorum* ssp.[t] *oryzifolium*
Sedum mexicanum	Smex	Whorls	Obtuse	Entire	Terete	Linear-lanceolate	Sessile	May–Jul.	Evergreen	USA, Mexico, C America, Colombia	—
Sedum album	Salb	Alternate	Obtuse	Entire	Terete	Linear-cylindrical	Sessile	May–Jun.	Evergreen	Except for parts of the N and E Europe	—
Sedum rupestre	Srup	Imbricate	Mucronate	Entire	Terete	Linear to oblong	Sessile	May–Jun.	Evergreen	Europe from Fennoscandia to Sicily, the Balkans to the Pyrenees	*Petrosedum rupestre*
Sedum acre	Sacr	Densely imbricate	Obtuse	Entire	Semiterete	Triangular-ovoid	Sessile	Apr.–May.	Evergreen	Whole Europe, N Africa, Turkey	—
Sedum sexangulare	Ssxa	Alternate	Obtuse	Entire	Terete	Linear-cylindrical	Sessile	Jun.–Jul.	Evergreen	C, S[u], SE Europe	—
Sedum japonicum	Sjap	Alternate	Rounded	Entire	Semiterete	Linear-ovate	Sessile	May–Jun.	Evergreen	China, Taiwan, Japan	*Sedum uniflorum* ssp. *japonicum*
Hylotelephium spectabile	Hspe	Opposite	Obtuse	Few undulate serrate	Flat	Ovate	Sessile	Aug.–Sep.	Deciduous	NE[v] China, Korea	*Sedum spectabile*
Hylotelephium telephium	Htel	Alternate	Obtuse	Dentate	Flat	Narrowly ovate	Sessile	Jul.–Sep.	Deciduous	Siberia, China, Korea, Japan	*Sedum telephium*
Hylotelephium sieboldii	Hsie	Whorled	Obtuse	Few low undulateserrate	Flat	Ovate	Petiolate	Oct.	Deciduous	China, Japan	*Sedum sieboldii*
Aeonium viscatum	Avis	Densely imbricate	Apiculate	Entire	Flat	Oblong-spatulate	Petiolate	Mar.–Jul.	Evergreen	Canary Islands	*Aeonium lindleyi* var.[s] viscatum
Greenovia aizoon	Gaiz	Densely imbricate	Apiculate	Entire	Flat	Oblong-spatulate	Sessile	Mar.–Jul.	Evergreen	Canary Islands	*Aeonium aizoon*

[z]: North; [y]: Central; [x]: East; [w]: South-Eastern; [v]: North-Eastern; [u]: South; [t]: subspecies; [s]: variety.

buffer at 135V for 18 min and then stained with EtBr. Amplification products (60 ng) were ligated into pGEM T-easy vector (Promega, USA), and the ligation mixture was transformed into *Escherichia coli*, strain DH5α. The recombinant plasmids were extracted by alkaline lysis and clones sequenced (Macrogen Inc., Korea) using T7/SP6 universal primers. A BLASTN search program in the National Center for Biotechnology Information (NCBI) database confirmed that these sequences were cpDNA.

Multiple alignment and generation of phylogeny based on cpDNA sequences

Nucleotide sequences of the cpDNA region in each sample were used to establish a distance matrix for analysis. Sequences of two outgroup species, *A. viscatum* and *G. aizoon*, were added to create a multiple alignment matrix; the sequences of *A. viscatum* and *G. aizoon* to be compared were retrieved from the NCBI database (Table 2). The cpDNA sequences were aligned using the CLUSTAL W multiple sequence alignment algorithm in MEGA6 with the following parameters: gap-opening penalty, 15; gap extension penalty, 6.66; transition weight, 0.5; delay divergence cut-off value, 30% (Tamura et al., 2013). A pairwise distance matrix was generated using the Kimura 2-parameter model (Kimura, 1980) and a phylogenetic tree was constructed using the Maximum-Likelihood (ML) method. Each bootstrap test was performed with 1000 replicates.

