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Phylogenetic analysis of Korean native *Chrysanthemum* species based on morphological characteristics

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ABSTRACT

In the analysis of genetic relationship among Korean native *Chrysanthemum* species, the morphological characteristics of 15 taxa of *Chrysanthemum* species were investigated. Principal component analysis (PCA) and cluster analysis were conducted for the grouping based on the morphological data. Fifteen taxa of *Chrysanthemum* species were classified into three groups through PCA and cluster analysis based on the general plant growth and flowering characteristics. Groups I and II included non-bushy type and big size flower plants, while Group III included bushy type and small size flower plants. Group I had nine *C. zawadskii* subspecies: *acutilobum*, *acutilobum* var. *tenuisectum*, *acutilobum* var. *alpinum*, *lucidum*, *coreanum*, *naktongense*, *yezoense*, *latilobum*, and *latilobum* var. *leiophyllum*. Group I was found to be desirable species as garden plants because of the white or pink flowers with a relatively large size (flower head diameter of 43.5–67.6 mm), good plant height (19.3–64.6 cm), and long flowering period (24–39 d). *C. lineare* was the only species included in Group II with unique cone head shaped seeds and no petiole. Group III included five *C. indicum* species and related species: *C. indicum*, *C. indicum* var. *albescens*, *C. indicum* var. *acuta*, *C. boreale*, and *C. makinoi*. Group III had great potentials as edible medicinal resources; e.g., chrysanthemum flower tea, which is abundant in number and has small sized flowers with white or yellow petals. The study provided a clear diagnostic key for the classification showing the species-specific plant form attributable to unique leaves, branch type, plant height, flower size, and flowering habits.

1. Introduction

The tribe Anthemideae of Asteraceae has 12 subtribes, 108 genera and 1741 species (Bremer and Humphries, 1993). The subtribe Artemisiinae has a total of 18 genera and 634 species and is characterized by disciform or discoid, commonly paniculate capitula, smooth or short spined pollen, obovoid, thin-walled, and ribless cypselae without pappus, and involucral bracts with dark brown margins (Fukai, 2003; Zhao et al., 2009). The genus *Chrysanthemum* named according to the International Code of Nomenclature, consists of 41 species and is distributed in temperate zones in eastern Asia and Siberia (Iwatsuki et al., 1997). Although most Korean native *Chrysanthemum* species are distributed throughout Korean Peninsula (Kim, 1999; Lee, 2006), some subspecies are found only in specific regions in Korea. *Chrysanthemum zawadskii* is a major and the most popular native plant in Korea, accounting for 8.1% (3.9 million pots) of Korean native plant production (KWPA, 2010) and 38% (US$ 6.3 million) of the total Korean native plant sales (KWFA, 2004).

The diagnostic key for identifying the morphological characteristics within the taxa of *Chrysanthemum* species was almost identical among previous studies, including the size of flower head, color of ray flowers, number and arrangement of head flowers, and leaf morphology, such as leaf shape, leaf thickness, leaf glossiness, and leaf color (Oh et al., 1994; Kim, 2003; Lee, 2006; Kim and Tobe, 2009; Zhao et al., 2009). There are a number of existing taxonomic studies identifying the morphological variations among Korean native *Chrysanthemum* species based on the characteristics of leaves (Oh et al., 1994; Kim and Tobe, 2009), flowers (Kim et al., 2011), roots (Oh et al., 1994), and genetic characteristics based on Random Amplified Polymorphic DNA (Lee and Kim, 2000), somatic chromosome and karyotype analysis (Kim, 2003) and chemical composition of petal (Park and Kwon, 1997).

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Recently, a multivariate analysis method for numerical taxonomy has been developed for identification within the species via the morphological and taxonomic characteristics of the species. The method uses not only quantitative but also qualitative data, thereby providing a better identification within the species (Kim and Lee, 1995; Kim et al., 1999; Sung, 2000).

Korean native *Chrysanthemum* species has garnered a lot of attention as an important medicinal and ornamental plant, and its market is expected to increase significantly. To establish the proper classification and usage of the species, better identification, understanding of the species, clear classification and grouping will be needed.

The study aimed to obtain a clear classification within the Korean native *Chrysanthemum* species using principal component analysis and cluster analysis to categorize the 15 taxa of *Chrysanthemum* species into groups by morphological characteristics.

2. Materials and methods

2.1. Plant materials and growing conditions

Lee (2006) reported that Korean native *Chrysanthemum* species included 18 taxa characterized by morphological criteria in New Flora of Korea. In accordance with the flower head diameter, flower size, leaf margin, seed shape, and others, Korean native *Chrysanthemum* species has been classified into 8 species, 9 subspecies and 1 variety, and listed 18 taxa: *C. zawadskii* ssp. *acutilobum*, *C. zawadskii* ssp. *acutilobum* var. *tenuisectum*, *C. zawadskii* ssp. *acutilobum* var. *alpinum*, *C. zawadskii* ssp. *lucidum*, *C. zawadskii* ssp. *coreanum*, *C. zawadskii* ssp. *naktongense*, *C. zawadskii* ssp. *yezoense*, *C. zawadskii* ssp. *latilobum*, *C. zawadskii* ssp. *latilobum* var. *leiophyllum*, *C. indicum*, *C. indicum* var. *albescens*, *C. boreale, C. morifolium, C. makinoi, C. coronarium, C. intermedium, C. pallasianum* and *C. lineare.* Of the 18 taxa, 15 taxa were investigated in the study: wherein four species, namely, *C. coronarium*, *C. morifolium*, *C. intermedium*, and *C. pallasianum* were excluded and one variety, *C. indicum* var. *acutum*, was added in accordance with Lee (1996).

