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Phylogenetic relationships of chrysanthemums in Korea based on novel SSR markers

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ABSTRACT. Chrysanthemums are well known for their esthetic and medicinal values. Characterization of chrysanthemums is vital for their conservation and management as well as for understanding their genetic relationships. We found 12 simple sequence repeat markers (SSRs) of 100 designed primers to be polymorphic. These novel SSR markers were used to evaluate 95 accessions of chrysanthemums (3 indigenous and 92 cultivated accessions). Two hundred alleles were identified, with an average of 16.7 alleles per locus. KNUCRY-77

gave the highest polymorphic information content value (0.879), while KNUCRY-10 gave the lowest (0.218). Similar patterns of grouping were observed with a distance-based dendrogram developed using PowerMarker and model-based clustering with Structure. Three clusters with some admixtures were identified by model-based clustering. These newly developed SSR markers will be useful for further studies of chrysanthemums, such as taxonomy and marker-assisted selection breeding.

Key words: Chrysanthemum; SSR; Marker development; Genotyping; Population structure

INTRODUCTION

Chrysanthemums, often called mums or chrysanths, belong to the genus *Chrysanthemum* and the family Asteraceae (Compositae). Mums are one of the most popular ornamentals in the world. They have diversified growth habits, inflorescence forms, and colors. Demand for chrysanthemum production, including cut flowers, gardens, potted plants, and ground-cover types, has increased worldwide.

In addition to ornamental effects, chrysanthemum flowers have antibacterial, antifungal, and antiviral activities (Yeung, 1983). They also have a calming, antihypertensive effect. Thus, chrysanthemum flowers have been used in traditional Chinese medicine for centuries. White chrysanthemum is considered slightly superior to other forms for nourishing the liver. Yellow chrysanthemum is used most often to treat eye redness and headache (Yeung, 1983). In addition to its medicinal properties, chrysanthemum extract can also be used as raw material in dye and tea production. In particular, simple chrysanthemum flower tea is a very common beverage in China and Korea. Chrysanthemum leaves are steamed or boiled and used as greens in Chinese cuisine. Although chrysanthemums have endless uses, relatively few systematic genetic analyses have been performed compared with other crops.

The genome of mums is composed of multiple sets of chromosomes that range from diploid to decaploid (Hartl and Jones, 2009). Although some pseudo self-incompatible plants have been discovered (Anderson et al., 1992), selfing is generally not possible because of a strong self-incompatibility system, common to members of the family Asteraceae (Richards, 1986). Moreover, a long period of cultivation, natural selection, and artificial crossing has led to numerous cultivars, abundant morphological variation, powerful suitability, wide distribution, and an extremely complex genetic background. Thus, developing a high-resolution technique is important for assessing and distinguishing the more complicated mum accessions.

High levels of polymorphisms at the DNA level in chrysanthemums have been determined (Wolff and Peters-Van Rijn, 1993), and Wolff et al. (1995) found identical DNA patterns in different accessions of the same chrysanthemum cultivar using random amplified polymorphic DNA (RAPD). Huang et al. (2000) detected molecular markers in three hybrid combinations of chrysanthemums using RAPD, and they were able to select 22 primers of the 45 random primers screened.

Most genetic studies in chrysanthemums have emphasized the ornamental traits of inflorescence, such as color (Li et al., 2005) and floret form (Chen et al., 2003), and vegetative traits, such as plant type (Jiang et al., 2003; Zhao et al., 2009; Zhang et al., 2010a). Flowering is photoperiod-, temperature-, and vernalization-dependent, and its genetic control is expected to be complex. Zhang et al. (2010b) investigated the inheritance of two flowering traits of chrysanthemums, the initial blooming time, and the duration of flowering using segregation within an F_1 population derived from a cross between the autumn-flowering "Yuhualuoying" and the summer-flowering "Aoyunhanxiao" cultivars. Their efforts regarding a marker - trait association analysis based on sequence-related amplified polymorphism (SRAP) genotyping uncovered 10 markers for initial blooming time and 12 for duration of flowering. Although a considerable number of DNA markers have been identified, substantially more DNA markers are needed to initiate genetic studies for these plant species.

