Genetic Diversity of Species of Chrysanthemum and Related Genera and Groundcover Cultivars Assessed by Amplified Fragment Length Polymorphic Markers

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Abstract. Chrysanthemums have beautiful flowers with high ornamental value and rich genetic diversity. Amplified fragment length polymorphism (AFLP) markers were used to detect the relationships among 12 wild accessions and 62 groundcover chrysanthemum cultivars. Nineteen EcoRI/MseI primer combinations revealed 452 informative polymorphic bands with a mean of 23.8 bands and 71.5% polymorphic rate per primer pair. Jaccard’s coefficient of similarity varied from 0.64 to 0.89, indicating much genetic variation in chrysanthemums. The 74 accessions were classified into two major groups by unweighted pair group method with the arithmetic averages (UPGMA). The dendrogram showed that AFLP variability was closely correlated with both geographic distribution and traditional classification of the wild accessions. Among all accessions, genetic relationship was the most relevant factor in AFLP-marker clustering, whereas petal type was also informative. AFLP technology could be very efficient for discriminating species of chrysanthemum and its related genera and reconstruct their genetic relatedness.

Chrysanthemum (Chrysanthemum spp.; Asteraceae, Anthemideae) is not only a traditional Chinese flower, but also one of the most important cut flower, garden flower, and potted ornamental floricultural crops in the world. Although a native from China (Editorial Committee of the Flora of China, 1983), chrysanthemums have been associated with various international cultures and have been widely cultivated for centuries for their beauty, fragrance, edibility, and medicinal values. Huge numbers of genotypes have been created by cultivation and breeding over thousands of years (Anderson, 2006). Chrysanthemums are among the most important and valuable fall-blooming plants for their diversity of flower shapes, colors, and forms. Chen et al. (1995) started to breed new garden chrysanthemums in 1961, hybridizing dwarf cultivars with wild species through mixed pollination or open pollination. After generations of hybridization and selection, groundcover chrysanthemums were developed, and many outstanding cultivars were introduced to urban landscapes (Chen et al., 1995, 2005; Wang and Chen, 1990). The distinguishing features of groundcover chrysanthemums are compact sizes, abundant flowers, long bloom duration, and rich colors. They are also highly drought-tolerant.

To breed desirable chrysanthemum cultivars, it is important to select appropriate parents that are beautiful and genetically diverse. In addition, in consideration of the self-incompatibility of most chrysanthemums, the crossability of parents is important for cross-breeding. Breeders would have much better success in creating new cultivars if they knew the genetic relatedness of the parents before initiating crosses (Saxena et al., 2010). Molecular technology has been widely used in analyzing genetic relationships and diversity. For example, random amplified polymorphic DNA (RAPD) markers were used to analyze 18 chrysanthemum cultivars (Chrysanthemum × grandiflora) (Qin et al., 2002). Miao et al. (2007) classified 85 popular chrysanthemum cultivars into six groups using intersimple sequence repeat analysis. In contrast, the AFLP technique is a much more highly polymorphic and more efficient method that has been widely used to detect genetic variation. Evolutionary relationships in the genus Chrysanthemum were analyzed by AFLPs (Zhou and Dai, 2002), and genetic variation in potted and garden chrysanthemum cultivars was also tested (Han et al., 2007; Liu et al., 2008; Wu et al., 2007). However, no reports of genetic variation between wild species and groundcover chrysanthemum cultivars have been published. This article reports the genetic relatedness of 12 wild species and 62 groundcover chrysanthemum cultivars as revealed through AFLP analyses.

Materials and Methods

Plant materials. A total of 74 accessions were assessed in this study, including eight wild species of Chrysanthemum, four wild species of Ajania pacifica (Nakai) K. Bremer & Humphries, Crossoptistem chinense (L.) Makino, Opisthopappus longilobus Shih, and O. taihagensis (Ling C. Shih) closely related to Chrysanthemum, and 62 groundcover cultivars of Chrysanthemum × morifolium with high ornamental value (Table 1). Chrysanthemum × morifolium have multiple evolutionary origins (Dai et al., 1998; Dowrick, 1952). Previous work suggested that C. indicum might be its primary ancestor (Fukai et al., 2002) with additional genetic contributions from C. vestitum and C. lavandulifolium (Zhou and Dai, 2002). These three species, as well as C. chaneitii and C. nankingense, were sampled as wild representatives of groundcover chrysanthemum ancestors. Ajania pacifica, Crossoptistem chinense, Opisthopappus longilobus, and O. taihagensis were included because they have been frequently hybridized with Chrysanthemum in recent years. Chrysanthemum indicum was obtained from Korea in July 2009 and the other wild species were received for publication 28 Dec. 2012. Accepted for publication 27 Feb. 2013.

