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A comparative analysis of genetic diversity in medicinal *Chrysanthemum morifolium* based on morphology, ISSR and SRAP markers

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ABSTRACT

The diversity and genetic relationship among 29 populations of *Chrysanthemum morifolium*, one of *Chrysanthemum indicum* and one of *Chrysanthemum nankingense* from China were analyzed using morphological traits and molecular markers. Twenty morphological traits were scored as well as 182 ISSR marker-fragments, as amplified by 22 primers [the percentage of polymorphic bands (PPB): 81.87%], and 243 SRAP marker-fragments as generated by 26 primer pairs (PPB: 75.72%). Mantel's test indicated significant correlation ($r = 0.624$) of morphological trait and SRAP. By contrast, the morphological trait showed low correlation with ISSR ($r = 0.246$). Cluster analysis showed groupings of the accessions according to all four methods correlated well with their geographic region of origin, and most populations from the south of China were classified into one cluster and most populations from the north of China were classified into another cluster. Finally, an appropriate strategy for conserving the *C. morifolium* germplasm was proposed.

1. Introduction

Various methods have been employed to evaluate genetic diversity. Morphological trait measurement is a commonly used index since it provides a simple technique of quantifying genetic variation while simultaneously assessing genotype performance under normal growing environments (Fu et al., 2008). Furthermore, measuring morphological traits is a technologically and financially low demand system of evaluating accessions. However, investigation of morphological traits is labor-intensive and the phenotypic plasticity of plants makes environmental variation a major problem (Van Beuningen and Busch, 1997). Molecular markers have a number of perceived advantages over the morphological measurement for the assessment of genetic diversity. Currently, ISSR and SRAP markers have been shown to be effective for DNA fingerprinting, genetic diversity analyses and germplasm evaluation (Hess et al., 2000; Li and Quiros, 2001; Qian et al., 2001; Budak et al., 2004; Ruiz and García-Martínez, 2005; Liu et al., 2008). *Chrysanthemum morifolium* is a common medicinal plant with notable curative effects on treating common cold of wind-heat, headache, and dizziness (Pharmacopoeia of the People's Republic of China, 2005). Pharmacological research indicates that extracts of *C. morifolium* influence cholesterol metabolism and have anti-bacterial, anti-virus, anti-inflammatory, anti-mutagenic and anti-neoplastic activities (Jaime, 2003; Liu et al., 2007; Deng et al., 2010a). *C. morifolium* can be divided into "Hang-ju", "Bo-ju", "Qi-ju", "Gong-ju", "Ji-ju", "Chu-ju", "Huai-ju" and "Chuan-ju" according to different growing regions and processing methods. In China, some cities or counties have become the main producing areas such as Tongxiang city from Zhejiang province, Yancheng city from Jiangsu province,

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Chuzhou and Sexian cities of An'hui province, Wuzhi county from Henan province. As one of the most important medicinal herbs, most studies to date have focused on breeding and cultivation technology (Mandal et al., 2000; Willits and Bailey, 2000; Deng et al., 2010b) and the effective ingredients and pharmacology of *C. morifolium* (Pinder et al., 1999; Clifford et al., 2005; Lin and James, 2009). Very little has been done on the genetic relationships among the different geographical populations of the species using molecular markers. For example, Xu et al. (2006) revealed a high genetic diversity of *C. morifolium* in several cultivated populations by RAPD markers; Lu et al. (2008) studied the genetic diversity and relationships of *C. morifolium* and divided the populations into two groups according to geographic origins by ISSR markers. However, the number of both the investigated populations and the molecular markers used in these studies was too small. In this survey, we evaluated genetic diversity among 29 *C. morifolium* populations obtained from diverse locations on the basis of 20 morphological traits and three molecular markers (ISSR, SRAP, ISSR + SRAP). Additionally, these four analyzing methods were compared to each other for the compatibility of the results and the suitability of the data sets for assessing genetic diversity in *C. morifolium* populations. Our objectives were to: (i) compare Morphology analysis and three molecular markers (ISSR, SRAP, ISSR + SRAP) of *C. morifolium*, and provide molecular data to assess genetic relationships among accessions; (ii) reveal the genetic diversity among populations; (iii) propose an appropriate conservation strategy of *C. morifolium*.

2. Materials and methods

2.1. Plant materials

A total of 31 germplasms, including samples from 29 populations of *C. morifolium*, one of *Chrysanthemum indicum* and one of *Chrysanthemum nankingense* were studied (Table 1 and Fig. 1). In each of the 31 populations, 12 individuals were selected randomly and about 5 g young and clean leaves per plant were sampled. Since *C. morifolium* is a clonal plant, in order to minimize collecting the ramet of the same clone, each individual sample from the same population was collected from different locations at least 150 m apart. In total, 372 samples were collected and immediately stored in liquid nitrogen for genomic DNA extraction.

2.2. Morphological traits

Twenty quantitative morphological traits were assessed across the populations. From these measurements, the mean, maximum, minimum, standard deviation (S.D.) and coefficient of variation (CV) were calculated for each of the 20

Table 1
Details of samples detected in the study.