Simple sequence repeat markers (SSRs) have become one of the most widely used molecular markers for genetic studies, because they offer several advantages, such as technical simplicity, relatively low cost, high genetic resolution power, and high polymorphism. SSRs, or microsatellite markers, have been used to study genetic diversity, phylogenetic relationships, classification, evolutionary processes, and quantitative trait loci in many crops (Dixit et al., 2010; Cho et al., 2010, 2011; Lu and Park, 2012; Moe et al., 2010, 2012; Yoon et al., 2012; Zhao et al., 2012; Khaing et al., 2013). Moreover, they are reliable and easy to score (Gupta and Varshney, 2000). Thus, Moe et al. (2011) constructed an SSR-enriched library using a modified biotin-streptavidin capture method and determined the distribution of SSR motifs in the sequence data of the chrysanthemum library. However, they did not screen with polymerase chain reaction (PCR) amplification for polymorphism with the primers designed. There is a need to screen and confirm the amplification and level of polymorphism to assess whether the designed primers are useful DNA markers for identifying individuals in a population.

Thus the present study focused on screening PCR amplification and polymorphism of the primers using pooled chrysanthemum DNAs and then the genotyping and population structure of chrysanthemums-cultivated (*Dendranthema grandiflora* syn. *Chrysanthemum morifolium* Ramat.) and indigenous [*Dendranthema zawadskii* var. *latilobum* (Maxim.) Kitam.]-using the resulting polymorphic SSR markers.

MATERIAL AND METHODS

Plant materials and DNA extraction

In total, 95 *Chrysanthemum* accessions representing two species, *Chrysanthemum morifolium* (Nos. 1-91, 95) and *D. zawadskii* var. *latilobum* (Maxim.) Kitam. (Nos. 92-94), were used to screen for SSR polymorphisms (Table 1). All accessions were kindly provided by the Chrysanthemum Research Station, Yesan, Republic of Korea. DNA was extracted from fresh leaves using the Qiagen DNA extraction kit (Qiagen, Hilden, Germany). The relative purity and concentration of extracted DNA was estimated with the NanoDrop ND-1000 (NanoDrop Technologies, Inc., Wilmington, DE, USA). The final concentration of each DNA sample was adjusted to 50 ng/µL.

Table 1. Information on the 95 chrysanthemums used and their inferred cluster.

No.	Accessions	Type	Usage type	Inferred cluster
1	Gukyagukhyang	Cultivated	Decoration type	1
2	Gaeul-agassi	Cultivated	Decoration type	1
3	Ganghohwangpaljang	Cultivated	Decoration type	1
4	Gyeomyukbaekguk	Cultivated	Decoration type	1
5	Gukhwagaeun	Cultivated	Decoration type	1
6	Gukhwagiun	Cultivated	Decoration type	1
7	Busanseol	Cultivated	Decoration type	1
8	Okpiri	Cultivated	Decoration type	1
9	Ibaek	Cultivated	Decoration type	1
10	Jaunjeon	Cultivated	Decoration type	1
11	Ipalhong	Cultivated	Decoration type	1
12	Hongeojeon	Cultivated	Decoration type	1
13	Cheonhyangmaehwa	Cultivated	Decoration type	1
14	Sinma	Cultivated	Standard type	1
15	Hi Maya	Cultivated	Standard type	1
16	Jeong Heung Seong	Cultivated	Standard type	1
17	Su I-Sin	Cultivated	Standard type	1
18	Eul-nyeo	Cultivated	Standard type	1
19	Jeong Heung Chu	Cultivated	Standard type	1
20	Yongma	Cultivated	Standard type	1
21	Jeong Heung Sin Nyeon	Cultivated	Standard type	1
22	ST09-173-01	Cultivated	Standard type	Admixture
23	Baekgwang	Cultivated	Standard type	1
24	Baekseon	Cultivated	Standard type	1
25	Baekma	Cultivated	Standard type	2
26	Seinonami	Cultivated	Standard type	2
27	Yuka	Cultivated	Standard type	2
28	Jeongju	Cultivated	Standard type	2
29	Seikonoilse	Cultivated	Standard type	2
30	Jangsuhwang	Cultivated	Standard type	Admixture
31	Seinoisami	Cultivated	Standard type	2
32	ST06-03-01	Cultivated	Standard type	2
33	ST09-148-04	Cultivated	Standard type	1
34	ST09-50-01	Cultivated	Standard type	1
35	ST09-40-02	Cultivated	Standard type	1
36	ST09-149-01	Cultivated	Standard type	1
37	ST09-136-01	Cultivated	Standard type	1
38	ST09-99-02	Cultivated	Standard type	1
39	ST09-173-11	Cultivated	Standard type	1
40	Gold coast	Cultivated	Spray type	1
41	Green Day	Cultivated	Spray type	Admixture
42	Monaliza	Cultivated	Spray type	2
43	Shamlook	Cultivated	Spray type	2
44	Aniasia	Cultivated	Spray type	2
45	Artic Queen	Cultivated	Spray type	2
46	Euro	Cultivated	Spray type	2
47	Besu Bio	Cultivated	Spray type	1
48	Sterion	Cultivated	Spray type	2
49	Yes Together	Cultivated	Spray type	1
50	Nice	Cultivated	Spray type	2
51	Loyal Aridillo	Cultivated	Spray type	2
52	Yes Nuri	Cultivated	Spray type	2
53	Arigos	Cultivated	Spray type	2
54	Yes Life	Cultivated	Spray type	1
55	Penny Line	Cultivated	Spray type	2
56	Puma	Cultivated	Spray type	2
57	luli puf	Cultivated	Spray type	2
58	Yes Miso	Cultivated	Spray type	2
59	Chilo	Cultivated	Spray type	2
60	Yes Day	Cultivated	Spray type	2
61	Frog	Cultivated	Spray type	Admixture
62	Ligan Orange	Cultivated	Spray type	Admixture

