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Disaccordance of Genetic Relationship between Randomly Amplyfied Polymorphic DNA and their Morphological Characteristics of Korean Native *Aster*

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ABSTRACT

This study were focused on the genetic relationship using RAPDs and some morphological characteristics among eleven taxa Aster collected in Korea. Twenty random primers were selected, a total 216 DNA bands were generated and 213 bands were shown polymorphism among species. The collected eleven taxa were clustered into five groups at 0.609 similarity index. The first group was A. glehni, A. ageratoides, A. maackii and A. scaber was clustered at 0.713 of genetic distance. The second group was A. tataricus and A. koraiensis and the third group was A. spathulifolius, the forth group was A. yomena, A. hayatae and A. hispidus and the fifth was A. tripolium.

Key Words : Achene, Compositae, Morphology, Pappus, Taxa, UPGMA

INTRODUCTION

The genus of *Aster* belongs to the *Compositae* family. *Asters* are native to Europe, Asia and North America, with a few in South Africa and America. More than 250 species are included in *Aster* genus over the world (Picton, 1999) and 25 species are found in Korea (Yoon, 1995),

This genus was called '*Aster*' (Lee, 1979) in Korea but there have been an dispute on the classification of the genus. So some scholar regard *Aster* as *Heteroppapus*, *Kalimeris*, and *Gymnaster* (Kitamura, 1937). To date, the classification of genus *Aster* carried out mainly observation of external shape such as, pappus, receptacle, epidermal cells of the lingulate florets, capitulum morphology, somatic choromosome numbers (Chung and Kim, 1991a, 1991b, 1991c, 1993, 1997) and leaf, achene morphology (Chung and Jeong, 1999, 2000) were investigated to estimate its taxanomic values. But to determined the limits of genus *Aster* and the others and to decided the taxanomic position distributed in Korea, we require further examination.

RAPD have been carried out to analyze the genetic similarity among cultivars, or intra-specific variation (Baek et al., 1997, Jeon et al., 1994, Jung et al., 1997, Kim et al., 1998, Kim et al.,. 2000, Kim and Hyun, 2000, Kim and Kim, 2000, Lee et al., 1996), to determine the genetic releationships of allied groups (Kim et al., 1999, Yoo et al., 1996) and to develop the marker for detecting the cultivar and useful character (Geraci et al., 2001).

Therefore, this study was focused on the genetic relationships between RAPDs and morphological

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characteristics among eleven taxa *Aster* collected in Korea.

MATERIALS AND METHODS

Plant materials and morphological characteristics : Eleven accessions of *Aster* were collected during year 2000~2001. Collection data was shown in Table 1 and Fig. 1. Collected *Aster* were planted in the field and green house and the leaf tissues were stored at -80℃ for DNA extraction. The morphological characteristics were investigated in accordance with UPOV.

DNA extraction : 100mg of the leaf tissues was ground in liquid nitrogen and DNA extracted using DNA extraction kit (QIAGEN Co., Germany). Extracted DNA was diluted with distilled water to 10ng/$\mu\ell$ and stored at -20℃

Amplification conditions : Amplification reactions were performed in volumes of 20$\mu\ell$ including 20ng of template DNA, 100nM of arbitrary random primer (OPERON Inc, USA), 200uM of dNTP, 10×reaction buffer, 1.0U of Taq DNA polymerase (Bioneer Co. Korea) and ddH_2O. Amplification was performed in DNA Thermal Cycler (iCycler, Bio-RAD) programed for 45cycles of 30 sec at 94℃, 30 sec at 40℃, and 1 min at 72℃ for denaturing, annealing and primer extension, respectively. The last cycle was followed by incubation at 72℃ for 10 min. Amplicons were

