J Korean Soc Appl Biol Chem (2014) 57(6), 789–796
DOI 10.1007/s13765-014-4162-5

Online ISSN 2234-344X
Print ISSN 1738-2203

ARTICLE

Classification of Korean *Chrysanthemum* Species based on Volatile Compounds Using Cluster Analysis and Principal Component Analysis

Su Jeong Kim · Tae Joung Ha · Jongyun Kim · Dong Chil Chang · Ki Sun Kim

Received: 16 May 2014 / Accepted: 22 September 2014 / Published Online: 31 December 2014

Abstract In the analysis of chemotype relationship among Korean *Chrysanthemum* species, the volatile compounds in the leaves of 15 taxa were analyzed and identified by gas chromatograph/mass spectrometry. Principal component analysis and cluster analysis used for the grouping based on the volatile compounds. Fifteen taxa of *Charysanthemum* species were categorized into three groups. Groups I and II included higher ketones than Group III. Group I had five *C. zawadskii* subspecies: *acutilobum*, *acutilobum* var. *tenuisectum*, *acutilobum* var. *alpinum*, *lucidum*, and *coreanum*. Five *C. zawadskii* subspecies were analyzed with main volatile compounds of D-limonene and *m*-thymol. Group II consisted of four *C. zawadskii* subspecies including *naktongense*, *yezoense*, latilobum, and *latilobum* var. *leiophyllum*, and one species *C. makinoi*. They consisted of main compounds of linalool, *cis*-chrysanthenol, eugenol, and chrysanthenone. Group III included five *C. indicum* species and related species: *C. indicum*, var. *albescens*, var. *acuta*, *C. boreale*, and *C. lineare*. The present study was able to classify volatile compounds of Korean *Chrysanthemum* species attributable to major compounds, such as hydrocarbons (sabinene, cymene, β-selinene), alcohols (1-octen-3-ol, *cis*-chrysanthenol, hinesol), ketones (chrysanthenone, camphor), and esters (*cis*-sabiene hydrate, *trans*-chrysanthenyl acetate).

Keywords borneol · camphor · chrysanthemum · cluster analysis · principal component analysis

Introduction

Terpenoid contained in the volatile compounds is a substance where two or more isoprenes are combined, and its main ingredients are monoterpene ($C_{10}H_{16}$) and sesquiterpene ($C_{15}H_{24}$) (Kondjoyan and Berdague, 1996). The volatile compounds of Korean *Chrysanthemum* species were composed of terpenoids and their oxygen derivatives (Choi et al., 2006). The main volatile compounds were repored to be monoterpenoid compounds such as 1,8-cineole, α-pinene and camphene, and sesquiterpenoid compounds such as β-caryophyllene, germacrene D (Kim et al., 1998; Hong, 2002).

Previous studies on the classification of Korean *Chrysanthemum* species were performed via karyotype analysis of somatic chromosome (Kim, 2003), morphological phylogeny (Oh et al., 1994), isozyme genetic studies (Park and Lee, 2004), and genetic characteristics (Lee and Kim, 2000). Whereas, a few studies on the comparison and classification of various Korean *Chrysanthemum* species based on volatile compounds have also been conducted (Hong, 2002; Woo et al., 2008). Regarding studies on the volatile compounds of *Chrysanthemum* species, some attempts have been made mainly on the comparisons of volatile compounds according to cultivation conditions (Wang et al., 2008), volatile composition depending on extraction methods (Wang and Yang, 2006), and volatile compounds for developing chrysanthemum tea (Choi et al., 2006).

Recently, plants were studied on numerical taxonomic classification using multivariate data based on the morphological, biochemical, and molecular characteristics (Sung, 2000; Kim et al., 2014). In particular, on the basis of the chemical components like the volatile compounds in plant, multivariate analyses such as principal component analysis (PCA) and cluster analysis have been performed (Chung, 1999; Yun et al., 2002). Cluster analysis is a type of multivariate analysis that divides data with close similarities into groups (clusters) that are meaningful and useful. If meaningful groups are the goal, then the clusters should capture the natural structure of the data. Therefore, as a technique for grouping clusters of similar traits based on the diverse characteristics of certain entities or subjects, cluster analysis can be utilized in

S. J. Kim (✉) · T. J. Ha · J. Kim · D. C. Chang · K. S. Kim
Highland Agriculture Research Center, National Institute of Crop Science, Rural Development Administration, 5481 Gyeonggangro, Pyeongchang 232-955, Republic of Korea
E-mail: sjkim30@korea.kr

situations where there are no clear or known classification criteria (Sung, 2000). However, little research has been performed on the Korean *Chrysanthemum* species such as the classification of species based on volatile compounds.

The present study was conducted to obtain clear inter-species classification by using multivariate analysis methods such as PCA and cluster analysis, as well as by analyzing and comparing the volatile compounds of 15 taxa of Korean *Chrysanthemum* species.