Continued on next page

Table 1. Continued.

No.	Accessions	Type	Usage type	Inferred cluster
63	Marble Orange	Cultivated	Spray type	2
64	Mujigae	Cultivated	Spray type	2
65	Moon Light	Cultivated	Spray type	2
66	Biking	Cultivated	Spray type	2
67	Bophi	Cultivated	Spray type	2
68	Borami	Cultivated	Spray type	2
69	Bimini	Cultivated	Spray type	Admixture
70	Yes Swan	Cultivated	Spray type	Admixture
71	Yes Line	Cultivated	Spray type	2
72	Yes Morning	Cultivated	Spray type	2
73	Uno Ivory	Cultivated	Spray type	2
74	Yes Star	Cultivated	Spray type	2
75	Hwiparam	Cultivated	Spray type	2
76	Green Berry	Cultivated	Pot mum	Admixture
77	Leema honey	Cultivated	Pot mum	Admixture
78	Monroe	Cultivated	Pot mum	Admixture
79	Peace Angel	Cultivated	Pot mum	3
80	Peace Yellow	Cultivated	Pot mum	3
81	Peace Pink	Cultivated	Pot mum	3
82	Saskia	Cultivated	Pot mum	3
83	Phikho Salto	Cultivated	Pot mum	3
84	Peace Copper	Cultivated	Pot mum	3
85	Phasoa Red	Cultivated	Pot mum	3
86	Geumbangul	Cultivated	Garden mum	3
87	Dohwa Ball	Cultivated	Garden mum	3
88	Popcorn Ball	Cultivated	Garden mum	3
89	Bright Ball	Cultivated	Garden mum	2
90	G-20	Cultivated	Garden mum	3
91	G-28	Cultivated	Garden mum	3
92	Gujeolcho	Indigenous		3
93	Ulreung Gukhwa	Indigenous		3
94	Gwandong Gukhwa	Indigenous		3
95	G31	Cultivated	Garden mum	3

SSR marker screening

In total, 100 SSR primer pairs designed by Moe et al. (2011) were screened for polymorphisms using the SSR MANAGER program (Kim, 2004); 40 DNA samples were used to screen the PCR amplification of the 100 designed primer pairs. PCR products were examined by electrophoresis (3% agarose gel). Of the 100 primer pairs, 12 were successful in providing PCR products. The M13-tail PCR method of Schuelke (2000) was used to measure the size of the PCR products. Forward sequences of the 12 primers were attached to the M13-tail (TGTAAAACGACGGCCAGT), and PCRs were run together with the respective normal reverse primers labeled with fluorescent dyes (FAM, VIC, NED and PET). The fragments of PCR products were analyzed with the ABI-3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the GeneMapper 4.1 software (Applied Biosystems) and sized precisely using the GeneScan 600 LIZ Size Standard v2.0 (Applied Biosystems). Conditions for PCR amplification were 94°C for 3 min, 35 cycles each at 94°C for 30 s, 55°C (varied according to annealing temperature requirements of primers in Table 2) for 45 s, and 72°C for 1 min, followed by a final extension at 72°C for 10 min.