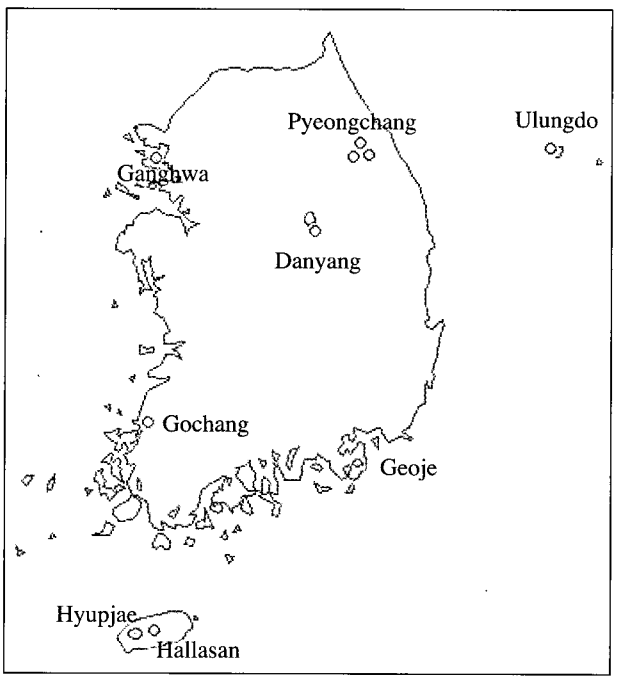

Fig. 1. Sampling localities of *Aster* and its allied texa in South Korea.

Table 1. Materials and collection data of 11 Korean *Aster* L.

Scientific Name	Kitamura(1937)	Collecting site	Sample code
Aster glehni F. Schmidt	*Aster glehni* F. Schmidt	GB: Ulungdo	1
A. maackii Regel	*A. maackii* Regel	GW: Pyeongchang	2
A. ageratoides Turcz	*A. ageratoides* Turcz	JN: Gochang	3
A. scaber Thunb	*A. scaber* Thunb	GW: Pyeongchang	4
A. yomena Makai	*Kalimeris yomena Kitamura*	CB: Danyang	5
A. spathulifolius Max.	*A. spathulifolius* Max.	GN: Geoje	6
A. tripolium L.	*A. tripolium* L.	IC: Ganghwa	7
A. hayatae Lev. et Van't	*A. hayatae Lev. et Van't*	JJ: Hallasan	8
A. tataricus L.	*A. tataricus* L.	GW: Pyeongchang	9
A. hispidus Thunb	*Heterppappus hispidus Less.*	JJ: Hyupjae	10
A. koraiensis Makai	*Gymnaster koraiensis* Kitamura	CB: Danyang	11

GB : Gyeongbuk, GW : Gangwon, JN : Jeonnam, CB : Chungbuk, GN : Gyeongnam, IC : Incheon, JJ : Jejudo.

Table 2. Morphological characteristics of 11 Korean *Aster* L.

Sample code	Flower color	Flowering date	No. of floret /plant	Pappus			The ratio of achene length and width	Leaf shape
				presence	length of tubulate floret (mm)	length of ligulate floret (mm)		
1	white	Mid. Aug.~Mid. Sep.	45.3±19.7	present	3.5±0.3	3.2±0.3	2.5±0.4	elliptic
2	light violet	Mid. Sep~Mid. Oct.	4.7±1.5	present	5.4±0.3	5.5±0.0	2.7±0.5	lanceolate
3	ligth violet	Mid. Aug.~Mid. Sep.	101.5±10.6	present	2.7±0.3	2.3±0.2	2.9±0.5	elliptic
4	white	Late Aug~Mid. Oct.	32.9±12.5	present	3.7±0.5	3.6±0.5	2.8±0.6	deltoid
5	light violet	Mid. Aug~Early Oct.	54.6±17.6	present	0.7±0.2	0.6±0.2	1.4±0.1	elliptic
6	light violet	Mid. Sep.~Late Oct.	4.3±1.8	present	5.1±0.5	4.6±0.4	3.3±0.5	spathulate
7	light violet	Mid. Sep.~Mid. Oct.	38.4±11.2	present	8.7±0.6	8.7±0.9	5.2±1.1	lanceolate
8	light violet	Mid. Sep.~Mid. Nov.	7.9±2.6	present	3.8±0.4	3.3±0.3	1.6±0.2	spathulate
9	light violet	Late Jul.~Early Sep.	46.7±22.8	present	6.6±0.3	6.8±0.4	2.0±0.3	elliptic
10	light violet	Early Sep.~Late Oct.	18.0±8.3	present	4.9±0.3	1.1±0.4	2.1±0.3	elliptic
11	light violet	Late Jul.~Early Sep.	7.9±5.3	absent	-	-	2.9±0.6	lanceolate