Results

Newly determined sequences of plant samples used in this study were approximately 1.4 kb long, but only the region of one spacer and *trn*L (UAA) gene of approximately 800 bp was investigated and compared between plants (Table 2). Sequences obtained from the cpDNA regions in 19 plants were analyzed and assigned the accession numbers shown in Table 2. Variations in the size of the sequenced region were observed with nucleotide site variation. The sequence of this region has been frequently used to investigate interspecific relationships in many species belonging to Crassulaceae and other plants because of the high and low intraspecific variations that occur at intervals in the noncoding regions (Taberlet et al., 2007). The length of *trn*L ranged from 568 bp in *S. sexangulare* to 617 bp in *S. album* (Table 2). The length of the *trn*L-F ranged from 246 bp in three *Phedimus* species to 286 bp in *H. spectabile* (Table 2). Most of the variations in length were caused by nucleotide insertion events, including some serial insertions. Serial insertions of nucleotides in the *trn*L sequenced region were found in *S. album*, where 19 and 29 nucleotides were inserted, respectively, in the middle of the gene (data not shown). An insertion of 23 nucleotides was observed in the *trn*L-F region of *H. spectabile*. Interestingly, serial deletions were also observed. Sixteen nucleotides in the *trn*L-F were missing at the same position in *P. kamtschaticus*,

Table 2. Nucleotide length analyzed from the region of the *trn*L(UAA) gene and *trn*L-F spacer located in the cpDNA of Sedoideae plants used in this study.

Scientific name	*trn*L(UAA) gene (nt)	*trn*L-F intergenic spacer (nt)	Total (nt)	Accession No.
P. kamtschaticus	579	246	861	KX510095 (In this study)
P. middendorffianus	578	246	860	KX510096 (In this study)
P. takesimense	579	246	861	KX510097 (In this study)
P. spurius	581	247	864	KX510098 (In this study)
P. spurius 'Tricolor'	581	255	872	KX510099 (In this study)
S. sarmentosum	581	269	886	KX510101 (In this study)
S. lineare	581	267	884	KX510102 (In this study)
S. makinoi	578	265	879	KX510100 (In this study)
S. polystichoides	576	260	872	KX510104 (In this study)
S. oryzifolium	578	256	870	KX510105 (In this study)
S. mexicanum	585	258	879	KX510103 (In this study)
S. album	617	252	905	KY207452 (In this study)
S. rupestre	590	257	882	KX510108 (In this study)
S. acre	574	271	881	KX510109 (In this study)
S. sexangulare	568	262	866	KX510110 (In this study)
S. japonicum	578	256	870	KX510111 (In this study)
H. spectabile	577	286	899	KX510112 (In this study)
H. telephium	577	261	874	KX510113 (In this study)
H. sieboldii	577	260	873	KX510114 (In this study)
A. viscatum	574	238	848	AY082299.1
G. aizoon	574	238	848	AY082229.1

Table 3. Pairwise distances of *Sedum* and related Sedoideae species estimated by the Kimura 2-parameter model.