Table 2. Characterization of 12 novel simple sequence repeat markers.

No.	ID	Forward (above) and reverse (below)	Repeat motif	A_T[1] (°C)
1	KNUCRY-10	GTGTCTTCATCCCACCACCA TGTGAGAGAGTGAGTGTAGTGTGAG	$(CA)_{24}$	60
2	KNUCRY-16	TGTTCACCCATTCACAGCTC CACATGTATGACTAGGTGAGGTGA	$(AC)_3$, $(AC)_4$	53
3	KNUCRY-35	CCTCGCACTACTTCCAAATGA GGAGATTGTTTGTTCGTATCCTT	$(CA)_{27}$, $(CA)_{16}$	50
4	KNUCRY-58	GGTGGAATTGCTCCTTGTTG CCATCATCAACACAAGCTTCA	$(TTG)_5$, $(TTG)_6$	60
5	KNUCRY-59	CGGTCCTCTCAGCCTTATTG GGTGTGTGTGTGAAGGTGCT	$(CA)_5$	57
6	KNUCRY-75	GTGGTTGAGCCATTTGAGGT TTGGCTATTGTGATTTCTACGC	$(TTG)_7$	55
7	KNUCRY-76	TTGAGGTTGTGGAAATGCAG CGCGTTAACTTTGGTGTTTTT	$(TTG)_7$	55
8	KNUCRY-77	CCCGGTTATCATGTGTATGC CGTATTTAAAGGTTTTCCTTTCG	$(CACG)_3$, $(CA)_{28}$	50
9	KNUCRY-84	CTAGGCTCCTTCAGCCCTCT TCTGGACTAGCCGTCAGTTG	$(CA)_{15}$	50
10	KNUCRY-85	GACCAACAAAACGGAATGCT GTTGTCGTCCGTTGGCTAGT	$(CA)_4$	50
11	KNUCRY-94	CATTCAGGACAGTCATACAAGTG CAACACACACACACACAGGAAT	$(GAA)_4$	55
12	KNUCRY-98	TCACATCACACATCACTGCAA TGTGTGTGAGGGACACATGA	$(AC)_4$	55

[1]Annealing temperature.

Genotyping of 95 *Chrysanthemum* accessions

Twelve newly identified polymorphic SSR markers were used for genotyping and population structure analysis of 95 *Chrysanthemum* accessions. The SSR alleles of 12 markers were resolved on a 3500 Genetic Analyzer (Applied Biosystems). The variability at each locus was measured in terms of the number of alleles, rare alleles and specific alleles, genotype number, heterozygosity (H), major allele frequency (MAF), gene diversity (GD), and polymorphic information content (PIC). These variables were measured in terms of the shared allele frequencies using PowerMarker 3.25 (Liu and Muse, 2005). The neighbor- joining method was used to construct a phylogram from a distance matrix using MEGA4 software (Tamura et al., 2007) embedded in PowerMarker. POPGENE (ver. 1.32) (Yeh et al., 1999) was used to test for Hardy-Weinberg equilibrium (HWE).

Population structure analysis

The model-based program Structure 2.2 (Pritchard et al., 2000; Falush et al., 2003) was used to determine the population structure for 95 accessions using a burn-in of 100,000, a run length of 200,000, and a model allowing for admixture and correlated allele frequencies. At least three runs of the Structure program were performed by setting the number of populations (*K*) from 2 to 10. The model choice criterion to detect the most probable value of *K* was ΔK, an ad hoc quantity related to the second-order change of the log probability of data with respect to the number of clusters inferred in Structure (Evanno et al., 2005).