analyzed by gel electrophoresis in 1.5% agarose gel (1 × TBE buffer, pH 8.0) and detected by UV transilluminator, staining in ethidium bromide. As molecular weight marker, 100bp ladder (Bioneer Co. Korea) was used.

Data analysis : RAPD bands were scored as 0 and absent of 1 for present of band in each population. Genetic similarity coefficients were computed using NTSYS-pc (ver. 2.10b). Similarity measures were using UPGMA (unweighted pair group method with arithmetic averages).

RESULTS AND DISCUSSION

Morphological characteristics :

Morphological characteristics of some Korean *Aster* were shown in Table 2. The flower color of A. glehni and A. *scaber* was white and that of the others was light violet. In flowering date, A. *tataricus* and A. *koraiensis* were from July to September, these species began to early bloom and the others came into flowers from August to October. The flowering period was the longest in A. *hayatae*. There was a wide difference in investigated species the number of flower per plant were varied to 4.3~101.5. Kitamura(1937) reported that pappus was key factor to classify the *Aster*. Only A. *koraienssis* was no pappus and in the length of tubulate floret and ligulate floret, A. *yomena* was 0.7mm and 0.6mm, respectively. But A. *hispidus* was different length, 4.9mm and 1.1mm, respectively. So, we can classified Aster, *Kalimeris*, *Heteropappus* and *Gymnaster* using presence and length of pappus. In th ratio of achene length and width, most of species were obovate or obovate-oblong type, the ratio were 1.4~2.9 and the upper(1/3) part was widest. A. *tripolium* was oblong type, it was flat in both sides and A. *koraiensis* was oblanceolate-oblong type, with widest in the upper part.

Analysis of RAPDs :

RAPDs analysis was performed in order to illustrate genetic relationships to eleven taxa of the Korean *Aster* and to population of each species. For the selection of

the primer, we used 114 of arbitrary random primers. The 20 primer sets were selected considering reproductivies and specificities of generated band. A considerable degree of polymorphic was detected at the specific level with all twenty primers (Table 3). These primers generated 216 bands. In the present study all the primers have a GC content >60%. The individual primers produced between 4 (OPJ-12) and 19 (OPA-04) and average bands were 10.8 per primer (Fig. 2). Of which 98.6% (213 bands) were polymorphic, only 3 bands were monomorphic. This seems to be relatively high when compared to the reports of other RAPD studies, e.g. in *Adenophora* species (14.4%), *Companula* species (28.1%) (Yoo et al., 1996) and among varieties of *Hibiscus syriacus* (86.8%) (Lee et al. 1996).

Analysis of genetic similarity :

A total of 216 polymorphic bands were used for analysis of genetic similarity between *Aster* (Table 4). A cluster analysis was performed using the UPGMA. The genetic similarity index was range from 0.546 to 0.764 (Fig. 3). RAPD's of 11 Aster plants revealed that A. *hayatae* and A. *hispidus* were most closely related

Table 3. List of arbitrary 10-mer primers used and the number of different DNA fragments and profiles observed among 11 *Aster* species.

