

Evaluation of genetic diversity of *Chrysanthemum* germplasm resources based on floral phenotypic traits

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Abstract

Chrysanthemum germplasms are valued for their beauty as an ornamental plant and are increasingly recognized as a source of medicinal products. The floral traits of *Chrysanthemum* × *morifolium* are of considerable importance and exhibit substantial genetic diversity. However, systematic studies on floral trait phenotypic diversity in the genus *Chrysanthemum*, based on representative germplasm resources, are scarce. In this study, the diversity and variations of 14 floral-related phenotypic traits in 103 *Chrysanthemum* genus germplasm resources, collected from key *Chrysanthemum* origin and the main production areas of China, Japan, and Europe covering wild species and all application types of *C. morifolium* cultivars, were analyzed by using the coefficient of variation, Shannon-Wiener index, analysis of variance, and cluster analysis, and screened by correlation analysis, principal component analysis, and regression analysis. The results showed that *Chrysanthemum* accessions exhibited notable phenotypic variation and higher genetic diversity in floral traits. Comparison of cultivated and wild chrysanthemums revealed that the diversity indices of cultivated species were generally greater than those of wild-type, especially for petal number and ray floret length. Cluster analysis classified the 103 chrysanthemum accessions into five groups. Through principal component and stepwise regression analyses, eight traits were identified from 14, accounting for 95.3% of the total variation and can serve as a comprehensive evaluation index for chrysanthemum floral morphology. This study provides a systematic assessment of the diversity of floral traits in chrysanthemum germplasm resources, and lays a solid foundation for future chrysanthemum breeding, variety improvement, and conservation of genetic diversity.

Citation: Yuan Q, Liu R, Song X, Wang Y, Wu H, et al. 2025. Evaluation of genetic diversity of *Chrysanthemum* germplasm resources based on floral phenotypic traits. *Ornamental Plant Research* 5: e044 <https://doi.org/10.48130/opr-0025-0044>

Introduction

Chrysanthemum × *morifolium*, perennial herbs of the Asteraceae family and *Chrysanthemum* genus, originated in China, where cultivation records date back to the 15th century BC. They spread to Japan around the 8th century AD during the Tang Dynasty through cultural exchanges and reached Europe in the 17th century via the Silk Road and maritime trade^[1,2]. As one of China's top ten traditional flowers, and the world's four major cut flowers, their excellent floral traits enhance ornamental value, market competitiveness, and economic benefits in the floriculture industry^[3]. One of the critical endeavors undertaken by breeders involves delving into genetic variation, enhancing variety quality, and driving the advancement of their respective industries through the strategic exploitation of abundant plant germplasm resources^[4,5]. An important task of breeders is to study genetic variation, improve varieties, and ultimately promote their industries by utilizing the rich plant germplasm. As posited by Alcazar, the effective conservation and utilization of biodiversity are contingent upon an in-depth analysis and comprehensive assessment of extant biodiversity^[6]. Currently, researchers often employ a range of molecular marker techniques to explore the genetic diversity of chrysanthemums. These techniques encompass RAPD (Random Amplified Polymorphic DNA), CDDP (Copia-type Retrotransposon-Derived Polymorphism), SSR (Simple Sequence Repeat), SRAP (Sequence-Related Amplification Polymorphism), among others^[7–13]. Although molecular marker technology can more accurately reveal the genetic diversity of plants, it must

ultimately be combined with phenotypic trait analysis. This is because morphological methods are the most straightforward and fundamental approaches for studying plant genetic diversity^[14]. For instance, a study investigated eight qualitative traits and 14 quantitative traits related to growth and flower attributes across 54 chrysanthemum varieties. Principal component analysis indicated that the phenotypic coefficient of variation ranged from 10.49% (leaf length-to-width ratio) to 106.38% (the length of the corolla tube of ray florets), showing significant variation. Chen et al. collected 29 medicinal and tea chrysanthemum germplasm resources from Macheng, Hubei Province, China, and related provinces, and used variance analysis, correlation, principal component analysis, and cluster analysis. Their results demonstrated that the number of tertiary branches and the number of ligulate florets exhibited abundant genetic variation^[15]. In another study, 70 tea chrysanthemum samples were comprehensively evaluated based on 19 agronomic traits to identify superior germplasms. The results revealed that the agronomic traits with a diversity index greater than 2.00 were first-branch height, number of branches per plant, number of ligulate flower whorls, number of ligulate florets, tubular flower diameter, and number of tubular petals. These traits can serve as evaluation indicators for chrysanthemum variety and selection genetic improvement^[16].

Studying phenotypic trait genetic diversity is crucial for germplasm resource identification, evaluation, and protection^[17,18]. It helps determine local variety adaptability, mine important agronomic trait genes, and develop new genetic germplasms. However,

phenotypic traits are not independent. Uncovering relationships between them allows fewer traits to describe germplasm, enhancing breeding efficiency^[19]. *Chrysanthemum* have a diverse germplasm. Flower type and color are key focuses of appreciation, and flower type is influenced by ligulate florets^[2,20]. Previous research only analyzed single types and limited *Chrysanthemum* germplasm phenotypic traits. The genetic diversity of floral traits in germplasm resources of the genus *Chrysanthemum* has not been systematically studied. Thus, this study will systematically analyze floral traits (flower head diameter, flower center diameter, ligulate floret width and length, etc.) of 103 representative germplasm resources in the genus of *Chrysanthemum*, including wild species, disbud chrysanthemum, spray chrysanthemum, traditional chrysanthemum, and potted and ground-cover chrysanthemum cultivars. The goal is to reveal their diversity, clarify floral characteristics of different *chrysanthemum* germplasm resources, and provide a reference for efficient chrysanthemum germplasm resource utilization, genetic basis expansion, and new variety development.