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Table 1. Twelve wild species and 62 chrysanthemum (*Chrysanthemum × morifolium* Ramat.) cultivars used in this study and their parentage, origins (to be consistent, sources for collecting cultivars are presented as origins), and key characteristics.  

| Taxon Name | Origin | Parentage | Key characteristics |
|------------|--------|-----------|---------------------|
| **Ajania pacifica** (Nakai) K. Bremer & Humphries | Beijing, China | — | Subshrub; compact type; leaves adaxially green with silver margin; axially white, yellow capitulum |
| **Chrysanthemum chanetii** (Levl.) Shih | Beijing, China | — | Stem erect or slightly curved in lower part; loose type; white, pink or lavender ligulate flower, single |
| **C. indicum** (L.) Des Moul. | Hubei, China | — | Stem prostrate at base; yellow ligulate flower, single |
| **C. lavandulifolium** (Fisch. ex Trautv.) Beppu | Beijing, China | — | Creeping stem; dark green leaves turn red in late autumn; yellow ligulate flower, single |
| **C. nankingense** | Fujian, China | — | Dwarf; whole plant with sparse white trichome; yellow ligulate flower; single |
| **C. vestitum** (Hems.) Stapf | Henan, China | — | Dwarf; whole plant with sparse white trichome; yellow ligulate flower; single |
| **Opisthopappus longilobus** | Hebei, China | — | Dwarf; pinnatisect leaves; white ligulate flower narrowly linear; single; fragrant |
| **O. taihangensis** (Ling) C. Shih | Hebei, China | — | Dwarf; pinnatisect leaves; white ligulate flower narrowly linear; single; fragrant |
| **Chong Ban Zao Huang** | Beijing, China | Seedling of ‘Mei Ai Huang’ × ‘Mei Gui Hong’ | Dwarf; crimson ligulate flower, double |
| **Chong Yang Huang** | Beijing, China | Seedling of ‘Pu Di Jin’ × ‘Mei Ai Fen’ | Dwarf; yellow ligulate flower, double |
| **D11** | Beijing, China | Seedling of ‘Xiang Yu’ × ‘Pu Di Jin’ | Dwarf; yellow ligulate flower, double |
| **Die Ying** | Beijing, China | Seedling of ‘Ql5’ × ‘Si Ji Huang’ | Dwarf; light pink ligulate flower, double; light fragrant |
| **Dong Fang Hong** | Beijing, China | Seedling of ‘Qi Pao’ × ‘Fan Hua Si Jin’ | Dwarf; red ligulate flower, double; fragrant |
| **Fan Bai Lu’ | Beijing, China | Seedling of ‘Pu Di Jin’ × ‘Mei Yi’ | Dwarf; white ligulate flower, double |
| **Fan Hua Si Jin’ | Beijing, China | Seedling of ‘Pu Di Jin’ × ‘Mei Yi’ | Dwarf; white ligulate flower, double; fragrant |
| **Han Lu Huang’ | Beijing, China | Seedling of ‘Pu Di Jin’ × ‘Mei Yi’ | Dwarf; yellow ligulate flower, double; fragrant |
| **Huang Fu’ | Beijing, China | Seedling of ‘Pu Di Jin’ × ‘Mei Yi’ | Dwarf; yellow ligulate flower, double; fragrant |
| **Huang Jin Jia’ | Beijing, China | Seedling of ‘Dan Dan De Huang’ × ‘Zhu Hai Jin Xin’ | Dwarf; yellow ligulate flower, double; fragrant |
| **Jiao Yang Hong’ | Beijing, China | Seedling of ‘Qi Pao’ × ‘VL8’ | Dwarf; red ligulate flower, single; fragrant |
| **Jin Bu Huang’ | Beijing, China | ‘Mei Ai Fen’ open-pollinated | Dwarf; yellow ligulate flower, double; fragrant |
| **Jin Qian Zao’ | Beijing, China | ‘Mei Ai Fen’ open-pollinated | Dwarf; yellow ligulate flower, double; fragrant |
| **Jin Zhan’ | Beijing, China | ‘Mei Ai Fen’ open-pollinated | Dwarf; yellow ligulate flower, double; fragrant |
| **Jin Zhu’ | Beijing, China | ‘Mei Ai Fen’ open-pollinated | Dwarf; yellow ligulate flower, double; fragrant |