RESULTS

SSR polymorphisms

In total, 200 alleles were detected, with an average of 16.7 alleles per locus. KNUCRY-84 produced the highest number of alleles (35), whereas only four alleles were observed at KNUCRY-10 and KNUCRY-75 (Table 3). Allele size ranged from 134 to 500 bp. The allele frequency database showed that rare alleles (with a frequency <0.05) comprised 77.5% of all alleles, while intermediate (0.05< frequency <0.50) and abundant alleles (frequency >0.50) comprised 19.5 and 3%, respectively, of all detected alleles, showing that most alleles were of low frequency (Figure 1). The specific and rare alleles detected ranged from 0 to 18 and 1 to 30, respectively, and the maximum numbers of both alleles were detected with KNUCRY-84. Values for heterozygosity ranged from 0.187 to 0.926 (mean, 0.588). The average gene diversity and PIC values were 0.662 and 0.635, with a range from 0.229 (KNUCRY-10) to 0.888 (KNUCRY-77) and from 0.218 (KNUCRY-10) to 0.879 (KNUCRY-77), respectively. Eleven loci deviated from HWE and showed highly significant linkage disequilibrium ($P < 0.01$; Table 3). A correlation analysis revealed that allelic richness was significantly and positively associated with gene diversity ($r = 0.743$, $P < 0.01$) and PIC value ($r = 0.776$, $P < 0.01$; data not shown). Moe et al. (2010), however, found no significant correlation between allelic richness and the number of repeats in the SSR loci.

Table 3. Genetic variables of 12 novel simple sequence repeat markers.

Marker	Size range (bp)	N_A[1]	MAF[2]	RA[3]	SA[4]	GD[5]	H[6]	PIC[7]	P[8]
KNUCRY-10**	160-410	4	0.874	2	1	0.229	0.187	0.218	0.0057
KNUCRY-16**	151-500	29	0.382	27	18	0.742	0.236	0.708	0.0000
KNUCRY-35**	160-435	14	0.315	8	5	0.818	0.865	0.797	0.0000
KNUCRY-58**	150-254	5	0.620	1	1	0.548	0.759	0.495	0.0007
KNUCRY-59**	134-500	27	0.437	23	11	0.779	0.517	0.767	0.0000
KNUCRY-75**	165-293	4	0.516	1	0	0.621	0.926	0.555	0.0000
KNUCRY-76	150-286	8	0.817	6	5	0.315	0.367	0.290	0.9999
KNUCRY-77**	257-390	31	0.213	27	12	0.888	0.632	0.879	0.0000
KNUCRY-84**	152-500	35	0.332	30	18	0.843	0.348	0.832	0.0000
KNUCRY-85**	157-268	13	0.403	7	3	0.773	0.682	0.749	0.0000
KNUCRY-94**	160-197	7	0.617	4	1	0.566	0.713	0.524	0.0000
KNUCRY-98**	157-457	23	0.335	19	9	0.824	0.819	0.807	0.0000
Mean		16.7	0.488	5	7	0.662	0.588	0.635	

[1]Number of alleles; [2]major allele frequency; [3]rare allele; [4]specific allele; [5]gene diversity; [6]heterozygosity; [7]polymorphic information content; [8]probability value of chi-square test for HWE. **Loci deviating from Hardy-Weinberg equilibrium (HWE) at the 0.01 significance level.

Genetic relationship and cluster analysis

A genetic distance-based analysis was performed to evaluate the usefulness of these newly developed SSRs for the study of genetic variations and phylogenetic relationships among a large germplasm collection of chrysanthemums. To determine the population structure of 95 accessions of *Chrysanthemum*, the model-based program Structure was used. Although the distribution of L(K) showed the highest peak, $K = 6$ (Figure 2a), clusters could not be differentiated because one cluster showed inferred values that were lower than 70%. Also, the slope at $K = 3$ was sharper and steeper than that at $K = 6$. Thus, when a further ad hoc quantity (ΔK) was used to overcome the difficulty of

interpreting the real *K* values (Evanno et al., 2005), the highest value of *ΔK* for the 95 accessions was found to be at *K* = 3 (Figure 2b). The *Chrysanthemum* accessions tested were distributed into three clusters with 89.4% (shared >70%) membership (85 accessions: 34 for cluster 1, 35 for cluster 2, 16 for cluster 3) showing 10.6% admixture (10 accessions) (Figure 3, Table 1). All decoration-type accessions and most standard-type accessions were found together in cluster 1. Among the standard-type accessions, some were found to be members of cluster 2. Most of the spray-type accessions belonged to cluster 2 and some were related to cluster 1. Three indigenous accessions (Gujeolcho, Ulreung Gukhwa and Gwandong Gukhwa), pot mums and garden mums, were found in cluster 3.