Materials and methods

Plant materials

This study utilized 103 germplasm resources collected from various global origins of the genus of *Chrysanthemum*, as well as the main production areas in China, Japan, and Europe (Fig. 1a, Supplementary Table S1). The collection encompasses all application types of both wild and cultivated species within the genus *Chrysanthemum*. It includes 23 wild species, featuring significant species such as *C. indicum*, *C. lavandulifolium*, *C. vestitum*, and *C. naktongense*. Additionally, the collection consists of 18 disbud (*Disb*) *C. morifolium*, 13 spray (*Spra*) *C. morifolium*, 22 traditional *C. morifolium* (*Trad*), 25 potted and ground-cover (*PoGc*) *C. morifolium*, and two medicinal varieties of *C. morifolium*. These materials effectively represent the genetic diversity of the genus *Chrysanthemum* germplasm resources. All materials are preserved in the Chrysanthemum Germplasm Resource Nursery at the National Engineering Research Center for Floriculture. For specific samples, see Supplementary Table S1.

Data collection

For each germplasm accession, three biological replicates were performed. Each replicate comprised 10 sample plants, all of which were cultivated through cutting propagation techniques. After 15 d of root development, the cuttings were transferred to 21 cm diameter pots. The plants were arranged in random blocks and then cultivated in the greenhouse of the National Engineering Research Center for Floriculture, Beijing, China.

For the collection of color data, three flowering plants for each cultivar were randomly selected, and from each plant, three fully blooming flowers were randomly chosen. The L^* , a^* , and b^* values of the inner and outer of ligulate petals were then measured separately using a colorimeter (NF555, Nippon Denshoku Industries Co. Ltd., Japan) under light source D50/2° conditions^[21,22].

For the acquisition of floral traits, fully open flowers at the blooming stage were randomly selected. Photos were taken with the camera in a fixed-focus state, along with a scale. Subsequently, Image J software was used to precisely measure the floral-related traits, including flower head diameter (FD), pistil diameter (PD), ray florets width (RFW), and ray florets length (RFL) (Fig. 1b). For each trait, the average value was calculated based on the 54 measured data points.

Data analysis

Statistical analyses were performed using Excel 2021 and SPSS 27.0 software.

Diversity analysis: The Shannon-Wiener genetic diversity index and coefficient of variation for each trait were calculated. SPSS 27.0 (IBM, Inc., Armonk, NY, USA) was used for variance analysis, and the squared Euclidean distance method for cluster analysis to obtain an approximate matrix. Dendrograms of clustering results were plotted using Origin Pro 2024.

Shannon-Wiener genetic diversity index (H): Indicates trait diversity in a group using the formula: $H = -\sum_{i=1}^s P_i \ln P_i$ ($i = 1, 2, 3, 4 \dots n$). In the formula, s is the total number of materials, P_i is the percentage of materials in the i -th level of a trait relative to the total, and \ln denotes the natural logarithm.

The coefficient of variation (CV) was used to represent the degree of dispersion of a trait, according to the formula: $CV = \delta/\mu$, where δ is the standard deviation, and μ is the mean value.

Screening of phenotypic trait integrated evaluation index: Correlation and principal component analyses of phenotypic data were conducted using the method described by Khadivi et al.^[23]. Raw phenotypic data were standardized, and the fuzzy membership function method confined the function values of 14 phenotypic traits for 103 pear germplasm materials within the interval [0, 1]. The calculation formula is $X_{ij} = (Y_{ij} - Y_{jmin}) / (Y_{jmax} - Y_{jmin})$, where X_{ij} is the membership function value of the j th trait for the i th material, Y_{ij} is the measured value, and Y_{jmin} and Y_{jmax} are the minimum and maximum values of the j th trait across materials^[24].

The weight coefficient is the ratio of the eigenvalue of each principal component to the sum of the eigenvalues of the extracted principal components. The calculation formula is: $\lambda_k = B_k / \sum_{k=1}^n B_k$, where k denotes the extracted principal components, λ_k is the weight

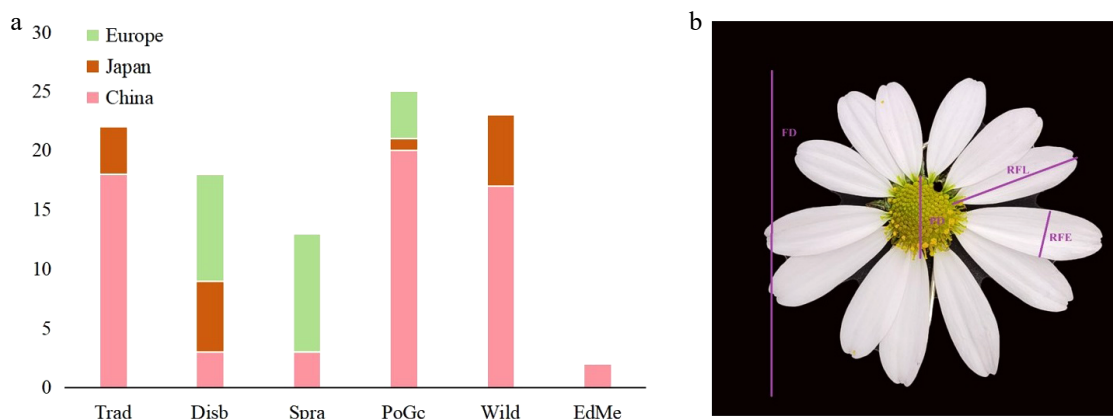


Fig. 1 (a) Distribution map of *Chrysanthemum* varieties across different regions. (b) Schematic diagram of measurement indicators.

coefficient of the k^{th} principal component, and B_k is the eigenvalue of the k -th principal component.