Materials and Methods

Plant materials. Fifteen taxa of Korean 5 *Chrysanthemum* species with 5 sub-species and 5 varieties were used at the Highland Agriculture Research Center of the National Institute of Crop Science, Rural Development Administration in Pyeongchang, Korea since 2010 (Table 1). Leaf samples of four individual plants were randomly selected and used in July 2011. These samples were stored at −70°C (deep freezer) and freeze-dried in July 2011. The leaf samples were freeze-dried using a freesing dryer (EDT-12012, Operon Co., Korea).

Extraction of volatile compounds. To extract the volatile compounds, Likens and Nickerson type simultaneous steam distillation and extraction (SDE) apparatus with round flask at each end were used in accordance with the method of Schultz et al. (1977). In one of the two round flasks, 2 g freeze-dried samples were added with 500 mL distilled water and heat-refluxed in a 100°C heating mantle for 2 h. Subsequently, 50 mL *n*-pentane: diethyl ether mixture (1:1= v:v) was added to the other round flask, and the volatile compounds were extracted by heat-refluxing at 40°C. Anhydrous sodium sulfate was used for dehydration and after concentration using 99.9% nitrogen gas by filtering through a filter paper (No. 1, Whatman International, UK), gas chromatograph/mass spectrophotometer (GC/MS) was used for analysis by dissolving in 0.2 mL diethyl ether.

Analysis of volatile compounds. The extracted volatile compounds were analyzed GC/MS (7890A, Agilent Co., USA) with a HP-5MS capillary column (30 m ×0.25 mm i.d., 0.25 μm film thickness) via split mode (split ratio =50:1) and identified via gas chromatograph/mass selective detector (GC/MSD) (Figs. S1, S2, and S3). In the GC analysis, the inlet and detector temperatures were maintained at 250°C, and the helium carrier gas flow rate was kept at 1.0 mL/min. The oven temperature was kept at 50°C for 5 min, raised the temperature by 7 per min, and maintained at 250°C for 30 min. In the GC/MSD conditions, the electron ionization energy was 70 eV, the ion source temperature was 250°C and the mass range was 20–400 a.m.u. Regarding verification of the compounds, tentative identification was made by comparing the mass spectrum obtained using GC/MSD with Wiley 70 database (Wiley 70, Agilent Co., USA) and mass spectral data from the literature (Kondjoyan and Berdague, 1996). Alkane (Aldrich Chemical Co., USA) of C_5-C_{20} was used as the volatile compound reference material and expressed as percentage (%) of relative peak area.

Statistical analysis. Based on the results of the analysis using GC/MSD of the volatile compounds in 15 taxa of *Chrysanthemum* species, ANOVA and multivariate analyses were performed using SAS program (SAS institute, ver. 9.2, USA). PCA of multivariate analysis methods was conducted to detect the differences among the 15 taxa of *Chrysanthemum* species with simultaneous consideration of the 45 volatile compounds using *proc factor* with SAS software. The first three principal components were analyzed through PCA and determined with the two-dimensional plot. Cluster analysis was performed using the volatile compounds of the taxa based on quantitative and qualitative variations, including

Table 1 List of 15 taxa of Korean *Chrysanthemum* species investigated in the study

Scientific name	KMRH voucher[a]	Cites (Natural habitats)
C. zawadskii Herbich ssp. *acutilobum* (DC.) Kitagawa	MPS003031	Mt. Yumyeong, Gyeonggi, Korea
C. zawadskii Herbich ssp. *acutilobum* (DC.) Kitagawa var. *tenuisectum* (Kitagawa) Y. Lee	MPS003032	Pocheon, Gyeonggi, Korea
C. zawadskii Herbich ssp. *acutilobum* (DC.) Kitagawa var. *alpinum* (Nak.) Y. Lee	MPS003033	Mt. Baekdu, Hambuk, Korea
C. zawadskii Herbich ssp. *lucidum* (NAK.) Y .Lee	MPS003034	Ullung, Gyeongnam, Korea
C. zawadskii Herbich ssp. *coreanum* (NAK.) Y. Lee	MPS003035	Mt. Halla, Jeju, Korea
C. zawadskii Herbich ssp. *naktongense* (NAK.) Y. Lee	MPS003037	Gimhae, Gyeongbuk, Korea
C. zawadskii Herbich ssp. *yezoense* (Maekawa.) Y. Lee	MPS003039	Goheung, Jeonnam, Korea
C. zawadskii Herbich ssp. *latilobum* (Maxim.) Kitagawa	MPS003041	Wando, Jeonnam, Korea
C. zawadskii Herbich ssp. *latilobum* (Maxim.) Kitagawa var. *leiophyllum* (Nak.) Y. Lee	MPS003043	Gangneung, Gangwon, Korea
C. indicum Linné	MPS003044	Anmyeondo, Chungnam, Korea
C. indicum Linné var. *albescens* Makino	MPS003046	Jeongseon, Gangwon, Korea
C. indicum var. *acuta* (Uyeki) Kitam.	MPS003047	Byeonsanbando, Jeonbuk, Korea
C. boreale (Mak.) Makino	MPS003049	Pyeongchang, Gangwon, Korea
C. lineare Matsumura	MPS003050	Mt. Chilbo, Gyeonggi, Korea
C. makinoi Matsumura et Nakai	MPS003051	Daegu, Korea

[a]KMRH, Korea Medicinal Resouces Herbarium at Rural Development Administration in Korea.