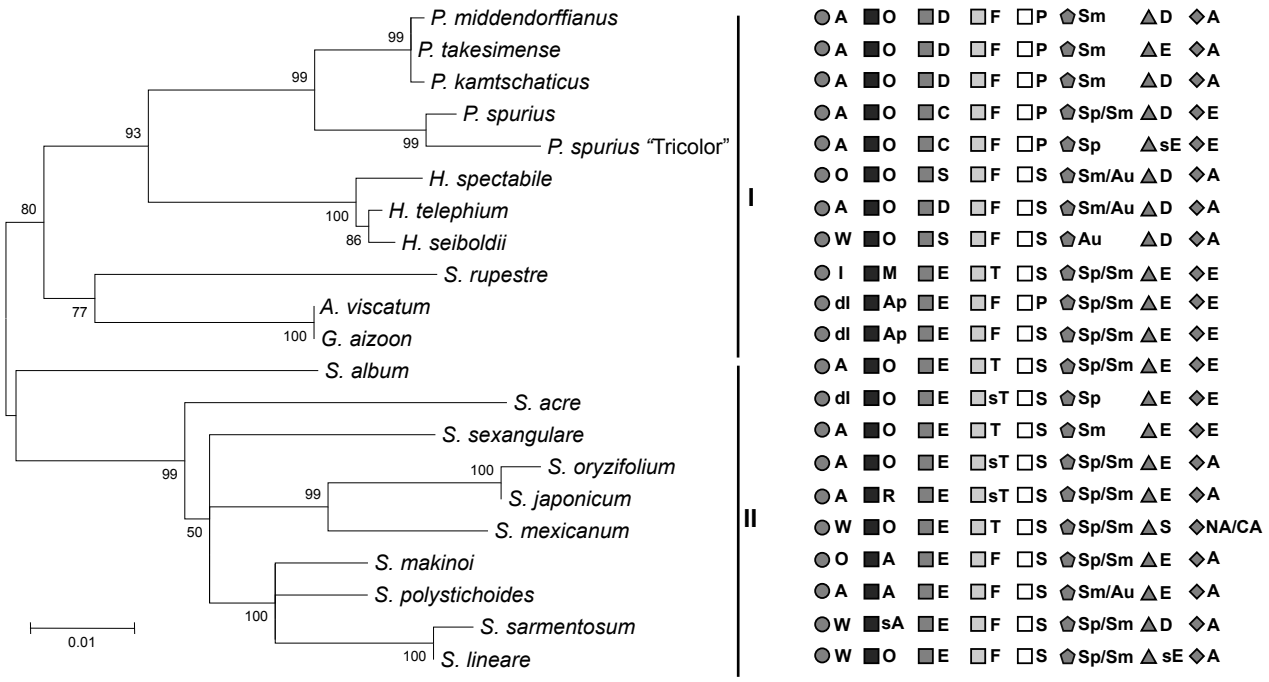
	Pkam	Pmid	Ptak	Pspu	PspuT	Ssar	Slin	Smak	Spol	Sorg	Smex	Salb	Srup	Sacr	Ssxa	Sjap	Hspe	Htel	Hsie	Avis	Gaiz
Pkam		0.002	0.002	0.005	0.006	0.011	0.010	0.010	0.009	0.010	0.010	0.009	0.009	0.012	0.010	0.010	0.008	0.008	0.008	0.009	0.009
Pmid	0.003		0.002	0.005	0.006	0.011	0.010	0.010	0.009	0.011	0.010	0.009	0.009	0.012	0.010	0.010	0.009	0.008	0.008	0.009	0.009
Ptak	0.002	0.003		0.005	0.006	0.010	0.010	0.010	0.009	0.010	0.010	0.009	0.009	0.012	0.010	0.010	0.008	0.008	0.008	0.009	0.009
Pspu	0.026	0.027	0.024		0.004	0.011	0.010	0.010	0.009	0.010	0.010	0.009	0.009	0.012	0.010	0.010	0.009	0.008	0.008	0.009	0.009
PspuT	0.033	0.032	0.031	0.014		0.011	0.011	0.011	0.010	0.011	0.010	0.010	0.010	0.012	0.010	0.011	0.009	0.009	0.009	0.010	0.010
Ssar	0.090	0.090	0.087	0.088	0.098		0.002	0.006	0.005	0.008	0.008	0.010	0.010	0.009	0.008	0.008	0.011	0.011	0.011	0.010	0.010
Slin	0.083	0.083	0.081	0.082	0.091	0.006		0.005	0.005	0.007	0.008	0.010	0.010	0.009	0.007	0.007	0.011	0.011	0.011	0.009	0.009
Smak	0.071	0.074	0.071	0.079	0.089	0.028	0.023		0.004	0.007	0.007	0.010	0.009	0.008	0.007	0.007	0.010	0.010	0.010	0.009	0.009
Spol	0.072	0.072	0.069	0.073	0.082	0.026	0.021	0.017		0.007	0.007	0.009	0.009	0.007	0.007	0.007	0.010	0.010	0.010	0.009	0.009
Sorg	0.092	0.092	0.090	0.092	0.100	0.060	0.056	0.053	0.049		0.006	0.010	0.011	0.009	0.008	0.002	0.012	0.011	0.011	0.010	0.010
Smex	0.085	0.085	0.082	0.086	0.097	0.058	0.053	0.049	0.045	0.040		0.010	0.010	0.009	0.008	0.006	0.011	0.011	0.011	0.009	0.009
Salb	0.068	0.069	0.065	0.067	0.080	0.088	0.082	0.075	0.073	0.089	0.083		0.009	0.011	0.009	0.010	0.010	0.009	0.009	0.009	0.009
Srup	0.069	0.070	0.066	0.071	0.082	0.093	0.087	0.084	0.078	0.097	0.086	0.075		0.011	0.010	0.011	0.010	0.009	0.009	0.008	0.008
Sacr	0.095	0.095	0.093	0.097	0.107	0.070	0.066	0.055	0.054	0.073	0.068	0.091	0.094		0.009	0.009	0.012	0.011	0.011	0.010	0.010
Ssxa	0.074	0.074	0.072	0.073	0.082	0.050	0.044	0.041	0.038	0.060	0.056	0.074	0.075	0.061		0.008	0.011	0.011	0.011	0.009	0.009
Sjap	0.088	0.088	0.086	0.089	0.097	0.055	0.052	0.048	0.044	0.005	0.035	0.084	0.092	0.068	0.055		0.011	0.011	0.011	0.010	0.010
Hspe	0.055	0.056	0.052	0.060	0.068	0.101	0.096	0.090	0.086	0.108	0.100	0.073	0.072	0.098	0.089	0.102		0.003	0.003	0.009	0.009
Htel	0.050	0.051	0.048	0.054	0.062	0.095	0.089	0.084	0.080	0.100	0.094	0.068	0.066	0.092	0.082	0.096	0.008		0.002	0.009	0.009
Hsie	0.051	0.053	0.049	0.055	0.064	0.096	0.091	0.086	0.081	0.101	0.095	0.069	0.068	0.093	0.083	0.096	0.009	0.003		0.009	0.009
Avis	0.067	0.069	0.065	0.068	0.076	0.082	0.076	0.069	0.063	0.083	0.073	0.064	0.057	0.085	0.059	0.078	0.068	0.062	0.064		0.000
Gaiz	0.067	0.069	0.065	0.068	0.076	0.082	0.076	0.069	0.063	0.083	0.073	0.064	0.057	0.085	0.059	0.078	0.068	0.062	0.064	0.000	