The coefficients of the principal components are calculated as eigenvector coefficients based on the eigenvalues and the loading matrix of the principal components. The calculation formula is:

$Z_{kj} = A_{kj}/B_k$, where Z_{kj} is the principal component coefficient of the j^{th} trait in the k^{th} principal component, and A_{kj} is the loading of the j^{th} trait in the k^{th} principal component^[25].

The principal component score for each trait is calculated as: $P_{ik} = Z_{kj} X_{aj}$, where P_{ik} is the principal component score of the k^{th} principal component for the i^{th} accession^[26,27]. The comprehensive score (F value) for each accession is then calculated as: $F_i = P_{ik} \lambda_{ik}$. Finally, comprehensive evaluation indices are selected through stepwise regression analysis.

Results

Genetic diversity of the *Chrysanthemum* germplasm

In diversity analysis and evaluation, the Shannon-Wiener index also reflects the diversity among varieties, which is influenced by the number of trait groups and the evenness of distribution of individuals within the groups. The higher the value, the richer the diversity of the trait^[28,29]. To investigate the genetic diversity of phenotypic traits, the Shannon-Wiener diversity index (H') was used

to compare the diversity of eight phenotypes of the *chrysanthemum* germplasm (Fig. 2a, b). The results showed that although the indices of the five chrysanthemum groups varied, the overall trend was similar. Notably, the RFL exhibited the lowest diversity index, whereas the a^* and b^* values related to both petal inner and outer color demonstrated higher indices, indicating greater diversity in these traits. Overall, the genetic diversity indices showed significant differences, ranging from 0.17 (RFL) to 1.72 (OC b^*). The average was 1.01. The traits with a diversity index exceeding 1.5 were as follows: the OC b^* of potted and ground-cover chrysanthemums, the IC a^* of wild chrysanthemums, the OC a^* of potted and ground-cover chrysanthemums, the IC a^* of spray chrysanthemums, and the b^* value of flower center color (PC b^*) of spray chrysanthemums. Their values were 1.72, 1.57, 1.53, 1.52, 1.52, and 1.52, respectively. This indicates that these traits have greater variation and richer phenotypic diversity compared to other traits. In addition, a comparative analysis of wild and cultivated species indicates that cultivated species exhibit a higher overall diversity index (Fig. 2c). Notably, the variation coefficients for petal number, RFL, and OC b^* in cultivated species were significantly higher than those in wild species.

Genetic variation of each trait

The coefficient of variation reflects inherent characteristics and individual differences, and is an indicator of genetic diversity. A higher coefficient of variation in a trait suggests a richer genetic