Ward's minimum variance clustering method displayed dendrogram, the cubic clustering criterion and the *Pseudo* option that display *Pseudo F* and t^2 (PST2) statistics. The number of groups in cluster analysis was decided based on the cluster history.

Results and Discussion

Compositions of volatile compounds of Korean *Chrysanthemum* species. The range and average values of percentage compositions of the 45 volatile compounds analyzed from the 15 taxa of Korean *Chrysanthemum* species are shown in Table 2. There were variations among taxa with major compounds of relative peak areas: 20.01 camphor, 12.31 *cis*-chrysanthenol, 10.95 phytol, and 7.92% borneol.

The previously reported compositions of volatile compounds of Asteraceae differed slightly as determined by several researchers. Borneol, 1,8-cineole, α-thujone, and camphor, reported as the main volatile compounds of *Artemisia* species, were similar to the volatile compounds of *Chrysanthemum* species (Morteza-Semnani et al., 2005; Chung, 2009). However, examining the volatile compounds of plants in other genus of the same Asteraceae, the commonly detected compounds in *Ligularia fischeri* var. *spiciformis*, *Ligularia fischeri* var. *spiciformis*, *Synurus deltoids*, and *Aster scaber* were detected to be caryophyllene, terpinole, α-cubebene, which showed different volatile compounds (Lee et al., 2012).

Even though camphor was found to be the main volatile compound in the genus *Chrysanthemum* species, the other volatile compounds were reported differently according to the taxa. Matsuo et al. (1973) reported *cis*-chrysanthenol acetate, *p*-cymene, camphor, borneol, and bornyl acetate as the main volatile compounds of *C. shiwogiku,* and camphor, bornyl acetate, 1,8-cineole, and chrysanthenone as the main volatile compounds of *C. japonense*. In addition, Nikerson and Likens (1996) reported that camphene, 1,8-cineole, camphor, borneol, and bornyl acetate were the main volatile compounds of *C. japonense* var. *debile*, and that α-pinene, 1,8-cineole, camphor, and *t*-muurolol were the main volatile compounds of *C. cuneifolium.* In *C. indicum* and *C. boreale*; the reported main volatile compounds were camphor, borneol, α-pinene, camphene, 1,8-cineole, and germacrene-D (Hong, 2002; Choi et al., 2006; Bae and Lee, 2008; Woo et al., 2008).

Principal component analysis (PCA) of volatile compounds. Volatile compounds were evaluated and coded by each graded value to classify 15 taxa of Korean *Chrysanthemum* species. Table 3 showed the eigenvalues and their contribution through the PCA using 45 volatile compounds. PCA is a method that can be used to identify patterns in a data set and to reduce dimentionality of multivariate data by removing inter-correlations among variables (Iezzoni and Pritts, 1991; Sung, 2000). Data was analyzed all variables, found the principal component and got meaning in that main component. We could obtain the eigenvalues of the matrix consisting of a component of the variance and convariance parameter. In the case of the first Principal component (PC1), the eigenvalue of each of the characteristics was 10.47, thereby showing a 23.28% contribution on the total variation. The second and third PCs had eigenvalues of 6.76 and 5.82, respectively, and the degree of contribution on the total variation was 51.25%. The first 10 PCs in Table 3 showed 91.33% cumulative proportion by PCA. Each of the 10 PCs was presented in one characteristic, because the eigenvalues were over 1.68, which was more informative than any single variable alone (Iezzoni and Pritts, 1991).

In addition, to estimate the characteristics contained in the various volatile compounds, correlations between the individual PCs were analyzed (Table 4). The eigenvalues of each PC were the total number of hypothetic variables analyzed by PCA, indicating that the first, second, and third PCs represented approximately 10, 7, and 6 variables among the 45 volatile compounds of Korean *Chrysanthemum* species, respectively. The first PC showed a highly correlated eigenvalue with 10 volatile compounds including sabinene, cymene, β-selinene, 1-octen-3-ol, *cis*-chrysanthenol, hinesol, chrysanthenone, camphor, *cis*-sabinene hydrate, and *tans*-chrysanthenyl acetate, among which sabinene showed the highest correlation based on loading of PC at 0.274976 followed by camphor at 0.259927 and *cis*-chrysanthenol at 0.246447. In the case of the second PC, *m*-thymol showed the highest correlation based on loading of PC at 0.370277 followed by borneol. The third PC was closely related with alcohols including 1-decanol, pentadecanoic acid, and α-cadinol.