Upper-right matrix indicates standard errors. Species abbreviations are given in Table 1.

P. middendorffianus, *P. takesimense*, and in two species of *P. spurius*. Yang et al. (1999) theorized that this phenomenon was caused by rapid evolution leading to the removal of non-functional sequences. In this study, spacer *trn*L-F was relatively uniform in size, unlike the *trn*L gene (Table 2). Multiple-alignment generated a corresponding matrix according to the algorithmic calculations used. Distance matrix analysis from cpDNA sequences were performed using the estimated sequence divergence values by the K80 substitution model in the MEGA6 program (Table 3). According to the distance matrix, genetic distance was observed from 0.002 between *P. kamtschaticus* and *P. takesimense* to 0.108 between *S. oryzifolium* and *H. spectabile*. The ML tree-building method was used to construct a phylogenetic tree. The clustering of species in the phylogenetic tree showed two main clades (Fig. 1). Clade I included three *Hylotelephium* species, five *Phedimus* species, *S. rupestre*, and outgroup species, while clade II included only nine *Sedum* species. In the phylogenetic tree, the compared *Sedum* species diverged from *S. album*, except for *S. rupestre* (Fig. 1). Interestingly, *S. rupestre* was clustered differently with other *Sedum* species, but it was clustered in the main group comprising *Hylotelephium* and *Phedimus* species (Fig. 1). *S. rupestre* is also known as '*Petrosedum rupestre*' or '*S. reflexum*' and this may explain its separation from other *Sedum* species. Five genera, *Hylotelephium*, *Petrosedum*, *Phedimus*, *Rhodiola*, and *Sinocrassula*, are thought to be separated from the genus *Sedum* (Horvath, 2014). In the phylogenetic tree, two different cultivars of *P. spurius* clustered together, as did *S. sarmentosum* and *S. lineare*. In Korea, these latter two species have been called 'Dollamul', meaning stone plant. The phylogenetic tree showed that three *Phedimus* species, *P. kamtschaticus*, *P. middendorffianus*, and *P. takesimense*, were grouped together and these species are classified as the 'Kirincho' group in the common classification method used in Korea. The 'Kirincho' group comprises nine species, including *P. aizoon* and its two varieties and *P. kamtschaticus*, *P. takesimense*, *P. latiovalifolium*, *P. middendorffianus*, *P. zokuriense*, and *P. selskianum* according to the classification of Korean natural resources database (http://www.nature.go.kr). However, in some studies and in commercial markets, these species are still typically named and recorded as *Sedum*, not *Phedimus*, despite the fact that these are traditionally thought to be different from other types of *Sedum* plants. *Hylotelephium* species are easily distinguished from other *Sedum* species by their taller upright and leafy stems (Horvath, 2014). In addition, the three species of *Hylotelephium* used here are indigenous to Korea, and these three *Hylotelephium* species were grouped together in Group I (Fig. 1). In the main group II, outgroup species were isolated from other Sedoideae species and positioned at a basal level.