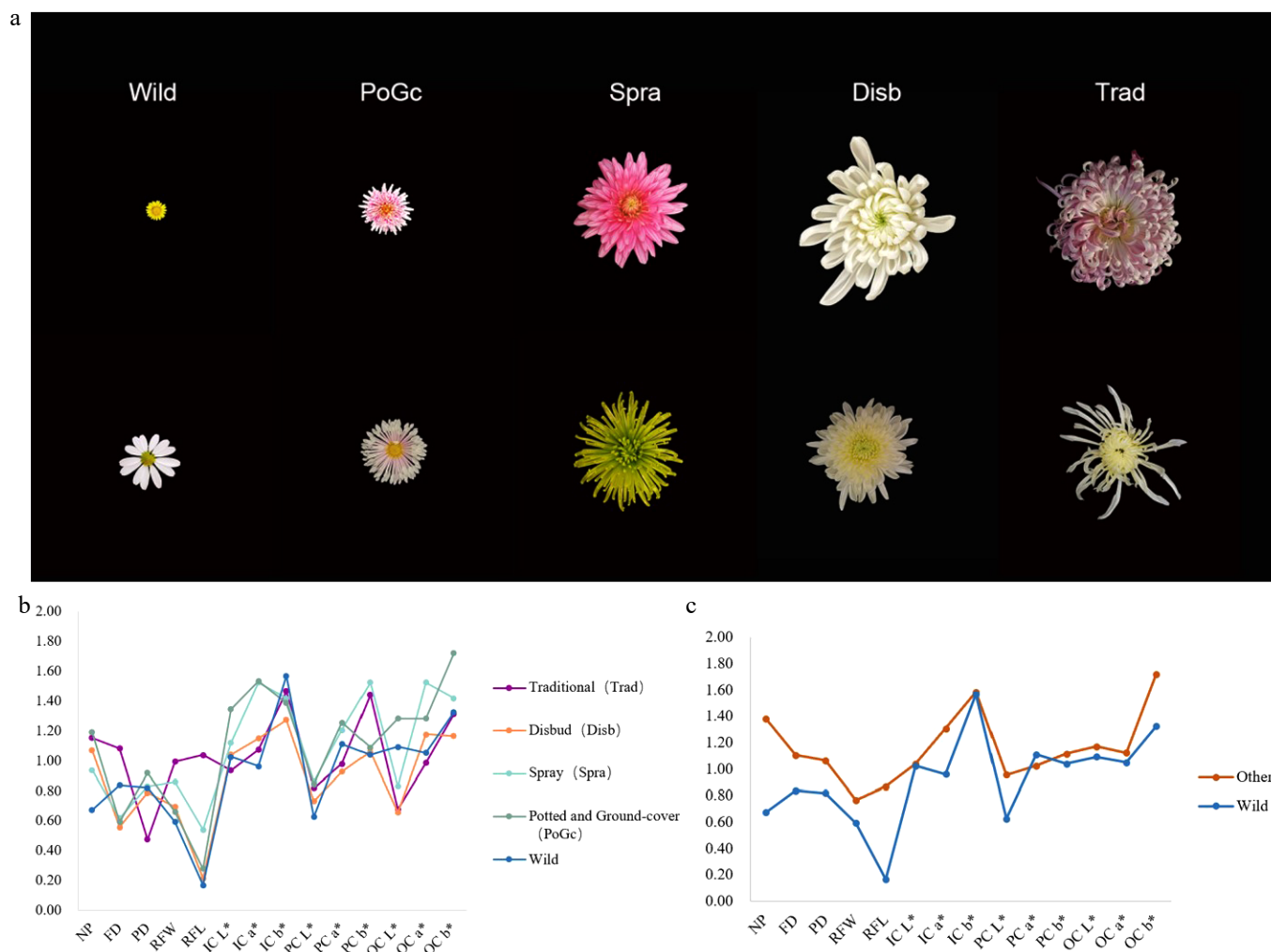


Fig. 2 (a) Different flower types of the chrysanthemum germplasm. (b) Shannon-Wiener of phenotypic traits of different types of chrysanthemum germplasm. (c) Shannon-Wiener of phenotypic traits of wild type and other types of chrysanthemum germplasm.

background, which is more conducive to variety identification^[30]. Tests and analyses were conducted on five floral traits of 103 chrysanthemum accessions. Table 1 shows the mean, standard deviation, and coefficient of variation for these five traits. Among the traits studied, chrysanthemum pistil diameter exhibited the greatest variation at 104.79%, indicating it better reflects differences among varieties.

To analyze differences between and within *chrysanthemum* populations, 103 varieties across six groups were analysed, including traditional, disbud, spray, potted and ground-cover, wild, edible and medicinal chrysanthemums. Nested analysis of variance (ANOVA) was used to assess variation in chrysanthemum floral quantitative traits at between—and within—group levels. Fourteen phenotypic traits showed highly significant variation between and within groups (Table 2), indicating widespread phenotypic variation. Mean and multiple comparisons revealed significant differences in 14 traits across the six groups (Table 2). For example, the number of petals (NP) was high in traditional and disbud chrysanthemums, whereas it was low in wild chrysanthemums; FD was larger in traditional chrysanthemums, whereas it was smaller in wild chrysanthemums; and the IC *a** value was larger in potted and groundcover chrysanthemums, whereas it was smaller in disbud chrysanthemums. The coefficient of variation for a trait can also vary across different populations (Table 3). Three cut-chrysanthemum groups had higher coefficients of variation for disk floret diameter at 236.65%, 180.53%, and 181.28%, indicating greater genetic diversity in traditional large and cut chrysanthemums. In contrast, wild chrysanthemums had the lowest coefficient of variation for petal number at just 25.47%, suggesting less diversity in this trait.

Cluster analysis based on six floral traits

Based on six floral traits (NP, FD, PD, RFW, RFL, and flower colour), 103 chrysanthemum germplasm accessions were classified into five groups using cluster analysis (Fig. 3). Group I comprised 24 accessions, predominantly wild and traditional chrysanthemums. These typically exhibited higher *b** than *a** values, with yellow observed in inner petals, pistils, and outer petals. Group II included potted and

ground-cover, traditional, wild, and spray chrysanthemums, characterized by small flower head diameter and pistil colors with *b** > *a**. Group III primarily consisted of disbud and wild chrysanthemums, displaying yellow or white petals, and green or yellow pistils. Group IV contained traditional and disbud chrysanthemums, featuring high petal counts and large flower head diameter. Group V was dominated by potted and ground-cover, and wild chrysanthemums, most exhibiting small flower head diameter.

Correlation analysis of six floral traits

Analysis of six floral traits across 103 *chrysanthemum* accessions revealed significant correlations (Fig. 4a), including: (1) NP exhibited negative correlations with PD and PC *b**, but positive correlations with OC *L**, RFL, and FD; (2) FD showed positive correlations with both RFL and RFW; (3) PD correlated negatively with RFL, IC *L**, and OC *L**, while positively with PC *a** and *b**; (4) RFW and RFL were mutually positively correlated yet both negatively correlated with IC *b** and OC *b**; (5) RFL demonstrated positive correlations with IC *L** and OC *L** but negative correlations with PC *a** and *b**; (6) Significant negative correlations emerged between *L** values of IC, OC and PC; (7) Within color parameters, *a**/*b** values among IC, OC, and PC displayed strong positive inter-correlations.

Principal component analysis (PCA) was conducted on six major phenotypes and 14 minor traits across 103 chrysanthemum accessions (Table 4). The first five principal components cumulatively accounted for 80.923% of the total variation, representing the majority of phenotypic trait variation in the core germplasm. The first principal component accounted for the largest proportion of variation, representing 30.353% of the total genetic information. This component was primarily defined by high and positive loadings of both IC *L** and OC *L**, indicating its strong association with petal lightness. The second principal component explained 20.075% of the contribution rate and was mainly characterized by high absolute loadings of OC *b** and IC *b**. The third principal component contributed 11.698% and was dominated by higher absolute loadings for the NP and PD, indicating that the third principal component is mainly determined by the structural traits.

Table 1. Analysis of genetic diversity for floral traits of *Chrysanthemum*.

Trait	Min.	Max.	Median	Mean	Standard difference	Coefficient of variation (CV; %)
Number of petals (NP)	11.00	528.43	108.22	137.77	111.83	81.17
Flower head diameter (FD; cm)	1.47	53.03	5.44	6.90	6.38	92.48
Pistil diameter (PD; cm)	0.00	2.24	0.53	0.55	0.57	104.79
Ray florets width (RFW; cm)	0.11	1.06	0.36	0.42	0.22	51.78
Ray florets length (RFL; cm)	0.43	8.42	2.05	2.60	1.77	68.06

Table 2. Comparative analysis of *Chrysanthemum* populations.