Primer	Sequence (5'-3')	GC content (%)	No. of observed bands(B)	No. of polymorphic products(A)	Polymorphism (A/B × 100) (%)
OPA-04	AATCGGGCTG	60	19	19	100.0
OPA-07	GAAACGGGTG	60	6	6	100.0
OPA-08	GTGACGTAGG	60	12	12	100.0
OPA-09	GGGTAACGCC	70	13	13	100.0
OPA-11	CAATCGCCGT	60	17	17	100.0
OPA-14	TCTGTGCTGG	60	9	9	100.0
OPA-15	TTCCGAACCC	60	11	11	100.0
OPI-03	CAGAAGCCCA	60	8	8	100.0
OPG-02	GGCACTGAGG	70	17	17	100.0
OPI-06	AAGGCGGCAG	70	14	14	100.0
OPI-07	CAGCGACAAG	60	7	7	100.0
OPI-09	TGGAGAGCAG	60	11	11	100.0
OPI-16	TCTCCGCCCT	70	17	17	100.0
OPI-18	TGCCCAGCCT	70	10	10	100.0
OPJ-12	GTCCCGTGGT	70	4	4	100.0
OPJ-17	ACGCCAGTTC	60	6	4	66.7
OPK-17	CCCAGCTGTG	70	6	5	83.3
OPM-02	ACAACGCCTC	60	9	9	100
OPN-02	ACCAGGGGCA	70	5	5	100
OPN-03	GGTACTCCCC	70	15	15	100
			216	213	98.6