Discussion

The habitats of *Sedum* species are widely distributed



Phyllotaxis: A, alernate; O, opposite; W, whorls; I, imbricate; dI, densely imbricate.
Leaf apices: O, obtuse; sA, subacute; A, acute; M, mucronate; R, rounded; Ap, Apiculate.
Leaf margin: D, dentate; C, crenate; E, entire; S, serrate.
Leaf cross-section: F, flat; sT, semiterete; T, terete.
Leaf petiole: P, petiolate; S, sessile.
Flowering: Sp, Spring (March-May); Sm, Summer (June-August); Au, Autumn (September-October).
Leaf phenology: D, deciduous; sE, semievergree; E, evergreen.
Distribution: A, Asia; E, Europe; NA, North America; CA, Central America.

Fig. 1. Phylogenetic tree generated using the Maximum-Likelihood method based on sequences of the *trn*L(UAA) gene and *trn*L-F intergenic spacer of the analyzed Sedoideae species including two outgroups. Numbers at nodes indicate corresponding bootstrap values. Leaf morphology and distributional features are represented by symbols as characterized above.

throughout the Northern hemisphere, with species traditionally classified by morphological characteristics. However, some ambiguity still exists in the conventional classification and naming of *Sedum* species, especially in Korea, where both indigenous and species introduced from other countries exist. Therefore, standardized methods for *Sedum* classification are required. In some cases, discrepancies between RAPD analysis and morphological based classification have been observed, especially in polyploid plants, because of the presence of multiple alleles and environmental factors. Furthermore, cytological criteria for Sedoideae systematic treatment are not available due to the high degree of diversity (Mayuzumi and Ohba, 2004). A sequence-based method was therefore applied to generate the *Sedum* phylogeny in this study.

According to the distance matrix, *S. oryzifolium* and *H. spectabile* showed the highest distance values (Table 3). This indicates that there may be considerable genetic distance between these species as indicated by the differences in leaf morphology, type of phyllotaxis, and phenology (Table 1). *Phedimus spurius* and its cultivar 'Tricolor', which showed relatively low distance values, are very similar in their morphology except in their leaf colors. In contrast to the variegated leaf color of *P. spurius* 'Tricolor', *P. spurius* leaves are uniformly green. Based on the matrix, a phylogeny was generated, showing two major clades. Distinctive topological features of the tree were a basal branching of *S. album* and monophyletic grouping of other *Sedum* species with the exception of *S. rupestre* (Fig. 1). Using cpDNA genome sequence analysis of *Sedum*, Van Ham and 'T Hart (1998) analyzed four species, three of which were also used in the present study (*S. album*, *S. acre*, and *S. sarmentosum*). Interestingly, among the four species in that previous study, *S. acre* and *S. sarmentosum* were the least related to each other, consistent with our generated phylogeny. This may be due to the different biogeographic distribution, considering that *S. sarmentosum* is indigenous to Asian countries, including Korea, while *S. acre* originated from Europe (Table 1). *Sedum album* was shown to be distant from the other *Sedum* species in the phylogeny according to cpDNA (Van Ham and 'T Hart, 1998), which also consistent with our findings (Fig. 1). Additionally, *Phedimus* species were always grouped with *Hylotelephium* species in this study, with reliable bootstrapping values (50% majority rule), consistent with