Trait	Trad	Disb	Spra	PoGc	EdMe	Wild	F
NP	202.82 ± 135.63	229.73 ± 71.94	181.66 ± 79.58	96.13 ± 62.42	174.78 ± 1.26	20.51 ± 5.22	20.27**
FD	13.67 ± 10.82	7.57 ± 1.86	6.42 ± 2.08	4.66 ± 1.18	3.58 ± 0.51	2.92 ± 0.98	11.78**
PD	0.29 ± 0.70	0.20 ± 0.36	0.32 ± 0.58	0.78 ± 0.45	0.00 ± 0.00	0.94 ± 0.33	8.43**
RFW	0.44 ± 0.21	0.50 ± 0.20	0.60 ± 0.24	0.35 ± 0.21	0.27 ± 0.05	0.31 ± 0.14	5.35**
RFL	4.98 ± 1.58	3.02 ± 1.17	2.27 ± 1.23	1.75 ± 0.62	1.50 ± 0.27	0.94 ± 0.40	40.18**
IC <i>L</i> *	76.40 ± 12.23	77.48 ± 18.70	61.86 ± 15.18	56.18 ± 22.05	74.95 ± 2.19	69.14 ± 11.41	5.41**
IC <i>a</i> *	2.97 ± 8.20	0.59 ± 7.16	9.91 ± 13.28	23.15 ± 26.34	6.25 ± 0.49	1.81 ± 2.75	7.73**
IC <i>b</i> *	10.78 ± 24.83	4.78 ± 31.46	-0.02 ± 32.37	7.12 ± 30.95	61.30 ± 1.84	23.17 ± 31.99	2.51*
PC <i>L</i> *	59.76 ± 10.61	63.48 ± 11.54	51.85 ± 11.64	60.38 ± 11.30	69.64 ± 3.38	57.09 ± 4.16	2.85*
PC <i>a</i> *	1.43 ± 7.85	-2.96 ± 2.81	-2.91 ± 8.94	8.67 ± 9.03	1.73 ± 2.04	5.89 ± 3.54	9.21**
PC <i>b</i> *	32.61 ± 22.43	12.61 ± 14.93	25.21 ± 19.19	48.76 ± 21.74	58.12 ± 11.76	51.34 ± 14.09	12.68**
OC <i>L</i> *	81.01 ± 9.14	83.22 ± 15.08	65.71 ± 15.34	60.78 ± 18.30	72.10 ± 2.12	67.07 ± 11.73	8.26**
OC <i>a</i> *	2.48 ± 6.48	0.52 ± 7.60	9.21 ± 13.25	14.70 ± 11.93	4.93 ± 0.01	1.42 ± 2.63	8.80**
OC <i>b</i> *	11.21 ± 24.78	0.45 ± 24.70	-2.02 ± 28.10	1.80 ± 24.74	38.30 ± 0.99	18.97 ± 25.62	2.62*

* *p* < 0.05; ** *p* < 0.01.

Table 3. Coefficients of variation of floral traits of the five populations of *Chrysanthemum* (CV; %).

Traits	Trad	Disb	Spra	PoGc	Wild
NP	66.87	31.32	43.81	64.93	25.47
FD	79.14	24.53	32.36	25.45	33.54
PD	236.65	180.53	181.28	57.62	34.76
RFW	47.09	39.33	39.52	58.62	46.48
RFL	31.67	38.86	45.47	35.39	42.95

The fourth principal component, with a contribution of 10.532%, showed higher loadings for PC a^* and PC L^* , indicating that it was mainly composed of the pistil colour. Finally, the fifth principal component accounted for 8.265%, with a high loading for FD, indicating this component is predominantly defined by FD.

The biplot was drawn by taking the first principal component as the X-axis and the second principal component as the Y-axis. The 103 *chrysanthemum* accessions were divided into four groups (Fig. 4b). The first group is mainly wild chrysanthemum, including Ind SNYJZ, Ind ZFDYJ, XNKN, etc; the second group is distributed in all but the wild type; the third group is mainly spray, and potted and ground-cover chrysanthemums, including HONG7, HDT, HLH, etc; and the fourth group consists of the traditional and disbud chrysanthemums, including ZYFB, QXSC, and GHYQD.

Screening of the comprehensive indices of phenotypic traits

Based on the eigenvalues and the load matrix of five principal components, the eigenvectors were calculated and used as the coefficients of each principal component. Based on the coefficients of each principal component and standardized phenotypic data, the calculation formula for the principal component score was obtained. The linear equation of the first principal component score was as follows:
$$P1 = 0.277U_1 + 0.196U_2 - 0.278U_3 + 0.161U_4 + 0.311U_5 + 0.383U_6 - 0.307U_7 - 0.076U_8 + 0.174U_9 - 0.251U_{10} - 0.306U_{11} + 0.401U_{12} - 0.291U_{13} - 0.079U_{14}$$

Since the five principal components can represent most of the information of the traits, five variables were used to replace the original 14 phenotypic traits to form a linear combination. The proportion of the eigenvalues corresponding to each principal component to the sum of the total eigenvalues of the extracted principal components was used as the weighting coefficients to obtain the mathematical model of the composite score: $F = 0.375P_1 + 0.248P_2 + 0.145P_3 + 0.130P_4 + 0.102P_5$. The comprehensive score (F value) was calculated to evaluate the phenotypic combination of various qualitative materials, with a higher F value indicating a more favorable combination of traits. Among the 103 accessions analyzed, the top five varieties ranked by F value were JZ, WJWW, RDSTH, QXSC, and