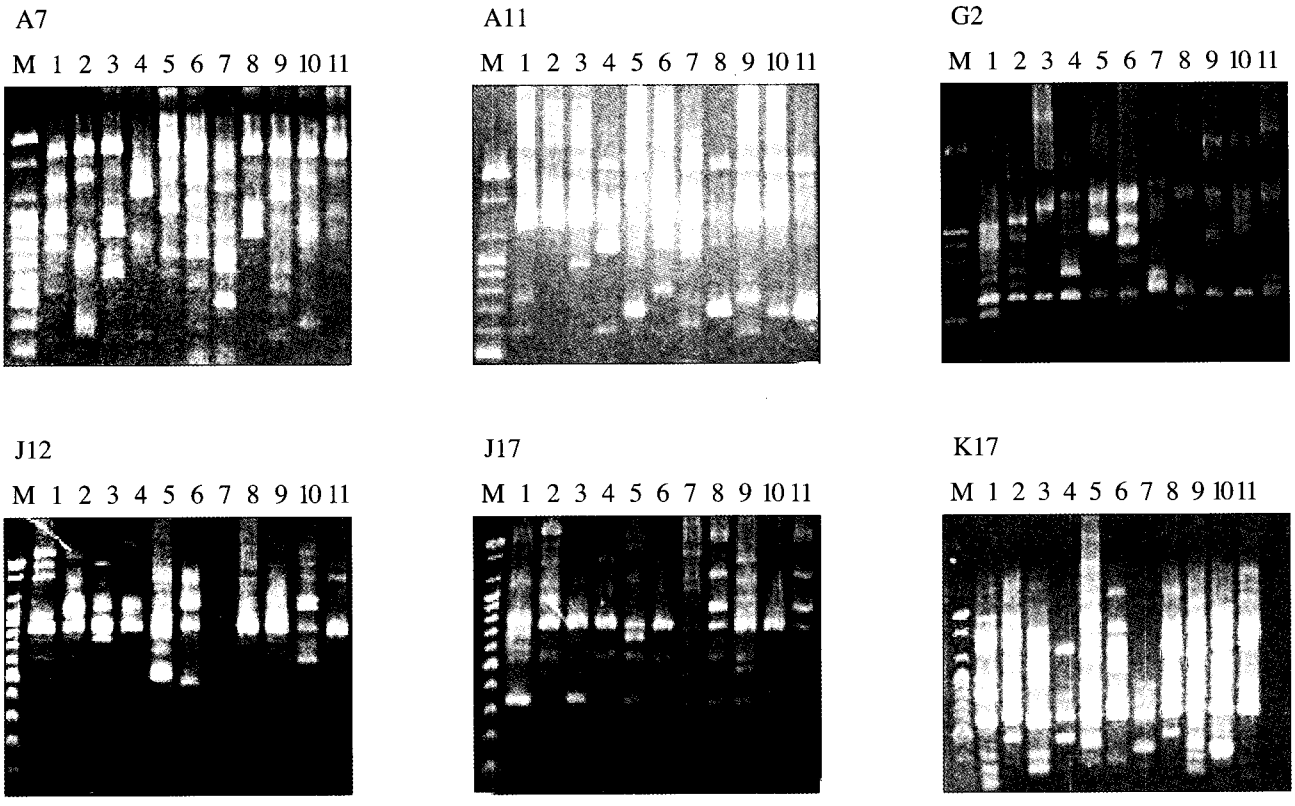

Fig. 2. RAPD band profiles of the analyzed plants.
M=100bp DNA ladder. Lane 1: *Aster glehni* 2: *A. maackii* 3: *A. ageratoides* 4: *A. scaber* 5: *A. yomena* 6: *A. spathulifolius* 7: *A. tripolium* 8: *A. hayatae* 9: *A. tataricus* 10: *A. koraiensis* 11: *A. hispidus*

Table 4. Similarity matrix for 11 Korean *Aster* L. and its allied taxa

	A. glehni	*A. maackii*	*A. ageratoides*	*A. scaber*	*A. yomena*	*A. spathulifolius*	*A. tripolium*	*A. hayatae*	*A. tataricus*	*A. koraiensis*	*A hispidus*
Aster glehni	1.000										
A. maackii	0.699	1.000									
A. ageratoides	0.694	0.690	1.000								
A. scaber	0.657	0.708	0.639	1.000							
A. yomena	0.602	0.607	0.574	0.620	1.000						
A. spathulifolius	0.546	0.662	0.620	0.602	0.565	1.000					
A. tripolium	0.528	0.588	0.556	0.574	0.574	0.546	1.000				
A. hayatae	0.593	0.625	0.565	0.620	0.741	0.528	0.546	1.000			
A. tataricus	0.565	0.616	0.593	0.620	0.583	0.602	0.528	0.583	1.000		
A. koraiensis	0.551	0.630		0.634	0.662	0.625	0.607	0.532	0.588	0.644	1.000
A. hispidus	0.542	0.565	0.532	0.560	0.673	0.532	0.486	0.764	0.532	0.611	1.000