(Continued on next page)
| Taxon Name                  | Origin          | Parentage                                      | Key characteristics                                                                 |
|----------------------------|-----------------|-----------------------------------------------|--------------------------------------------------------------------------------------|
| 38 'Jing Qi'               | Beijing, China  | Seedling of 'Wan Xia' × 'Si Ji Huang'          | Dwarf; yellow ligulate flower adaxially red, double, fragrant                        |
| 39 'Jing Tian Hong'        | Beijing, China  | Seedling of 'VL8' × 'DC'                      | Compact type; dark red ligulate flower, double; light fragrant                        |
| 40 'JuZi Zhou'             | Beijing, China  | 'Mei Ai Fen' open-pollinated                 | Dwarf; orange ligulate flower, double                                                |
| 41 'Lian Yu'               | Beijing, China  | Seedling of 'Fan Hua Si Jin' × 'DC'           | Dwarf; red ligulate flower, double; fragrant                                          |
| 42 'Mao Xiang Yu'          | Beijing, China  | 'Xiang Yu' open-pollinated                    | Dwarf; white outer ligulate and light yellow inner tubular flowers, anemone-shaped; light fragrant |
| 43 'Mei Ai Fen'            | Beijing, China  | Unknown                                       | Dwarf; pink ligulate flower, double                                                 |
| 44 'Mei Ai Huang'          | Beijing, China  | 'Mei Ai Fen' open-pollinated                 | Dwarf; yellow ligulate flower, double                                               |
| 45 'Mei Ai Zi'             | Beijing, China  | 'Mei Ai Fen' open-pollinated                 | Dwarf; aubergine ligulate flower, double                                             |
| 46 'Mi Bai Zao'            | Beijing, China  | Unknown                                       | Dwarf; white ligulate flower, double                                                |
| 47 'Mi Ban Chu Long'       | Beijing, China  | Unknown                                       | Dwarf; pink ligulate flower, double                                                 |
| 48 'Mu Ai'                 | Beijing, China  | Unknown                                       | Dwarf; light and dark pink bicolor ligulate flower, anemone-shaped                   |
| 49 'Ni Shang'              | Beijing, China  | Seedling of 'Qi Pao' × 'Fan Hua Si Jin'       | Dwarf; aubergine ligulate flower, double; fragrant                                   |
| 50 'Nong Fen Zhao Xia'     | Beijing, China  | Seedling of 'Qi Pao' × 'Fan Hua Si Jin'       | Dwarf; purplish red ligulate flower, double; fragrant                                |
| 51 'Pu Di Fen Fei'         | Beijing, China  | Seedling of 'Xiang Yu' × 'Mao Xiang Yu'       | Dwarf; light and dark pink bicolor ligulate flower, anemone-shaped                   |
| 52 'Pu Di Fen Dai'         | Beijing, China  | Seedling of 'Wan Xia' × 'Zao Huang Jin'       | Dwarf; compact type; pink ligulate flower, double                                    |
| 53 'Pu Di Jin'             | Beijing, China  | 'Mei Ai Fen' open-pollinated                 | Dwarf; orange-yellow ligulate flower, double                                         |
| 54 'Qi Yue Tao Hua'        | Beijing, China  | Unknown                                       | Dwarf; light and dark pink bicolor ligulate flower, double                          |
| 55 'Qing Chun'             | Beijing, China  | Seedling of 'ZWYC' × 'Si Ji Huang'            | Dwarf; orange-yellow ligulate flower, anemone-shaped                                |
| 56 'QuShui'                | Beijing, China  | Unknown                                       | Dwarf; orange ligulate flower, single                                               |
| 57 'VL5'                   | Beijing, China  | Unknown                                       | Dwarf; pink ligulate flower, double; light fragrant                                 |
| 58 'Wei Xiang Fen Tuan'    | Beijing, China  | Seedling of 'Qi Pao' × 'Fan Hua Si Jin'       | Compact type; yellow ligulate flower, double; light fragrant                         |
| 59 'XA7'                   | Beijing, China  | Seedling of 'Huang Ying' × 'Pu Di Jin'        | Loose type; pink ligulate flower, double                                             |
| 60 'Xia Ri'                | Beijing, China  | Seedling of 'VL8' × 'Zao Huang Jin'           | Dwarf; red ligulate flower, double                                                  |
| 61 'Xian Hong'             | Beijing, China  | Unknown                                       | Dwarf; pink ligulate flower, double; fragrant                                       |
| 62 'Xiang Fei'             | Beijing, China  | Seedling of 'VL5' × 'Zao Huang Jin'           | Compact type; pink ligulate flower, double; fragrant                                |
| 63 'Xiang Yu'              | Australia       | Unknown                                       | Dwarf; light and dark pink bicolor ligulate flower, anemone-shaped                   |
| 64 'Xiao Yin Qiu'          | Beijing, China  | Unknown                                       | Dwarf; white ligulate flower, double                                                |
| 65 'Xin Hong'              | Beijing, China  | Unknown                                       | Dwarf; red ligulate flower, double                                                  |
| 66 'Yu Lin Long'           | Beijing, China  | Unknown                                       | Dwarf; white ligulate flower, double                                                |
| 67 'Yu Long'               | Beijing, China  | Unknown                                       | Loose type; white ligulate flower, double                                           |
| 68 'Yu Ren Mian'           | Beijing, China  | Unknown                                       | Dwarf; orange ligulate flower, double                                               |
| 69 'Yuan Guan Cheng'       | Beijing, China  | Unknown                                       | Compact type; orange-yellow ligulate flower, double                                 |
| 70 'Yuan Guan Huang'       | Beijing, China  | Unknown                                       | Compact type; orange-yellow ligulate flower, double                                 |
| 71 'Yue Yang Cheng'        | Beijing, China  | 'Jin Qian Zao' open-pollinated               | Compact type; orange-yellow ligulate flower, double                                 |
| 72 'Zao Huang Jin'         | Beijing, China  | Unknown                                       | Compact type; yellow ligulate flower, double                                        |
| 73 'Zi Rui Chang Duan He'   | Beijing, China  | Unknown                                       | Compact type; white outer ligulate flowers, purple and yellow inner tubular flowers, double |
| 74 'Zi Yun Qing Fang'      | Beijing, China  | Seedling of 'Xiang Yu' × 'Pu Di Jin'          | Dwarf; dark purple ligulate flower, double; light fragrant                           |