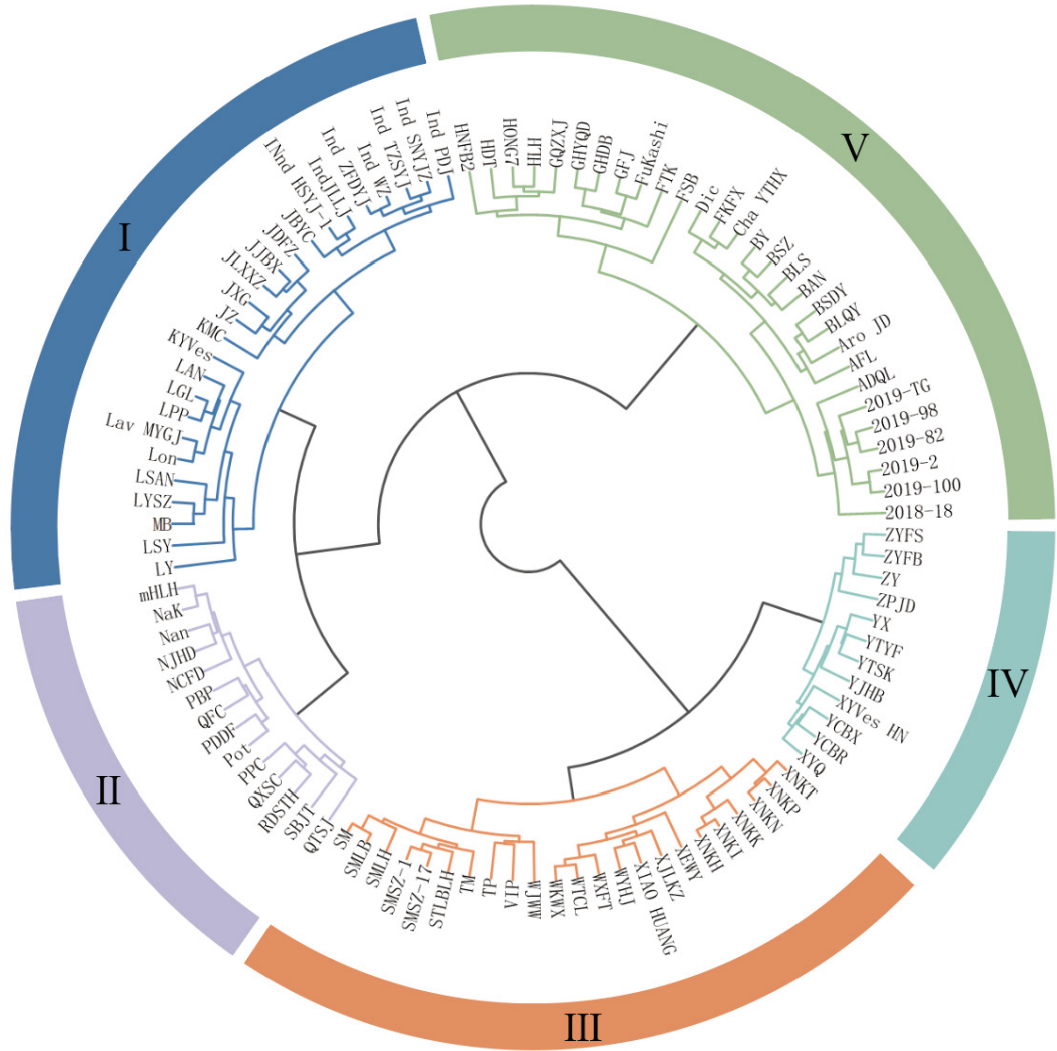


Fig. 3 Cluster analysis of 103 *Chrysanthemum* germplasm accessions.

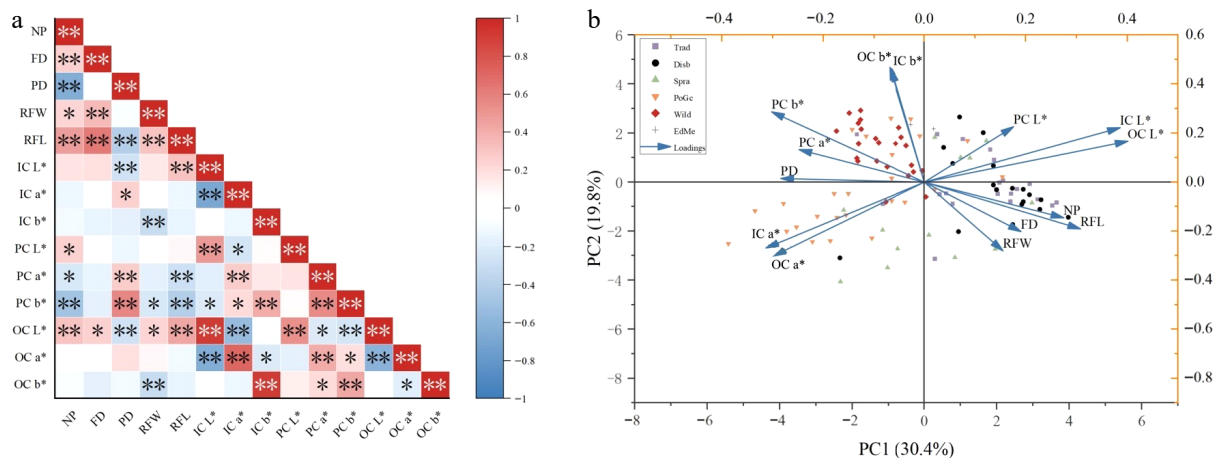


Fig. 4 (a) Heatmap of correlation analysis for 14 traits. (b) Biplot based on the principal component analysis of 103 *Chrysanthemum* accessions. Note: * means significant difference ($p < 0.05$); ** means extremely significant difference ($p < 0.01$).