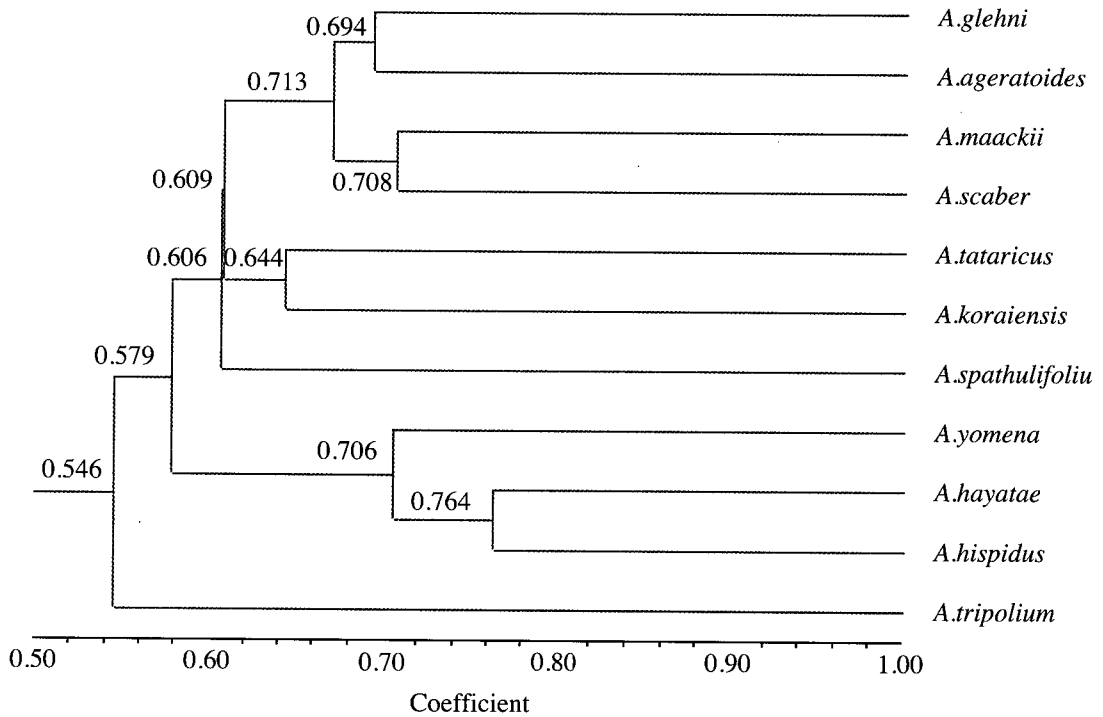

Fig. 3. Phenogram for Korean *Aster* L. and its allied taxa based on analysis of RAPD fragments.

taxa. These taxa were collected from Jeju Island, so genetic distance was closed by crossing each other in same region. The next were A. *maackii* and A. *scaber*, these taxa were collected from same region too. Genetic distance was 0.708 between them. While A. *tripolium* was most distant taxa among the 11 *Aster*. A. *tripolium* was collected from Seokmo Island, there is far away from Ganghwa Island, Suggesting variation was accumulated in this taxon by isolation.

The treated eleven taxa were clustered into five groups at 0.609 similarity index (Fig. 3). The first group composed of A. *glehni*, A. *ageratoides*, A. *maackii* and A. *scaber* was clustered at 0.713 of genetic distance. The second group was A. *tataricus* and A. *koraiensis* and the third group was A. *spathulifolius*, the forth group was A. *yomena*, A. *hayatae* and A. *hispidus* and the fifth was A. *tripolium*.

Somatic chromosome number is relatively stable, so this character is widely used for plant classification. Somatic chromosome number is similar if genetic relationship is close but chromosome number is a wide difference if genetic relationship is distant (Lee, 1999). Chung and Kim (1997) published the somatic chromosome number of Korean native *Aster*. According to the this paper, A. *glehni*, A. *ageratoides*, A. *maakii*, A. *scaber*, A. *spathulifolius*, and A. *tripolium* is included in diploid (2n=2X=18). A. *hayatae* and A. *hispidus* is included in tetraploid (2n=4X=36), A. *tataricus* is hexaploid (2n=6X=54), A. *yomena* is heptaploid (2n=7X=63) and A. *koraiensis* is sixteenploid (2n=16X=144). In this study, diploid is clustered on group except A. *spathulifolius* and A. *tripolium*. A. *tataricus* and A. *koraiensis* is grouped and A. *hayatae*, A. *hispidus* and A. *yomena* is grouped. This result is confirm to some extent that somatic chromosome number is related to genetic relationships.

Genetic relationship using RAPD and morphological characteristics differ. Because RAPD reflect more qualitative character than quantitative character (Lee and Chang, 2002).

Therefore, to classify of native Korean Aster and its allied taxa, on the basis of this study, more high level molecular assay is accomplished.

LITERATURES CITED

Baek, I.Y., Y.H. Yoon, D.C. Shin, G.H. Park, and Y.H. Hwang, D.U. Kim. 1997. Analysis of genetic relationships among *Glycine* species using RAPDs. Korean J. Breed. 29(3) : 308-317.

Chung, G.Y. and H.J. Jeong. 1999. Study on the leaf morphology of Korean Aster L. and its allied taxa. Korean J. Plant. Res. 12(1) : 50-61.

_____ and _____. 2000. Taxonomic study on the achene morphology of Korea *Aster* L. and its allied taxa. Korean J. Plant. Res. 13(3) : 179-187.

_____ and Y.S. Kim. 1991a. A taxonomic study of the pappus morphology on the genus *Aster* L. and its allied groups in Korea. Kor. J. Plant Tax. 21(3) : 197-209.