As a result of arranging the values of the first and second PCs on a 2-dimensional scatter diagram for 15 taxa of Korean *Chrysanthemum* species, these were categorized into three groups (Fig. 1). Five *C. zawadskii* subspecies having with main volatile compounds of D-limonene and *m*-thymol were located in the middle part of plot 1 and upper part of plot 2 (Fig. 1; Group I). The present study corresponded that D-limonene was reported as the main compounds in *Ligularia fischeri* var. *spiciformis* and *Ligularia fischeri* var. *spiciformis* in Asteraceae (Lee, 2012). However, this compound was neither detected in *Aster scaber* nor in *Synurus deltoid* (Lee, 2012). When the genus *Thymus* was analyzed, it was further observed that even though the main compound of *T. quinquecostatus* var. *quinquecostatus* was cymene, the main compound of *T. quinquecostatus* var. *japonica* was *m*-thymol. These results showed that each speices has different chemotypes, making it possible to completely distinguish the species (Kwon et al., 2006). Thus, the main compounds of *m*-thymol and limonene in 15 taxa of Korean *Chrysanthemum* species corresponded to *Thymus* based on classification results.

In the result of PCA, four *C. zawadskii* subspecies and *C. makinoi,* having main volatile compounds of linalool, *cis*-chrysanthenol, eugenol, and chrysanthenone, were located in the right part of plot 1 and lower part of plot 2 (Fig. 1; Group II). Using plants as basic materials in perfumes and cosmetics, among others (Arctander, 1969), linalool was contained in *Matricaria recutita* flower, *C. boreale* flower and *Camellia sinensis* leaf

Table 2 List of 45 volatile compounds and their range of values in Korean *Chrysanthemum* species

Functional group	Volatile compounds	R.T.	MW	Minimum Value	Maximum value	Mean±SD	*F* value[a]
Hydrocarbones	2,4-Dimethyl-heptene	5.80	126	0.02	0.57	0.20±0.18	1.51^{NS}
	α-Thujene	8.51	136	0.03	0.15	0.10±0.05	2.85^{NS}
	α-Pinene	8.72	136	0.01	1.33	0.67±0.40	0.66^{NS}
	Camphene	9.22	136	0.06	3.57	0.84±0.98	1.90^{NS}
	Sabinene	9.93	136	0.06	0.70	0.32±0.24	14.23***
	β-Pinene	10.01	136	0.05	1.41	0.37±0.44	1.83^{NS}
	α-Terpinene	13.22	136	0.02	1.30	0.39±0.44	3.72^{NS}
	Cymene	11.46	134	0.02	1.36	0.64±0.52	4.62*
	Limonene	11.51	136	0.06	0.97	0.28±0.34	2.34^{NS}
	γ-Terpinene	12.43	136	0.11	1.21	0.59±0.35	7.77**
	α-Thujone	13.93	152	0.05	0.28	0.17±0.12	5.46*
	trans-Caryophyllene	13.90	204	0.21	2.62	0.99±0.74	0.71^{NS}
	β-Selinene	21.63	204	0.19	1.79	0.84±0.60	4.21*
	Germacrene-D	21.73	204	0.18	3.71	1.61±1.09	1.72^{NS}
	α-Muurolene	21.97	204	0.15	3.80	1.72±1.80	3.15^{NS}
	δ-Cadinene	22.44	204	0.09	8.03	2.65±2.46	0.96^{NS}
	α-Longipinene	19.29	204	3.80	3.80	3.80±0.00	1.00^{NS}
	Caryophyllene oxide	23.59	220	1.11	3.90	2.49±1.00	0.14^{NS}
	γ-Gurjunene	23.67	204	0.65	2.84	1.34±0.73	1.28^{NS}
Alcohols	1-Octen-3-ol	10.13	128	0.06	0.58	0.27±0.17	7.51**
	1-Decanol	12.10	158	1.77	1.77	1.77±0.00	1.00^{NS}
	Linalool	13.41	154	0.07	0.76	0.36±0.27	9.04**
	cis-Chrysanthenol	13.90	152	9.24	14.81	12.31±2.75	100.47***
	Borneol	15.13	154	0.62	20.94	7.92±7.91	14.15***
	Epoxylinalol	15.26	170	0.03	0.03	0.03±0.00	1.00 ^{NS}
	α-Terpineol	15.63	154	0.28	1.53	0.88±0.44	10.10**
	Myrtenol	15.79	152	0.23	2.85	0.99±0.73	0.23^{NS}
	Piperitol	17.14	152	0.14	12.39	4.20±5.78	1.98^{NS}
	m-Thymol	17.74	150	1.97	4.26	3.09±0.84	68.62***
	Eugenol	19.25	164	0.06	1.80	0.84±0.86	3.24^{NS}
	Hinesol	24.51	222	2.95	6.73	4.49±1.68	10.49**
	α-Cadinol	24.52	222	2.62	2.62	2.62±0.00	1.00^{NS}
	Phytol	31.36	296	4.05	21.62	10.95±4.96	2.52^{NS}
Aldehydes	*trans*-2-Hexenal	6.25	98	0.01	0.80	0.25±0.23	2.95^{NS}
Ketones	Chrysanthenone	14.12	150	0.25	1.99	1.06±0.63	14.32***
	Camphor	14.61	152	6.44	33.22	20.01±9.03	13.34***
	Pinocarvone	15.05	150	0.02	1.07	0.56±0.39	0.04^{NS}
	Thyme camphor	17.75	150	0.26	2.98	1.20±1.08	6.18*
Acids	Pentadecanoic acid	28.73	270	0.31	0.31	0.31±0.00	1.00^{NS}
	Hexadecanoic acid	29.32	256	0.23	4.95	1.15±1.50	1.82^{NS}
Esters	1,8-Cineole	11.65	154	0.02	3.50	0.80±0.85	0.41^{NS}
	trans-Sabinene hydrate	12.60	154	0.04	6.97	2.16±2.66	3.59^{NS}
	cis-Sabiene hydrate	13.38	154	0.18	3.37	1.72±1.10	4.87*
	trans-Chrysanthenyl acetate	16.68	194	0.14	0.68	0.36±0.18	11.53**
	Bornyl acetate	17.74	196	0.40	2.66	1.28±0.85	3.31^{NS}