*Nos. 13 to 74 are cultivars of *Chrysanthemum × morifolium*. Plants are characterized as compact or loose based on the degree of stem scattering. Dwarf = plants less than 30 cm tall. Single = one whorl of ligulate flowers around the anthodium. Double = more than two to three whorls of ligulate flowers around the anthodium. Anemone-shaped = stamens in the center modified into petals.
collected from several provinces in China between 2005 and 2010 (Table 1, Nos. 1 to 12). Groundcover cultivars in this study included 53 cultivars bred and selected in Beijing between 2004 and 2011 and nine groundcover cultivars introduced earlier (Table 1, Nos. 13 to 74). All of these taxa were grown in both greenhouses and fields at the China National Engineering Research Center for Floriculture (Beijing, China).

**DNA extraction.** Fresh young leaves were collected from greenhouse-grown plants of each accession and dried in silica gel desiccant. Approximately 0.40 g silica gel-dried leaves of each sample were weighed and ground to a fine powder in liquid nitrogen using a pestle and mortar. Genomic DNA was extracted from the powder using the DNA Secure Plant Kit (Tiangen Biotech, Beijing, China) according to the manufacturer’s instructions. The quality and concentration of DNA were estimated with Smart Ladder (TaKaRa Biotechnology, Dalian, China) on a 1% agarose gel containing Gel Red (Biotium, China) according to the manufacturer’s instructions. The DNA of each sample was digested and performed as described by Vos et al. (1995).

**PCR amplification.** Polymerase chain reaction (PCR) primers were designed with the following criteria: (1) specific primer pairs spanned a minimum of 300 bp of genomic DNA of each accession and (2) the forward and reverse primers had opposite restriction sites (EcoR I and Mse I). A total of 452 AFLP bands across all plant samples were scored by visual inspection as present (1) or absent (0). There were 100-bp ladders on both sides of each gel as standards, and every polymorphic fragment was scored. Fragments smaller than 50 bp and larger than 600 bp were excluded from the analysis.