Code	Original accessions	Abbreviation	Longitude (E)	Latitude (N)	Source	Voucher numbers
1	*C. morifolium* 'Hangju' (Zaoxiaoyangju)	Zxyj	120°32′	30°38′	Tongxiang, Zhejiang province	Shao2005-Tx001
2	*C. morifolium* 'Hangju' (Wanxiaoyangju)	Wxyj	120°32′	30°38′	Tongxiang, Zhejiang province	Shao2005-Tx002
3	*C. morifolium* 'Hangju' (Dayangju)	Dyj	120°32′	30°38′	Tongxiang, Zhejiang province	Shao2005-Tx003
4	*C. morifolium* 'Hangju' (Yizhongdabaiju)	Yzdbj	120°32′	30°38′	Tongxiang, Zhejiang province	Shao2005-Tx004
5	*C. morifolium* 'Hangju' (Xiaotanghuang)	Xth	120°32′	30°38′	Tongxiang, Zhejiang province	Shao2005-Tx005
6	*C. morifolium* 'Hangju' (Xiaobaiju)	Xbj	120°13′	33°38′	Yancheng, Jiangsu province	Guo2004-Yc001
7	*C. morifolium* 'Hangju' (Hongxinju)	Hxj	120°13′	33°38′	Yancheng Jiangsu province	Guo2004-Yc002
8	*C. morifolium* 'Hangju' (Dabaiju)	Dbj1	120°13′	33°38′	Yancheng, Jiangsu province	Guo2004-Yc003
9	*C. morifolium* 'Hangju' (Changbanju)	Cbj	120°13′	33°38′	Yancheng, Jiangsu province	Guo2004-Yc004
10	*C. morifolium* 'Hangju' (Huangju)	Hj	120°13′	33°38′	Yancheng, Jiangsu province	Guo2004-Yc005
11	*C. morifolium* 'Hangju' (Dahuangju)	Dhj	120°13′	33°38′	Yancheng, Jiangsu province	Guo2004-Yc006
12	*C. morifolium* 'Hangju' (Xiangyiju)	Xyj	120°32′	30°38′	Tongxiang, Zhejiang province	Guo2004-Tx006
13	*C. morifolium* 'Hangju' (No. 1 of Jinju)	Jj1	120°32′	30°38′	Tongxiang, Zhejiang province	Guo2004-Tx007
14	*C. morifolium* 'Hangju' (No. 2 of Jinju)	Jj2	120°32′	30°38′	Tongxiang, Zhejiang province	Guo2004-Tx008
15	*C. morifolium* 'Hangju' (No. 3 of Jinju)	Jj3	120°32′	30°38′	Tongxiang, Zhejiang province	Guo2004-Tx009
16	*C. morifolium* 'Hangju' (No. 4 of Jinju)	Jj4	120°32′	30°38′	Tongxiang, Zhejiang province	Guo2004-Tx010
17	*C. morifolium* 'Gongju' (Zaogongju)	Zgj	118°44′	29°88′	Sexian, An'hui province	Shao2005-Sx001
18	*C. morifolium* 'Gongju' (Wangongju)	Wgj	118°44′	29°88′	Sexian, An'hui province	Shao2005-Sx002
19	*C. morifolium* 'Gongju' (Huangyaoju)	Hyj	118°44′	29°88′	Sexian, An'hui province	Shao2005-Sx003
20	*C. morifolium* 'Boju' (Xiaoboju)	Xbj	115°47′	33°52′	Bozhou, An'hui province	Guo2004-Bz001
21	*C. morifolium* 'Boju' (Daboju)	Dbj	115°47′	33°52′	Bozhou, An'hui province	Guo2004-Bz002
22	*C. morifolium* 'Boju' (Tezhongboju)	Tzbj	115°47′	33°52′	Bozhou, An'hui province	Guo2004-Bz003
23	*C. morifolium* 'Huaiju' (Huaixiaobaiju)	Hxbj	113°12′	35°14′	Wuzhi, Henan province	Guo2004-Wz001
24	*C. morifolium* 'Huaiju' (Huaidabaiju)	Hdbj	113°12′	35°14′	Wuzhi, Henan province	Guo2004-Wz002
25	*C. morifolium* 'Huaiju' (Huaixiaohuangiju)	Hxhj	113°12′	35°14′	Wuzhi, Henan province	Guo2004-Wz003
26	*C .morifolium* 'Chuju'	Cj	118°31′	32°33′	Chuzhou, An'hui province	Shao2005-Cz001
27	*C. morifolium* 'Jiju'	Jj	116°33′	35°23′	Jiaxiang, Shandong province	Shao2005-Jx001
28	*C. morifolium* 'Qiju'	Qj	115°20′	38°24′	An'guo, Hebei province	Shao2005-Ag001
29	*C. morifolium* 'Machengju'	Mcj	115°01′	31°10′	Macheng, Hubei province	Shao2005-Mc001
30	*C. indicum* L.	Yj	118°46′	32°03′	Nanjing, Jiangsu province	Guo2004-Nj001
31	*C. nankingense* Hm.	Jhn	118°46′	32°03′	Nanjing, Jiangsu province	Guo2004-Nj002