[a]The variables in 15 taxa of Korean *Chrysanthemum* species expressed ^{NS}non-significant, *Significant at $p \leq 0.05$, **Significant at $p < 0.01$, or ***Significant at $p < 0.001$.

Table 3 Eigenvalues and contributions of the first 10 principal components using 45 volatile compounds found in Korean *Chrysanthemum* species

Principal component	Eigenvalue	Difference	Contribution (%)	Cumulative contribution (%)
PC 1	10.47	3.71	23.28	23.28
PC 2	6.76	0.93	15.03	38.30
PC 3	5.82	2.22	12.95	51.25
PC 4	3.60	0.48	8.00	59.25
PC 5	3.11	0.25	6.92	66.17
PC 6	2.86	0.39	6.36	72.53
PC 7	2.47	0.18	5.50	78.03
PC 8	2.29	0.28	5.10	83.13
PC 9	2.01	0.33	4.47	87.60
PC 10	1.68	0.42	3.73	91.33

(Choi et al., 2006). It was further determined to be an index compound where taxa could be identified only in Group II. In addition, most compounds in Group II were detected quite similarly to the volatile compounds of Group I.

Five taxa were located in the left part of plot 1 and lower part of plot 2 (Fig. 1; Group III). *C. indicum*, *C. indicum* var. *albescens*, *C. indicum* var. *acuta*, *C. boreale*, and *C. lineare* had mainly α-thujene, α-muurolene, piperitol, and hinesol due to the different volatile compounds of Groups I and II. *C. lineare* contained 1-decanol, α-cadinol, pentadecanoic acid compounds, which were detected specifically in Group III, showed a different pattern. Even though α-terpineol was identified in Groups I and II, it was determined to be an index compound among taxa, because it was not identified in Group III.

Cluster analysis of compositions of volatile compounds. Cluster analysis was performed based on 45 volatile compounds and showed the dendrogram of relationships among 15 taxa of *Chrysanthemum* species. The number of clusters was distinguished through statistics such as cubic clustering criterion, PST2, *pseudo* F (PSF), among others (Fig. 2). When the volatile compounds were subjected to cluster analysis using an average distance of 0.15 semi-partial R square (RSQ) as the criterion, it was possible classify volatile compounds into 3 groups baed on the cluster history. Group I included *C. zawadskii* ssp. *acutilobum,* ssp. *acutilobum* var. *tenuisectum*, ssp. *acutilobum* var. *alpinum*, ssp. *lucidum*, and ssp. *coreanum*. In the case of *C. zawadskii* ssp. *lucidum,* it was distinguished within Group I due to observation of α-longipinene. This is deemed attributable to the unique volatile compounds generated in the leaf, because *C. zawadskii* ssp. *lucidum* grew only in the habitual environment of island in the East Sea of Korea.

Group II included 5 taxa that were mostly distributed geographically in the southern regions of the Korean Peninsula. These taxa were *C. zawadskii* ssp. *naktongense*, ssp. *yezoense*, ssp. *latilobum*, ssp. *latilobum* var. *leiophyllum,* and *C. makinoi.* However, *C. makinoi* was rather distant in terms of phylogenetic relationship with the other taxa contained in Group II.

Group III consisted of 5 taxa of *C. indicum,* var. *albescens*, var. *acuta*, *C. boreale*, and *C. lineare.* On the other hand, *C. lineare* was detected in 1-decanol, α-cadinol, and pentadecanoic acid which had not been seen in other taxa. In addition, δ-cadinene was detected in all 14 taxa of *Chrysanthemum* species except for *C. lineare*.