The similarity among plants was estimated with Jaccard’s similarity coefficient. Cluster analyses of similarity matrices were achieved by using the UPGMA in NTSYS-pc Version 2.1q (Rohlf, 2000).

**Results.** AFLP polymorphisms. Most selective E + 3/M+2 primer combinations yielded too few polymorphic bands. In contrast, E + 2/M+2 primers generated too many bands that overlapped and were difficult to distinguish. E + 3/M+2 and E + 2/M+3 combinations produced abundant amplification products with sufficient polymorphism. Ultimately, 19 primer combinations were selected to examine the 74 accessions of chrysanthemum. The 19 primer pairs produced a total of 452 AFLP bands across all plant samples (Table 3). There was wide variation in the number of polymorphic loci per primer pair, ranging from nine to 40 and averaging 23.8. Allele size ranged from 50 to 583 bp, which covered an adequate amount of the whole genome. The mean polymorphism rate was 71.5% with a range of 57.8% to 88.4%. The

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**Table 2. Sequences of oligonucleotide adaptors and primers used for amplified fragment length polymorphism analysis.**

| Name         | Sequence                                                                 |
|--------------|--------------------------------------------------------------------------|
| EcoRI        | 5′-CTCGTACTGGCTTCGAC-3′                                                  |
| MseI         | 3′-CTGAGCTGGTTGAT-5′                                                    |
| EcoRI-00     | 5′-GCTGGCTTGCAGTCTC-3′                                                  |
| MseI-00      | 3′-GTCGAATCTGGTAA-5′                                                    |
| EcoRI-NNN    | 5′-GTCGTCTTGCAGTCTC-3′                                                  |
| MseI-NNN     | 3′-GTCGCTGACAGATT-5′                                                    |

^{NNN} represents one to three random nucleotides.

| Primer combination | Number of polymorphic loci | Allele size range (bp) | Polymorphism rate (%) |
|--------------------|---------------------------|------------------------|-----------------------|
| E-AAG/M-CTG        | 9                         | 57–360                 | 62.3                  |
| E-ACA/M-CAG        | 27                        | 62–413                 | 63.9                  |
| E-ACC/M-CTG        | 24                        | 54–396                 | 73.9                  |
| E-ACG/M-CTG        | 23                        | 55–427                 | 86.7                  |
| E-ATT/M-ACC        | 16                        | 84–496                 | 66.3                  |
| E-ATA/M-CCC        | 12                        | 56–456                 | 87.5                  |
| E-ATA/M-CTG        | 14                        | 54–306                 | 79.1                  |
| E-ACA/M-CG         | 40                        | 55–343                 | 75.5                  |
| E-AGG/M-CG         | 19                        | 69–458                 | 74.7                  |
| E-ATG/M-CG         | 28                        | 74–428                 | 79.1                  |
| E-ATT/M-CC         | 19                        | 55–382                 | 70.6                  |
| E-AC/A-M-CTG       | 30                        | 59–376                 | 81.7                  |
| E-CCG/M-CAG        | 19                        | 66–564                 | 75.2                  |
| E-CT/M-CAG         | 26                        | 69–564                 | 66.7                  |
| E-GT/M-CTG         | 24                        | 50–361                 | 70.1                  |
| E-TA/M-CAG         | 40                        | 71–383                 | 88.4                  |
| E-TTT/M-CAG        | 32                        | 57–501                 | 80.5                  |
| E-TTT/M-CTG        | 34                        | 50–580                 | 76.4                  |
| E-CC/M-CG          | 16                        | 50–457                 | 75.8                  |
| Mean               | 23.8                      | —                      | 71.5                  |
| Total              | 452                       | 50–600                 | —                     |
primer combination E-TA/M-CAG had the highest polymorphism rate (88.4%) followed by E-ATA/M-CCC (87.5%) and E-ACG/M-CTG (86.7%). The lowest polymorphism rate was observed with primer combination E-CC/M-CG (57.8%), whereas E-AAG/M-CCC and E-ACA/M-CAG produced only 62.3% and 65.4% polymorphic bands, respectively.