Table 4. Eigenvector plot of PCA for 14 traits in *Chrysanthemum*.

Trait	PV1	PV2	PV3	PV4	PV5
NP	0.277	−0.146	0.411	0.154	−0.292
FD	0.196	−0.202	0.027	0.373	0.535
PD	−0.278	−0.004	−0.520	0.091	0.248
RFW	0.161	−0.276	−0.091	0.236	0.209
RFL	0.311	−0.189	0.193	0.265	0.272
IC L*	0.383	0.229	−0.276	0.091	−0.037
IC a*	−0.307	−0.279	0.155	0.200	−0.055
IC b*	−0.076	0.457	0.388	0.054	0.241
PC L*	0.174	0.228	−0.123	0.413	−0.461
PC a*	−0.251	0.129	−0.070	0.483	−0.248
PC b*	−0.306	0.279	−0.154	0.325	0.182
OC L*	0.401	0.168	−0.230	0.173	−0.052
OC a*	−0.291	−0.311	0.166	0.320	−0.183
OC b*	−0.079	0.464	0.374	0.104	0.208
E	4.249	2.811	1.638	1.474	1.157
CR %	30.353	20.075	11.698	10.532	8.265
CCR %	30.353	50.428	62.126	72.658	80.923

E: eigenvalues; CR: contribution rate; CCR: cumulative contribution rate.

ADQL. Correlation analysis based on the 14 traits and F values (Table 5) revealed that NP, FD, RFL, IC L*, IC b*, PC L*, OC L*, and OC b* were all significantly positively correlated with the composite F value. In addition, PD, IC a*, PC a*, PC b*, and OC a* also showed significant positive correlations with the F value. Conversely, PD, IC a*, PC b*, and OC a* were significantly negatively correlated with the F value. No significant correlation was found between RFW and the F value. Based on the calculated comprehensive F values and phenotypic traits, an optimal regression equation was constructed to screen evaluation indicators for comprehensive trait assessment of germplasm resources.

Taking the F value as the dependent variable and the 14 phenotypic traits as independent variables, the optimal regression equation was established by stepwise regression analysis as follows: $Y = -2.297 + 0.208x_1 + 0.100x_2 - 0.168x_5 + 0.524x_6 + 0.420x_8 + 0.099x_9 - 0.090x_{10} - 0.073x_{11}$, where the eight traits with their direct path coefficients given in parentheses: $x_1 = \text{NP}$ (0.208), $x_2 = \text{FD}$ (0.100), $x_5 = \text{RFL}$ (0.168), $x_6 = \text{IC L}^*$ (0.524), $x_8 = \text{IC b}^*$ (0.420), $x_9 = \text{PC L}^*$ (0.099), $x_{10} = \text{PC a}^*$ (−0.090), $x_{11} = \text{PC b}^*$ (−0.073). The correlation coefficient r and determination coefficient R^2 of the equation were 0.976** and 0.953, respectively, indicating that the eight phenotypic traits could determine 95.3% of the total variation. The F value was 243.533, and the regression equation reached a very significant

Table 5. Correlation coefficients (CC) between 14 phenotypic traits and the comprehensive value (F).

Trait	F	Trait	F
NP	0.472**	IC b*	0.359**
FD	0.340**	PC L*	0.470**
PD	−0.569**	PC a*	−0.287**
RFW	0.122	PC b*	−0.267**
RFL	0.538**	OC L*	0.794**
IC L*	0.782**	OC a*	−0.656**
IC a*	−0.670**	OC b*	0.361**

* $p < 0.05$; ** $p < 0.01$.

level, indicating that these eight phenotypic traits could be used as comprehensive evaluation indices of *chrysanthemum* germplasm resources. These results indicated that the eight floral traits (NP, FD, RFL, IC L*, IC b*, PC L*, PC a*, and PC b*) significantly influence the overall value of floral traits in *Chrysanthemum* plants. These indicators can be utilized as comprehensive evaluative measures for the floral morphology of *Chrysanthemum* species.

Discussion

Genetic diversity of floral traits in *Chrysanthemum* germplasm resources

Genetic diversity represents the fundamental basis for resource evaluation and utilization, providing essential insights for gene discovery in genetic resources. Phenotypic traits, while serving as a critical foundation for breeders conducting chrysanthemum improvement programs, also constitute a core reference for researchers investigating the molecular mechanisms underlying complex traits[31,32]. To facilitate effective utilization and innovation of chrysanthemum germplasm, genetic diversity within its phenotypic traits was quantified using the Shannon-Wiener diversity index. This study revealed that while genetic diversity levels vary across phenotypic traits in chrysanthemums, a similar trend emerged across different types. Both inner and outer chrysanthemum petals exhibited high diversity indices and substantial color variation, spanning white, yellow, pink, purple, green, red, muddy gold, and inky hues. Notably, many varieties displayed bicolored petals, producing complex variegation. Among the diversity indices greater than 1.5 were OC b* (1.72) for spray chrysanthemums, IC b* (1.57) for wild chrysanthemums, OC a* (1.53) for potted and

ground-cover chrysanthemums, IC a^* (1.52) for spray chrysanthemums, PC b^* (1.52) for spray chrysanthemums, and OC a^* (1.52) for spray chrysanthemums. It indicated that the phenotypic variation of *chrysanthemum* germplasm resources had rich genetic diversity. Comparing the diversity indices of cultivated species and wild type, it was found that each trait of cultivated species was basically higher than that of wild type, indicating that cultivated species were more genetically diverse, especially in the number of petals and the length of the ray floret.