the cpDNA genome analysis by Van Ham and 'T Hart (1998). Recently, cpDNA variations have been used to trace the origin of *Hosta* cultivars by Lee and Maki (2015) who previously analyzed two noncoding regions of cpDNA in wild populations of *Hosta* to identify specific regional features (Lee and Maki, 2013). Based on this analysis, they reported that the genetic diversity of wild species was lost, and hypothesized the occurrence of spontaneous mutations. The RAPD-based grouping of 31 *S. sarmentosum* ecotypes distributed in Korea was strongly related to morphological characteristics (Kim et al., 2008). This pattern has been found in many previous studies of *Sedum* phylogeny conducted using RAPD analysis (Kwon and Jeong, 1999), *mat*K sequence data (Mort et al., 2001), and chloroplast data (Van Ham and 'T Hart, 1998). In the *mat*K sequence analysis by Mort et al. (2001), the phylogeny revealed the polyphylic nature of *Sedum* species, with *S. sexangulare*, *S. oryzifolium*, and *S. sarmentosum* classified into the *Acre* clade in the Sedoideae subfamily, while *S. rupestre* was included in the *Sempervivum* clade in the Sedoideae subfamily. The polyphyletic position of *S. rupestre*, as observed in this study, may be explained as follows—in morphological, phenological, and biogeographical contexts, *S. rupestre* was distinguished with other *Sedum* species classified in the *Acre* clade. Based on cytogenetic features, species in the *Sempervivum* clade had a basic chromosome number of 28 (x = 28). However, the *Sedum* species examined in this study are classified in the *Acre* clade, and have various chromosome numbers, although the most common number is x = 10 (Mort et al., 2001; Uhl and Moran, 1973). In Korea, *P. takesimense*, confined to the island 'Uleung-do' in Korea, has been reported to have a similar phenotype to *P. aizoon*, and has similar branching patterns to *P. kamtschaticus* (Kwon and Jeong, 1999). However, *P. kamtschaticus* is decumbent while *P. takesimense* is procumbent. Our study also showed similar grouping features to this earlier report (Fig. 1). The phylogeny also indicated that groupings could reflect morphological characteristics, especially in leaf cross-section types. *Sedum* species with a flat leaf type can be grouped in a minor group of the main group II (Fig. 1). *Sedum* species distributed in the European region can also be differentiated from Asian and American originated species (Fig. 1). Overall, our study showed that *Sedum* species could be characterized by geographic origin or native habitat (Table 1; Fig. 1). These results, based on the cpDNA analysis (Fig. 1), suggest that phenological characteristics affected the genetic relationships between these species.

Classifications for pragmatic reasons have sometimes confused the true phylogeny or origin of plants, providing incorrect information for crossing, breeding, and other uses. In this study, data from cpDNA analysis supported the phylogenetic linkages among 19 species in the Sedoideae subfamily. In conclusion, the phylogeny generated in this study using species cultivated in Korea indicated that cultivated plants such as *Phedimus* or *Hylotelephium* classified in the *Telephium* clade could be distinctively differentiated from most *Sedum* species in the *Acre* clade, and classification using cpDNA analysis can be explained based on the morphological, phenological, and biogeographical features. This study found that cpDNA sequences obtained from nineteen Sedoideae species provided phylogenetic information that addressed the polyphylous (*Sedum*) and monophylous (*Hylotelephium* and *Phedimus*) nature of, and genetic variability among, the samples analyzed. However, further sampling with exact sampling locality information and further investigations combining nuclear information such as rDNA sequences or chromosome analysis are needed to better understand taxonomy and evolutionary divergence. We hope that the results and techniques revealed in this study will be useful in providing a better means of efficiently and accurately clarifying the taxonomic relationships among various *Sedum* species and related species that are native to Korea or introduced from abroad. Moreover, the DNA sequences analyzed here could be used as molecular markers for identification in breeding programs for new hybrid cultivars and for the conservation of horticultural crop resources.

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