Genetic diversity and cluster analysis within 12 wild accessions. Jaccard’s genetic similarity coefficients, calculated between accessions, varied from 0.66 to 0.82, and UPGMA cluster analysis resolved the genetic relationships among the 12 wild accessions (Fig. 1). The dendrogram divided the species into two main clusters split at a similarity of 0.66. Cluster A contained only one wild species from Fujian, *Crossostephium chinense*, whereas cluster B included the other 11 wild accessions collected in Korea and other provinces of China. Cluster B was further divided into six subclusters, from BI to BVI, at a similarity of 0.76. Furthermore, subcluster BI consisted of two subgroups, BI-I with *C. indicum* (two accessions) and *C. indicum var. aromaticum* and BI-II with *Opisthopappus longilobus* and *O. taihangensis*. *Chrysanthemum lavandulifolium var. seticuspe* and *C. nankingense* were grouped into subcluster BIV. The other four taxa, *A. pacifica*, *C. chanetii*, *C. lavandulifolium*, and *C. vestitum*, each comprised a monotypic subcluster.

The 12 accessions had different polymorphic fragments, indicating that the AFLP primer combinations had great discriminatory power and that the 12 wild species were highly variable. The dendrogram indicates that wild accessions were divided into different groups in accordance with both the geographic distributions and traditional classification.

Genetic diversity and cluster analysis within species and cultivars. As shown in Figure 2, Jaccard’s genetic similarity coefficient ranged from 0.64 to 0.89. All 74 accessions were divided into two clusters by UPGMA analysis. Cluster B contained only ‘Zi Rui Chang Duan He’, which appeared to be quite isolated from other accessions.

Cluster A contained the other 73 accessions and was divided into subclusters AI and AII at a similarity of 0.67. Subcluster AII contained only ‘Jin Qian Zao’, an old cultivar. Subcluster AI contained all 53 ground-cover cultivars and 12 wild species, indicating close relationships among them. This subcluster was further divided into groups AI-I and AI-II at a similarity of 0.68. ‘Han Lu Huang’ and ‘Chong Yang Huang’ were the most closely related accessions in this study and formed a small subgroup (Fig. 2) with ‘Mei Ai Zi’ and ‘Pu Di Jin’, these Beijing descendants of ‘Mei Ai Fen’ shared dwarf phenotypes with yellow, orange-yellow, or aubergine double flowers. There were four additional subgroups of closely related cultivars that shared parents and petal type. Subgroup 1 comprised ‘XA7’, ‘Dan Dan De Huang’, and ‘Dan Han Fen’, all dwarf or compact offspring of ‘Huang Ying’ × ‘Pu Di Jin’ with ligulate, double flowers. ‘Xiang Yu’ was the female parent of ‘Mao Xiang Yu’ and ‘Pu Di Dan Fen’, and these three cultivars with bicolor, anemone-shaped flowers formed subgroups 3, ‘Jing Qi’ and ‘Jin Zhan’, both descendants of ‘Wan Xia’ × ‘Si Ji Huang’, formed subgroup 4 with fragrant yellow, ligulate, double flowers. In subgroup 5, ‘Zao Huang Jin’ was the male parent of ‘Pu Di Fen Dai’. Finally, both ‘Jing Tian Hong’ and ‘Lian Yu’ (subgroup 6) had ‘DC’ as the male parent; both have red ligulate double flowers. In addition, all 53 ground-cover chrysanthemums were grouped with 12 wild species in subcluster AI, indicating a close relationship among them.

Discussion
Compared with traditional morphological identification, molecular markers are much more effective and reliable for analyzing genetic relationships and diversity. Morphological identification can be highly subjective and prone to human error. Molecular markers, on the other hand, provide objective and quantifiable data that can be used to accurately determine genetic relationships and diversity. This is particularly important in cases where traditional morphological identification is not possible or not accurate. Molecular markers can also be used to identify new cultivars and to track the spread of genetic material across different regions. This information can be used to inform conservation efforts and to improve the genetic diversity of the species.

![Image](image_url)
Fig. 2. Cluster analysis of 12 wild accessions and 62 *Chrysanthemum morifolium* cultivars based on the matrix of genetic similarity of amplified fragment length polymorphism markers. ▲ = wild species; ● = older cultivars of groundcover *Chrysanthemum*.
results were consistent with previous reports of the dendrogram of wild species. These re-
accessions of the total germplasm.