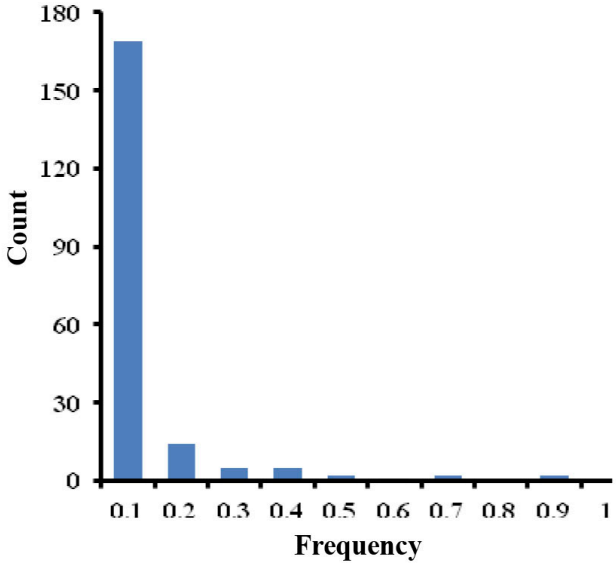

Figure 1. Histogram of allele frequencies for 200 alleles in the 95 chrysanthemum accessions.

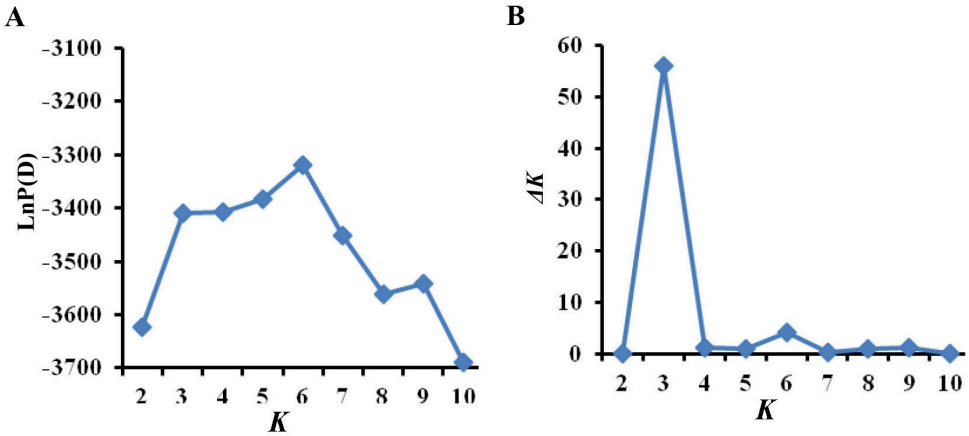

Figure 2. A. Likelihood of the data (N = 95), L(K), as a function of *K* (the number of groups used to stratify the sample) and **B.** values of *ΔK*, with its modal value detecting a true *K* of three groups (*K* = 3).

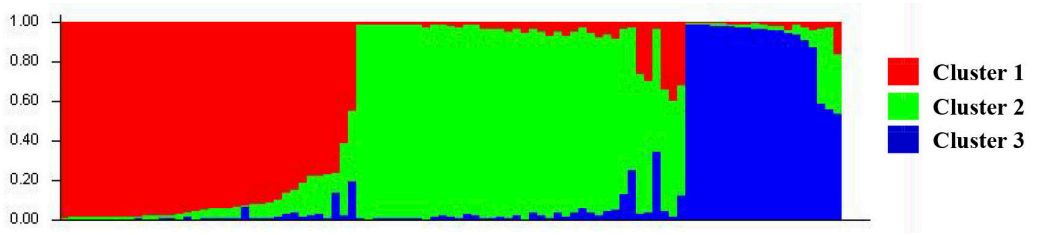

Figure 3. Model-based clustering for each of the 95 chrysanthemum accessions examined based on the 12 SSR markers used to build the Q matrix. Each accession is divided into several hypothetical populations based on the proportional membership.

The genetic distance between cluster 1 and cluster 3 (0.074) was the highest among the relationships of the populations, followed by the distance between cluster 2 and cluster 3 (0.058), and the lowest genetic distance (0.026) was between cluster 1 and cluster 2 (Table 4). The average genetic distance within the same population was highest in cluster 1 (0.638) and lowest in cluster 3 (0.596). The genetic diversity in each model-based population was measured (Table 5). Reduced genetic diversity, as measured by differences in the number of alleles, genetic diversity, and PIC values, was detected within subpopulations in comparison with the overall population. Among three subpopulations, cluster 1 showed the highest genetic parameter values except in heterozygosity, and cluster 3 showed the lowest values in all parameters.