Fifteen taxa of five Korean *Chrysanthemum* species with five subspecies and five varieties were collected from the natural habitats in different regions of Korean Peninsula from April to October during 2006–2009 (Table S1 and Fig. 1). The classification and nomenclature of species in *Chrysanthemum* were mainly in accordance with Lee (1996, 2006) and the International Code of Botanical Nomenclature (Trehane, 1995). Collected plants were transplanted into square plastic pots (20 cm × 20 cm × 16 cm) filled with a mixture (1:1, v:v) of commercial horticultural substrate (SsukSsukyi: 60% peat moss, 30% perlite, Nongwoo Co., Suwon, Korea) and sandy loam (Masato, Hwaboonworld, Chilgok, Korea). Voucher specimens were deposited in the Korea Medicinal Herbarium (KMH) at the Rural Development Administration (RDA), Korea. These species have been deposited in the herbarium at the Highland Agricultural Research Center (HARC, 37°40′N, 128°43′E, altitude 772 m). Individual plants were then grown in the greenhouse at HARC. Foliar spray of nutrient solution (6-10-5 New Hyponex, Hyponex Co., Osaka, Japan) was applied bi-weekly throughout the growing period from June to September, 2010, 2011, and 2012. When flowering occurred, the plants were sampled and preserved at the herbaria of RDA. Each of five individuals was randomly selected and investigated for morphological characteristics for three years from 2010 to 2012.

2.2. Meteorological data

Meteorological data were collected based on 30-year average value from Korea Meteorological Administration from 1981 to 2010 (KMA, 2012). The climatological data of the local areas were obtained from the national weather service website. Annual meteorological conditions, such as average, maximum and minimum temperatures, precipitations, wind speeds, humidity, and daylight durations were investigated (Table S2).

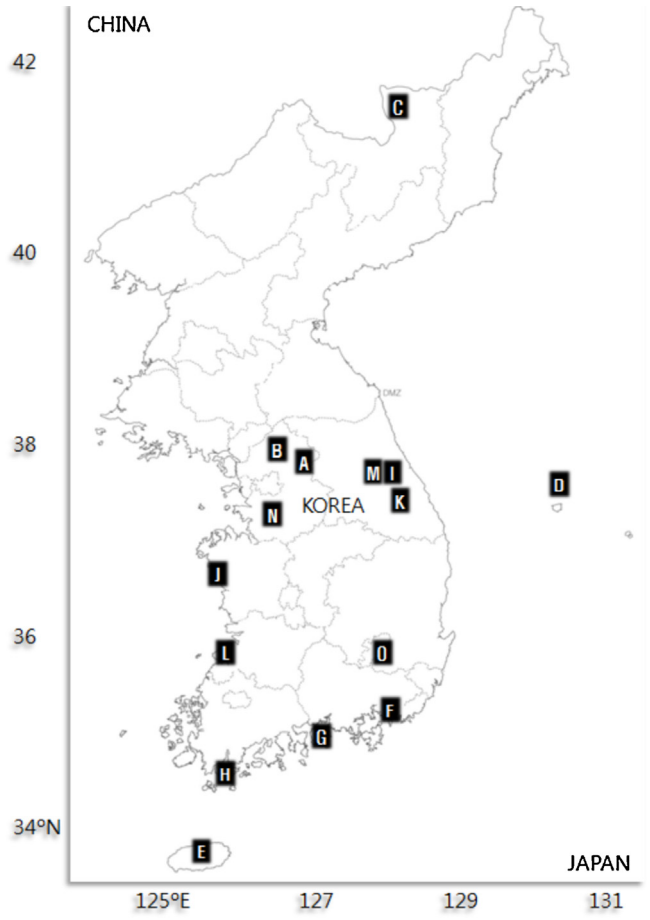

Fig. 1. Collection sites of the Korean native *Chrysanthemum* species from the 15 regions of Korea. Sites are indicated with black dots. A, *C. zawadskii* ssp. *acutilobum*; B, *C. zawadskii* ssp. *acutilobum* var. *tenuisectum*; C, *C. zawadskii* ssp. *acutilobum* var. *alpinum*; D, *C. zawadskii* ssp. *lucidum*; E, *C. zawadskii* ssp. *coreanum*; F, *C. zawadskii* ssp. *naktongense*; G, *C. zawadskii* ssp. *yezoense*; H, *C. zawadskii* ssp. *latilobum*; I, *C. zawadskii* ssp. *latilobum* var. *leiophyllum*; J, *C. indicum*; K, *C. indicum* var. *albescens*; L, *C. indicum* var. *acuta*; M, *C. boreale*; N, *C. lineare*; O, *C. makinoi.*

2.3. Measurement of anatomical characteristics

As shown in Fig. 2, the general morphological characteristics were investigated to determine the average plant growth and flowering based on the Chrysanthemum Test Guidelines criteria set by the International Union for the Protection of New Varieties of Plants (UPOV, 2008) and the Korea Seed and Variety Service (Song et al., 2000).

Plant height was measured from the tallest point of the canopy to the base of the plant. Winter suckers were photographed and the color was determined using the Royal Horticultural Society (RHS) chart with the visual appearance. Glossiness of winter suckers was visually rated as either strong, weak, or absent. Basal leaf morphology was measured during the growing period and cauline leaf morphology was measured during the flowering time. Leaf length was measured from the lamina tip to the intersection of lamina and petiole along the lamina midrib. Leaf width was measured from the widest lamina lobes. Petiole length was measured from the stem margin to the end of leaf lamina. To measure leaf thickness and cell