In this study, AFLP markers were used to evaluate the genetic relationships among wild species and cultivars of chrysanthemum. The results supported the conclusions of pre-
vious studies that the AFLPs could distin-
guish species and cultivars effectively, even when they had high similarity indices. The primer pairs used in this study had an average 71.5% polymorphic rate, whereas in a RAPD study of chrysanthemums by Dai et al. (1998), the markers had only 15% polymorphism. Other studies have also con-
cluded that AFLPs were a powerful tool for revealing genetic relationship in chrysanthemum cultivars (Han et al., 2007; Zhou and Dai, 2002). Obviously, the AFLP technique was an informative method for assaying genet-
ic diversity, genetic relationships, and cul-

tivar identity in chrysanthemums in this study.

The range of Jaccard’s similarity coeffi-
cients obtained from the AFLP data indicated that there was significant genetic diversity between chrysanthemum species and culti-
vars. It also indicated that there existed wide genetic variation among groundwork cover chrysanthemums. In this study, 62 groundwork chrysanthemums were analyzed, 30% of this group of cultivars. Ortiz et al. (1998) suggested that a core collection of 10% of the available Misao germplasm stored in vitro may contain most genetic diversity; our core collection represented a much larger fraction of the total germplasm.

The AFLP markers used in this study could clearly differentiate the wild species by geographic region and traditional taxo-
nomic delineation. For example, the three accessions of C. indicum clustered together, and the two accessions from Hubei, China, were more closely related. The two species of Opisthopappus also grouped together in the dendrogram of wild species. These re-
results were consistent with previous reports (Wu et al., 2007; Zhou and Dai, 2002; Zhu et al., 2011). However, the two accessions of C. lavidulifolium did not group together.

When AFLP data from the groundwork accessions were analyzed along with the wild species, the resulting dendrogram indi-
cated that most accessions were closely related both to other cultivars and to wild species. The older cultivars were the more closely related to wild species than were most recently bred groundwork cultivars. This finding was probably the result of the work of breeders, who created the oldest accessions by crossing cultivars with wild species, whereas the newer cultivars were mainly developed from older ones. The origin of C. morifolium remains controversial; how-
ever, the species is generally believed to be a hybrid complex derived from chance hybridization that occurred naturally among C. vestitum, C. indicum, C. lavidulifolium, and C. zawadskii Herbich (Chen et al., 1998; Dai et al., 1998; Wang et al., 2004). There-
fore, the older cultivars were closely related to these wild parents.

The dendrogram also indicated that rich genetic diversity exists in chrysanthemums. After generations of intraspecific hybridization, the group of groundcover chrysanthemums are heterogeneous and have diverse genetic background. Furthermore, more distant hybridization with wild spe-
cies can broaden the genetic base of chry-
santhemum cultivars. Consequently, AFLP markers can provide valuable molecular data for investigating genetic relationships among closely related groups to guide future crosses, although traditional methods of differenti-
ating varieties based on phenotypic charac-
ters should also be considered.

The results of this study were generally similar to those obtained by Miu et al. (2007) and Wu et al. (2007), which showed that genetic relationships within chrysanthemum were partly indicated by their petal types. The majority of cultivars had double flowers, but the accessions that shared anemone-
shaped petals, ‘Lu Di Dan Fen’, ‘Mao Xiang Yu’, and ‘Xiang Yu’, clustered together. Although flower color varies in chrysanthemums, there was no significant correlation between flower color and genetic as indicated by molecular markers. The relationships be-
tween the other morphological characters such as ligulate flower length, plant height, and flower diameter, and molecular markers need further testing and verification.

China is the center of chrysanthemum di-

erity with rich native germplasm resources. There are many species with desirable char-

acteristics such as prolonged florescence and resistance to drought, salinity, and dis-
 ease. These species have excellent potential to widen the genetic base for breeding new cultivars. Generally, parents that are more genetically distant would provide more var-

iation. However, genetic improvement of chrysanthemums is hampered by its genome complexity, high level of heterozygosity, and the occurrence of both inbreeding depres-
sion and self-incompatibility (Anderson and Ascher, 2000; Li et al., 2007; Xu et al., 2009). Understanding genetic relationships and di-

versity in chrysanthemum is critical. Our AFLP data will help breeders to choose appro-
priate parents while breeding new varieties.

In conclusion, this research showed that the AFLP technique is an efficient method for revealing genetic relationships and var-

tiation among chrysanthemum cultivars. Fur-

thermore, the results from this study will be useful for formulating effective breeding stra-
geries and characterizing germplasm. These re-

ults form the foundation for further analysis that will include more wild species to cover more chrysanthemum genetic diversity.

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