Table 4. Allele frequency divergence among clusters (net nucleotide distance, below diagonal) and average distance (expected heterozygosity) between individuals in same cluster (diagonal).

	Cluster 1	Cluster 2	Cluster 3
Cluster 1	0.683		
Cluster 2	0.026	0.663	
Cluster 3	0.074	0.058	0.596

Table 5. Genetic diversity of model-based populations for 95 chrysanthemum accessions.

	Sample size	N_A[1]	GD[2]	H[3]	PIC[4]
Overall	95	16.7	0.662	0.588	0.635
Cluster 1	34	10.2	0.639	0.596	0.608
Cluster 2	35	9.3	0.614	0.652	0.580
Cluster 3	16	4.6	0.549	0.397	0.500

[1]Number of alleles; [2]gene diversity; [3]heterozygosity; [4]polymorphic information content.

A GD-based analysis was performed by calculating the shared allele frequencies among the 95 accessions, and an unrooted phylogram (neighbor-joining tree) was computed using Powermarker 3.23 and MEGA4 (Tamura et al., 2007). A clustering pattern similar to that of the Structure analysis was observed (Figure 4). Colors were applied according to the model-based cluster analysis results.

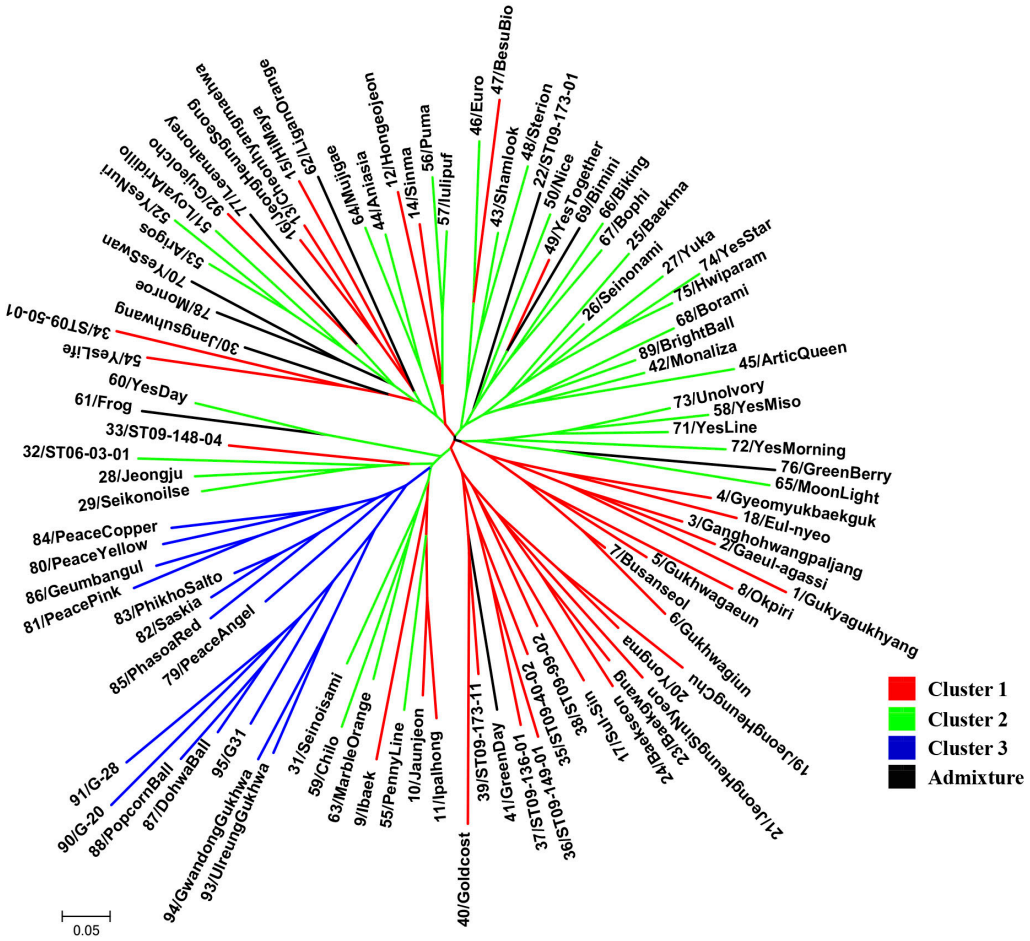

Figure 4. Neighbor-joining tree of 95 accessions using shared allele distances among 12 SSR markers.