_____ and _____. 1991b. A taxonomic study of the receptacle morphology on the genus *Aster* L. and its allied groups in Korea. Kor. J. Plant Tax. 21(4) : 229-238.

_____ and _____. 1991c. A study on epidermal cells of the ligulate florets of the genus Aster L. and its allied groups in Korea. Kor. J. Plant Tax. 21(4) : 239-250.

_____ and _____. 1993. A study on the capitulum morphology of the genus *Aster* L. and its allied taxa in Korea. Kor. J. Plant Tax. 23(3) : 105-118.

_____ and _____. 1997. Study on the somatic chromosome numbers of Korean *Aster* L. and its allied taxa. Korean J. Plant. Res. 10(4) : 292-299.

Geraci, A., I. Divaret, F.M. Raimond and A.M. Chevre. 2001. Genetic relationships between Sicilian wild populations of *Brassica* analysed with RAPD markers. Plant Breeding 120 : 193-196.

Jeon, H.J., C.G. Bean, and K.H. Hong. 1994. Identification of *Cucurbitaeceae* cultivars by using RAPD markers. J. Kor. Soc. Hort. Sci. 35(5) : 449-456.

Jung, Y.H., M.H. Ko, Y.S. Oh, G.O. Kim, Y.C. Chung, M.H. Kim, and M.Y. Oh. 1997. Phylogenetic relationship among selected taxa of Pruns in Mt. Halla and cultivated Prunus *yedoensis* by the RAPD analysis. Kor. J. Plant Tax. 27(4) : 415-428.

Kim, I.S. and J.O. Hyun. 2000. Genetic diversity of *Abies koreana* Wilson based on RAPD analysis. Korean J. Breed. 32(1) : 12-18.

Kim, J.H., C.Y. Son, H.Y. Kyung, and Y.J. Kim. 1999. Comparative studies on the *Hibiscus syriacus* and its allied species based on RAPD analysis. J. Kor. Hort. Sci. 40(2) : 241-244.

Kim, K.Y., D.Y. Hyun, S.J. Yun, C.H. Kim, and Y.P. Lim. 2000. Intra-specific variation and genetic relationsships in *Oenanthe javanica* DC. by RAPD analysis. Korean J. Breed. 32(1) : 74-82.

Kim, S., K.Y. Kim. M.S. Park, S.Y. Choi, and S.J. Yun. 1998. Intraspecific relationship of *Eleutherococcus senticosus* Max. by RAPD markers. Korean J. Medicinal Crop Sci. 6(3) : 165-169.

Kim, Y.D. and S.H. Kim. 2000. Interspecific relationship of *Weigela* based on RAPD analysis. Kor. J. Plant Tax. 30(1) : 17-33.

Kitamura, S. 1937. Compositae Japonicae. Mem. Coll. Sci. 8 : 299-377.

Lee, C. B. 1979. An illustrated Guide to Korean Flora. HyangMunSa, Korea

Lee, J.S. and Y.K. Chang. 2002. Morphological characteristics and analysis of genetic relationship

using RAPD in leaf variegated *Hosta minor* native to Korea. J. Kor. Soc. Hort. Sci. 43 : 373-378.

Lee, S.H., C.H. Kim, W.S. Song, and I.S. Nou. 1996. Phylogenetic relationship and genetic variation among varieties of *Hibiscus syriacus* based on RAPD analysis. Korean J. Breed. 28(4) : 445-456.

Lee, Y.S. 1999. Plant Taxonomy, Woo-Seong Publications co. LTD., Korea.

Picton, P. 1999. The Gaudener's Guide to Growing Asters. Timber Press. USA.

Yoo, K.O., W.T. Lee, N.S. Kim, J.H. Kim and H.T. Lim. 1996. Comparative studies on the *Hanabusaya asiatica* and its allied groups based on randomly amplified polymorphic DNA (RAPD) analysis. J. Kor. Soc. Hort. Sci. 37(2) : 324-328.

Yoon, P.S. 1995. Hortus Koreana. Jisik-Sanup Poblications co. LTD., Korea

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