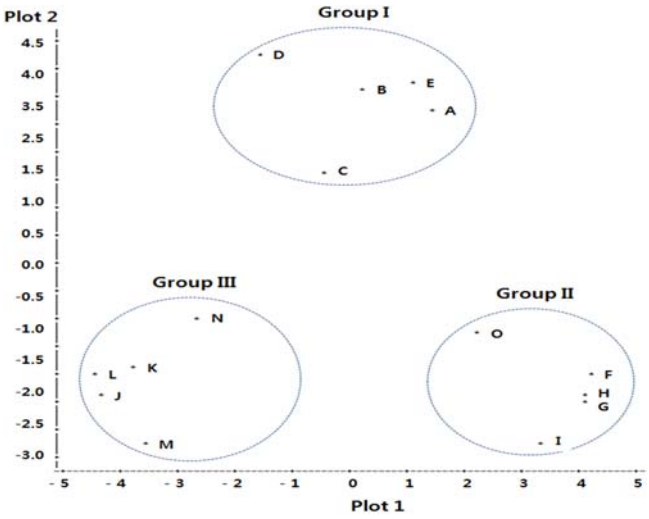

Fig. 1 Plot based on principal component analysis in 15 taxa of Korean *Chrysanthemum* species. *C. zawadskii* ssp. *acutilobum* (A), *C. zawadskii* ssp. *acutilobum* var. *tenuisectum* (B), *C. zawadskii* ssp. *acutilobum* var. *alpinum* (C), *C. zawadskii* ssp. *lucidum* (D), *C. zawadskii* ssp. *coreanum* (E), *C. zawadskii* ssp. *naktongense* (F), *C. zawadskii* ssp. *yezoense* (G), *C. zawadskii* ssp. *latilobum* (H), *C. zawadskii* ssp. *latilobum* var. *leiophyllum* (I), *C. indicum* (J), *C. indicum* var. *albescens* (K), *C. indicum* var. *acuta* (L), *C. boreale* (M), N; *C. lineare* (N), *C. makinoi* (O).

Comparing the inter-group characteristics of *Chrysanthemum* species through a cluster analysis, functional group of volatile compounds such as hydrocarbons, alcohols, aldehydes, ketones, acids, and esters showed differences (Fig. 3). In Group I, the composition of aldehyde was relatively low, whereas the compositions of alcohols and esters were high. In Group II, the composition of ketones was high whereas the compositions of alcohols and acids were relatively low. In Group III, the compositions of alcohols and acids were higher in relation to Groups I and II, whereas the compositions of ketones were lower

Table 4 Loadings of three principal components among 45 volatile compounds in Korean *Chrysanthemum* species

Functional group	Volatile compounds	Loading of principal component		
		PC 1	PC 2	PC 3
Hydrocarbones	2,4-Dimethyl-heptene	0.151169	-0.036358	-0.081854
	α-Thujene	0.156667	0.073682	0.133573
	α-Pinene	0.107073	0.033887	-0.131258
	Camphene	-0.112005	0.115246	0.005350
	Sabinene	**0.274976**[a]	-0.077003	0.031517
	β-Pinene	0.148728	0.005741	-0.017851
	α-Terpinene	0.042363	**0.246840**	-0.019599
	Cymene	**0.217660**	0.078302	0.023357
	Limonene	0.050832	**0.208936**	0.009159
	γ-Terpinene	0.186282	0.191088	0.087366
	α-Thujone	-0.187067	-0.147448	0.017357
	trans-Caryophyllene	-0.137563	0.018842	0.232685
	β-Selinene	**0.207477**	-0.071048	0.104086
	Germacrene-D	-0.040842	-0.169127	-0.085989
	α-Muurolene	-0.172210	-0.094069	0.155605
	δ-Cadinene	0.089344	0.032327	0.016232
	α-Longipinene	-0.038363	0.176341	-0.070457
	Caryophyllene oxide	-0.059811	-0.016015	**0.275720**
	γ-Gurjunene	-0.082181	0.068966	0.316431
Alcohols	1-Octen-3-ol	**0.232682**	0.031638	0.028810
	1-Decanol	-0.070376	-0.010000	**-0.326521**
	Linalool	0.179901	-0.165416	-0.016956
	cis-Chrysanthenol	**0.246447**	-0.203264	-0.005911
	Borneol	-0.087005	**0.299217**	0.057413
	Epoxylinalol	0.056441	-0.038169	-0.101769
	α-Terpineol	0.188122	0.186852	0.040418
	Myrtenol	-0.057896	-0.082279	**0.319047**
	Piperitol	-0.134099	-0.130623	0.036826
	m-Thymol	0.008844	**0.370277**	0.037989
	Eugenol	0.169627	-0.121044	0.019953
	Hinesol	**-0.232142**	-0.157992	0.177239
	α-Cadinol	-0.070376	-0.010000	**-0.326521**
	Phytol	-0.109881	-0.161784	-0.150459
Aldehydes	*trans*-2-Hexenal	0.131332	-0.185657	0.096000
Ketones	Chrysanthenone	**0.205682**	-0.183751	0.000285
	Camphor	**0.259927**	-0.022520	0.006863
	Pinocarvone	-0.009726	-0.104409	0.191614
	Thyme camphor	0.018116	**0.255284**	0.105630
Acids	Pentadecanoic acid	-0.070376	-0.010000	**-0.326521**
	Hexadecanoic acid	-0.134942	-0.131552	0.061932
Esters	1,8-Cineole	-0.057421	0.059248	**0.236369**
	trans-Sabinene hydrate	0.078373	**0.212838**	0.092349
	cis-Sabiene hydrate	**0.234741**	0.024207	0.071986
	trans-Chrysanthenyl acetate	**0.243119**	-0.130490	0.014068
	Bornyl acetate	-0.050397	**0.269687**	-0.209525

[a]Underlined loading was the trait that had higher correlation with principal component of column.