DISCUSSION

Chrysanthemum is one of the most important flowers worldwide, not only for ornamental purposes, but also for its medicinal utility. Because it has the ability to survive in and is adaptable to different ecosystems, chrysanthemums spread easily. It was domesticated long ago and artificial crossings have led to numerous cultivars. The genome of the chrysanthemum is composed of multiple sets of chromosomes that range from diploid to decaploid (Hartl and Jones, 2009). Moreover, the chrysanthemum has a strong self-incompatibility system, which leads to gene flow and makes for a genome complex. Difficulties in systematically classifying chrysanthemum were reported by Yang et al. (2006), who stated that lineage recombination was due to extensive sharing of chloroplast haplotypes among tetraploid populations of different origins. The characterization of chrysanthemums is vital for their conservation and management as well as for the understanding of their genetic relationships.

Several DNA works and genetic studies have investigated the chrysanthemum using different molecular markers, such as RAPDs (Wolff et al., 1995; Chatterjee et al., 2006; Zhang et al., 2010b), sequence-characterized amplified regions (Chatterjee et al., 2006), chloroplast SSR markers (Yang et al., 2006), amplified fragment length polymorphisms (Zhang et al., 2010b), and sequence-related amplified polymorphisms (Zhang et al., 2011). However, the results based on morphology, cytology, interspecific hybridization, and molecular systematics have indicated that a lot of work remains to be done on chrysanthemums (Silan et al., 2002). Thus, an intensive classification of inter- and intraspecies variation should be performed.

Moe et al. (2011) identified the distribution of SSR motifs in a chrysanthemum SSR-enriched library through 454 pyrosequencing technology to develop a more powerful analytical tool, SSR markers. Based on their work, 100 SSRs were developed in the present study and screened for polymorphisms. Twelve of the 100 SSR markers designed were polymorphic. KNUCRY-77 showed the greatest polymorphism and KNUCRY-10 showed the lowest polymorphisms among the 12 SSR loci. The wide variation in gene diversity, heterozygosity, rare alleles, and specific alleles revealed by SSRs among the different *Chrysanthemum* accessions reflected high polymorphism in the accessions of chrysanthemums. In the present study, three indigenous accessions were also used to detect the cross-amplification ability of the loci. These 12 noble SSR loci were found to be cross-amplified. However, intensive genotyping with many indigenous accessions is still needed to confirm the wide potential applicability of these loci for the study of interspecific genetic diversity.

In a model-based population, the *Chrysanthemum* accessions tested were distributed into three clusters, with 89.4% (shared >70%) membership showing 10.6% admixture. The high value of admixture indicates ancestral relatedness among the accessions tested. Almost all accessions having the same plant type clustered together, except spray types. Decoration-type accessions and most of the standard accessions were found in cluster 1. In Korea, the decoration type is popular for flower arrangements or training the plant to have a desired shape, such as a butterfly, star, or country map. Decoration types used in the present study were similar to the standard type in terms of flower size and plant type. Most of the spray types grouped in cluster 2 and some were related together in cluster 1. Flower sizes of spray types range from small to medium and some standard types have medium-sized flowers. This may be the reason why the standard type and spray type were found in cluster 1 and cluster 2. Pot mum and garden mum types were found together with three indigenous accessions in cluster 3. Generally, the pot mum type is a short plant type having many branches and many small- to medium-sized flowers, and the garden mum is a bush type with small-sized flowers on the whole plant. Based on the phenotype and cluster relationship, these 12 SSRs may be related to flower size and plant-architecture phenotypes.

According to the results of genetic distance, cluster 1 and cluster 3 had the highest genetic distance between them, and the phenotypes of the accessions between these two clusters were significantly different. Genetic diversity values for each subpopulation showed that cluster 1 and cluster 2 had greater genetic diversity values, suggesting more allelic richness and more diverse genotypes in these two clusters.

The distance-based dendrogram was basically similar to the clustering by model-based structure analysis. The SSRs of the present study were successfully used to measure genetic diversity and relationships within the chrysanthemum collection, and could be useful for determining genetic diversity, identification, conservation, core set construction, and crop improvement of chrysanthemum germplasm.

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