Cluster analysis results indicated that group I comprised varieties with monochromatic petals, predominantly yellow, where the L^* values of the petals were significantly higher than the a^* and b^* values. Group II included varieties characterized by small flower diameters and a yellow pistil. Group III consisted of varieties with similarly small flower diameters, mostly white or yellow petals, and featured either single or semi-double petals. Group IV was distinguished by large flower diameters and a high petal count, with most types being double-petaled. Varieties in Group V also had small flower diameters and were primarily single-petaled or semi-double-petaled. Overall, the 103 chrysanthemum varieties exhibited a high diversity index in petal color, although the actual variation was relatively low, implying that long-term selection has led to a general preference for and acceptance of brightly colored flowers. The high diversity indices for a^* and b^* values indicate rich flower color variation among chrysanthemums, likely driven by diverse aesthetic preferences. Furthermore, comparisons between cultivated and wild chrysanthemums revealed that cultivated varieties generally possessed higher diversity indices and coefficients of variation, suggesting that cultivated chrysanthemums have abundant genetic variation and broader phenotypic differences than wild types. The greater diversity of floral traits observed in cultivated chrysanthemums is likely significantly influenced by artificial selection and breeding practices. Additionally, factors such as the plant's self-incompatibility, high hybrid vigor, gene flow, and the common occurrence of polyploidy in cultivated varieties also play a role in this diversity^[33,34].

Screening of comprehensive indicators for phenotypic traits in *Chrysanthemum* germplasm resources

There are fewer studies on the screening of comprehensive indicators of phenotypic traits in chrysanthemum among the published studies on chrysanthemum core resources and genetic diversity^[35,36]. The Membership Function Method and Principal Component Analysis (PCA) was employed to identify integrated indices for evaluating phenotypic traits in chrysanthemum germplasm. This methodology has been previously applied in genetic diversity assessments of other plant species^[36,37]. The initial eigenvalues and cumulative contribution rate of principal components served as the basis for component selection, where eigenvalues > 1 were adopted as the standard extraction criterion^[38]. This study analyzed 14 phenotypic traits of chrysanthemum. The results showed that the initial eigenvalues of the first five principal components exceeded 1, with a cumulative contribution rate of 80.923%. The first principal component had the highest contribution rate at 30.353%, representing traits related to petal color brightness, highlighting the importance of these characteristics in floral trait evaluation and genetic studies of *chrysanthemum*. The F value of the comprehensive phenotypic trait score was subsequently used to evaluate the overall performance of various qualitative resources, providing breeders and researchers with an intuitive, convenient, and quantitative reference^[39]. The results indicated that the variety 'Jin Zun', classified as both a potted and groundcover chrysanthemum, had the highest composite value, while 'Lian Yu', also

categorized as potted and groundcover, had the lowest value. Therefore, combining these varieties could contribute to constructing a research population rich in genetic diversity. Correlation analysis revealed a significant positive correlation between the composite F value and traits such as NP, FD, RFL, IC L^* , IC b^* , PC L^* , OC L^* , and OC b^* . Higher composite values corresponded to elevated levels of these traits, thereby offering valuable guidance for selecting parental lines in breeding programs. Given the diverse phenotypic traits in *chrysanthemum* floral characteristics and their varying degrees of correlation, it is crucial to identify a minimal set of representative traits that capture the full spectrum of diversity. Through principal component analysis and stepwise regression analysis, this study integrated and distilled 14 phenotypic traits into eight core traits (NP, FD, RFL, IC L^* , IC b^* , PC L^* , PC a^* , PC b^*). This streamlined set serves as a comprehensive evaluation index system for *chrysanthemum* germplasm resources, retaining maximal phenotypic information, while reducing dimensionality.

Phenotyping has become one of the important directions of modern biological research. Without phenotypic data to complement genomic, transcriptomic, and other omics datasets, it remains challenging to systematically decipher the formation mechanisms and intrinsic principles governing breeding traits. In-depth analysis of phenomic data, combined with comprehensive exploitation of its biological value will significantly accelerate breeding progress.

Conclusions

The diversity of the genus of *Chrysanthemum* germplasm was assessed using 14 floral-related phenotypic traits across 103 accessions. The results revealed a high level of phenotypic diversity, with substantial variation among individual traits, although the overall patterns of variation were similar among different chrysanthemum types. Among all quantitative traits, PD exhibited the highest coefficient of variation, providing the most evidence for differences among chrysanthemum varieties. Cluster analysis grouped the 103 chrysanthemum accessions into five distinct clusters. Through principal component analysis and stepwise regression, eight representative traits (NP, FD, RFL, IC L^* , IC b^* , PC L^* , PC a^* , and PC b^*) were selected from the original 14 traits. Together, these eight traits accounted for 95.3% of the total phenotypic variation and may serve as comprehensive evaluation indices for chrysanthemum germplasm resources. This study enabled a better understanding of the genetic diversity of chrysanthemums, thus providing data to support the selection of new varieties and the development of breeding strategies.

Author contributions

The authors confirm their contributions to the paper as follows: conceived and designed the experiments: Yuan C, Cheng T, Zhang Q; contributed to the experiments: Yuan Q, Liu R, Song X, Wang Y, Wu H, Jiang Y, Sun T, Liu H, Cong T; analyzed the data and prepared the original draft: Yuan Q; reviewed and edited the paper: Yuan C. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

This work was supported by the National Key Research and Development Project of China (2023YFD1200105), the Fundamental Research Funds for the Central Universities (QNTD202503), the Science and Technology Innovation Project of the Xiongan New Area (2022XAGG0100).

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary information accompanies this paper at (<https://www.maxapress.com/article/doi/10.48130/opr-0025-0044>)

Dates

Received 15 August 2025; Revised 3 October 2025; Accepted 17 October 2025; Published online 27 November 2025

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