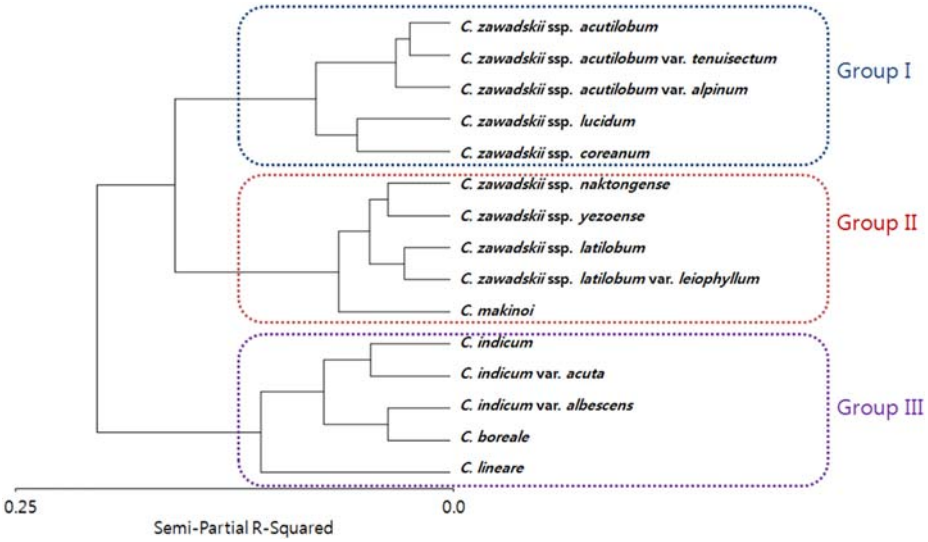

Fig. 2 Dendrogram of 15 taxa of Korean *Chrysanthemum* species classified by ward's minimum variance clustering method.

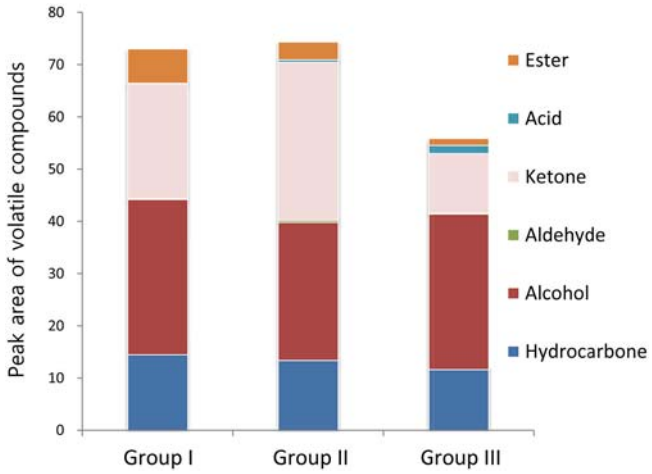

Fig. 3 Differnence in functional group of volatile compounds of Korean *Chrysanthemum* species classified by cluster analysis.

than Groups I and II. The present study also corresponded the previous report that mainly hydrocarbons and alcohols were contained as volatile compounds in *C. boreale* in large quantities (Choi et al., 2006).

Taxonomic studies of *Chrysanthemum* species have been conducted mainly through the morphological camparison (Oh et al., 1994), anatomical analysis (Kim et al., 2003) and chromosomal study (Kim, 2003). In the present study, the volatile compounds of 15 taxa were analyzed with GC/MSD as well as through multivariate analysis techniques of PCA and cluster analysis, thereby confirming the possibility of classifying the 15 taxa of *Chrysanthemum* species into three groups. Thus, it was determined that the chemo-taxonomy technique using the compositions of the volatile compounds of *Chrysanthemum* species would be useful in the classification and identification of the verified inter-species differences. Finally, the present study provided the basic materials needed for species selection and cultivation of *Chrysanthemum* species, which are useful for foods, cosmetics, and medicine.

Acknowledgment The work was supported by a grant from the Rural Development Administration (ATIS code PJ00646008 and PJ00876405) in Republic of Korea. The authors would like to thank Dr. Jeong-Hun Lee and Ms. Mi Yeong Park for their assistance in the herbaria voucher specimen production. The authors are also grateful to Ms. Hye-Rim Lee of the RDA for the critical review of the statistical analysis of the study.

References

Arctander S (1969) In *Flavor descriptions Perfume and flavor chemicals*, Allured Pub Corp, USA.

Bae SM and Lee SC (2008) Effect of rot-air drying temperature on volatile compounds in *Chrysanthemum boreale* M. flowers. *Kor J Food Technol* **40**, 466–9.

Choi SH, Im SI, and Bae JE (2006) Analysis of aroma components flower tea of German chamomile and *Chrysanthemum boreale* Makino. *Kor J Food Cookery Sci* **22**, 768–73.

Chung KH (1999) Morphological characteristics and principal component analysis of plums. *Kor J Hort Sci Technol* **17**, 23–8.

Chung MS (2009) Changes in the volatile compounds of *Artemisia princeps* var. *orientalis* essential oils during storage. *Food Sci Biotechnol* **18**, 481–7.

Hong CU (2002) Essential oil composition of *Chrysanthemum boreale* and *Chrysanthemum indicum*. *J Kor Soc Agric Chem Biotechnol* **45**, 108–13.

Iezzoni AF and Pritts MP (1991) Applications of principal component analysis to horticultural research. *HortScience* **26**, 334–8.

Kim JS (2003) A study of diversity and speciation in *Dendranthema zawadskii* and its related species (Asteraceae). Ph D Thesis, Kyoto University, Japan.

Kim JS, Park JH, Seo BB, and Tobe H (2003) Karyotypes of metaphase chromosomes in diploid populations of *Dendranthema zawadskii* and related species (Asteraceae) from Korea: diversity and evolutionary

implications. *J Plant Res* **116**, 47–55.
Kim SJ, Lee CH, Kim J, and Kim KS (2014) Phylogenetic analysis of Korean native *Chrysanthemum* species based on morphological characteristics. *Sci Hort* **175**, 278–89.
Kim SK, Lee SC, Kang DK, Chung SH, and Lee SD (1998) Essential oil content and composition of aromatic constituents in some medicinal plant. *Kor J Plant Res* **11**, 279–82.
Kondjoyan N and Berdague JL (1996) In *Aromatic compounds A compilation of relative retention for the analysis of aromatic compounds*, (1st ed.), Laboratire flaveur Press, France.
Kwon YH, Lee DS, Choi K, Oh SH, Shin YJ, Oh SY, Kim HJ, Park KW, Kim HJ, and Lee DS (2006) Characterization of fragrances from genus *Thymus* by GC-MS. *J Kor For Soc* 287–9.
Lee CH and Kim KS (2000) Genetic diversity of *Chrysanthemum zawadskii* Herb. and the related groups in Korea using RAPD. *J Kor Soc Hort Sci* **41**, 230–6.
Lee KC, Sa JY, Wang MH, and Han SS (2012) Comparison of volatile aroma compounds between *Synurus deltoids* and *Aster scaber* leaves. *J Med Crop Sci* **20**, 54–62.
Matsuo A, Uchio Y, Nakayama M, and Hayashi S (1973) The isolation of *trans*-chrysanthenyl acetate and chrysanthenone from the essential oil of *Chrysanthemum shiwogiku*. *Bull Chem Soc Jap* **46**, 1565–7.
Morteza-Semnani K, Akbarzadeh M, and Moshiri K (2005) Essential oil composition of *Artemisia fragrans* wild from Iran. *Flavour Fragr J* **20**, 330–1.
Nikerson GB and Likens ST (1996) Gas chromatographic evidence for occurrence of hop oil compounds in beer. *J Chromatogr* **21**, 1–5.
Oh IS, Yoo SO, and Bae JH (1994) Morphological characteristic of *Chrysanthemum zawadskii* Herb native to Korea. *J Kor Soc Hort Sci* **35**, 78–87.
Park KR and Lee CH (2004) The genetic evidence for polyploid origin of *Chrysanthemum zawadskii* complex. *J Basic Sci* **19**, 99–112.
Schultz TH, Flath RA, Mon TR, Eggling SB, and Teranishi R (1977) Isolation of volatile compounds from a model system. *J Agr Food Chem* **25**, 446–9.
Sung WH (2000) In *Principal Component Analysis Applied multivariate statistics*, (2nd ed.), Tamjin Press, Korea.
Wang YJ and Yang XW (2006) GC-MS analysis of essential oil of the flower of the *Chrysanthemum morifolium* by the different processing methods. *China J Chinese Material Medica* **31**, 456–9.
Wang YJ, Guo QS, Yang XW, Xu WB, and Tao HY (2008) Characterization of chemical components of essential oil from flowers of *Chrysanthemum morifolium* produced in Anhui province. *China J Chinese Materia Medica* **33**, 2207–11.
Woo KS, Yu JS, Hwang IG, Lee YR, Lee CH, Yoon HS, Lee JS, and Jeong HS (2008) Antioxidative activity of volatile compounds in flower of *Chrysanthemum indicum*, *C. morifolium*, and *C. zawadskii*. *J Kor Soc Food Sci Nutr* **37**, 805–9.
Yun JS, Son SY, Kim IH, Hong EY, and Yun T (2002) Classification of *Polygonatum* ssp. collections based on multivariate analysis. *Kor J Med Crop Sci* **10**, 333–9.