

Quantitative Classification of the Morphological Traits of Ray Florets in Large-flowered Chrysanthemum

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Abstract. The large-flowered Chinese chrysanthemum is one of the most morphologically complex ornamental plants, and its identification and classification requires a well-defined and reproducible system. The diversity of the capitulum is determined mainly by multiple shapes of ray florets. However, the existing classification systems for ray floret types are incomplete and unsystematic. In this study, 299 ray florets from 151 large-flowered chrysanthemum varieties in China, as well as 12 related traits of ray florets, were selected for quantitative classification. First, as one of the most important indices of ray floret shape, the corolla tube merged degree (CTMD) was defined as the corolla tube length/ray floret length (CTL/RFL). Combined with a probability grading method and linear regression analysis, the CTMD was divided into three groups, flat, spoon, and tubular, of which the CTL/RFL ranged from 0 to 0.20, 0.20 to 0.60, and 0.60 to 1.00, respectively. Second, Q-mode cluster analysis indicated that each group could be further categorized into three types (straight, curved, and atypical), based on other important variables in the ray floret. Finally, the ray floret was classified into nine types, including flat-straight, flat-curve, flat-atypical, spoon-straight, spoon-curve, spoon-atypical, tubular-straight, tubular-curve, and tubular-atypical. This ray floret classification system will be valuable in the classification of capitulum shape and has significance for the identification, breeding, and international standardization of chrysanthemum cultivars.

Chrysanthemum [*C. ×grandiflorum* Tzvelv. (= *C. ×morifolium* Ramat.)] is a valuable ornamental and commercial crop, and ≈20,000–30,000 cultivars have been developed date (Zhang and Dai, 2013). The outer ray florets and inner disc florets represent the basic elements of the chrysanthemum capitulum, with the former being more abundant in shape. Accordingly, there are many different flower types of chrysanthemum (Ackerson, 1957; Crook, 1942; Meek, 1968; Zhang et al., 2013), and the classification of chrysanthemum

cultivars is based mainly on the flower type. Meanwhile, the flower type of chrysanthemum is affected by the ray floret shape, ray floret orientation, and the number of ray florets (Ackerson, 1957; Anderson, 2006), especially ray floret shape (Zhang et al., 2014). However, existing classification systems for ray floret types are incomplete and unsystematic, and the identification and classification of the phenotypically complex cultivars would benefit from a well-defined system. The Chinese large-flowered chrysanthemum not only attracts the interest of breeders but is also an important potted, cut, and garden ornamental plant for the Chinese market (Zhang et al., 2013). It represents the most abundant group of chrysanthemums, with from dozens to thousands of ray florets with different shapes on the capitulum (Fig. 1). Because of the complexity variation of the ray floret, to define them accurately is very important for the study of chrysanthemum cultivar classification.

To aid in the classification, we defined the distance from the tip of corolla tube to the bottom of ray floret as CTL and defined the ratio of CTL to RFL as CTMD (Fig. 2). We

included the use of CTMD as a morphological index to aid in defining petal type. In general, the ray floret shape of chrysanthemum is referred to as petal type and includes not only the CTMD but also the bending posture of the ray floret, the shape of the tip of ray floret, and aristate on the abaxial surface of the ray floret. Previous studies have only examined the variation in CTMD to classify ray floret shape. For example, Dejong and Drennan (1984) used CTMD to classify petal type into five types: flat, spoon I, spoon II, spoon III, and tubular degree, whereas Anderson (2006) divided the petal type into three types (flat, spoon, and tubular). Other studies have classified the Chinese large-flowered chrysanthemum petal type into five types (flat, spoon, tubular, atypical, and anemone), whereas the flower head type was further divided into 30 forms based on the aforementioned petal type (Chinese Chrysanthemum Society, 1993). And then, Zhang et al. (2014) suggested that the petal type be divided into four types (flat-spoon, tubular, atypical, and anemone type) based on 735 Chinese large-flowered chrysanthemum cultivars. In other words, the differences between the chrysanthemum varieties are mainly based on the variation of CTMD. In addition, the Chinese large-flowered chrysanthemums still have atypical type (refers to appearing aristate on the abaxial surface of ray floret or atypical variations on the tip of ray floret) and anemone type (refers to the change of disc florets). However, almost all the existing ray floret classification systems have ignored other important aspects of the variation in ray florets, such as the bending posture of ray floret, which occur among Chinese large-flowered chrysanthemums. Moreover, until now, most of the classification studies of CTMD have been based on direct observation of morphological characters but lack uniform measurement and classification criteria. The measurement of CTMD and classification of ray florets would therefore benefit from a well-defined and uniform standard.

An important method in plant classification is multivariate statistical analysis, which represents a comprehensive method to assess statistical patterns involving multiple objects and includes variance analysis, regression analysis, correlation analysis, discriminant analysis, and cluster analysis (Kachigan, 1991). At the beginning of the last century, many biostatisticians started to apply multivariate statistical analysis to taxonomic studies (Alpatov and Boschko-Stepanenko, 1928; Anderson and Abbe, 1934; Forbes, 1933; Pearson, 1926; Smirnov, 1925); since then, plant classification research has progressed from qualitative descriptions to more precise quantitative analysis (Gu et al., 2017). These methods also have been widely applied in the classification of ornamental cultivars (Borba et al., 2002; Chang et al., 2014; Dai and Zhong, 1995; Du et al., 2018; Generoso et al., 2016; Lopez Laphitz and Semple, 2015; Maiti et al., 2016; Osman, 2011;

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Fig. 1. Different shapes of the capitulum (consisting of different ray floret shapes) among the large-flowered Chinese chrysanthemums.

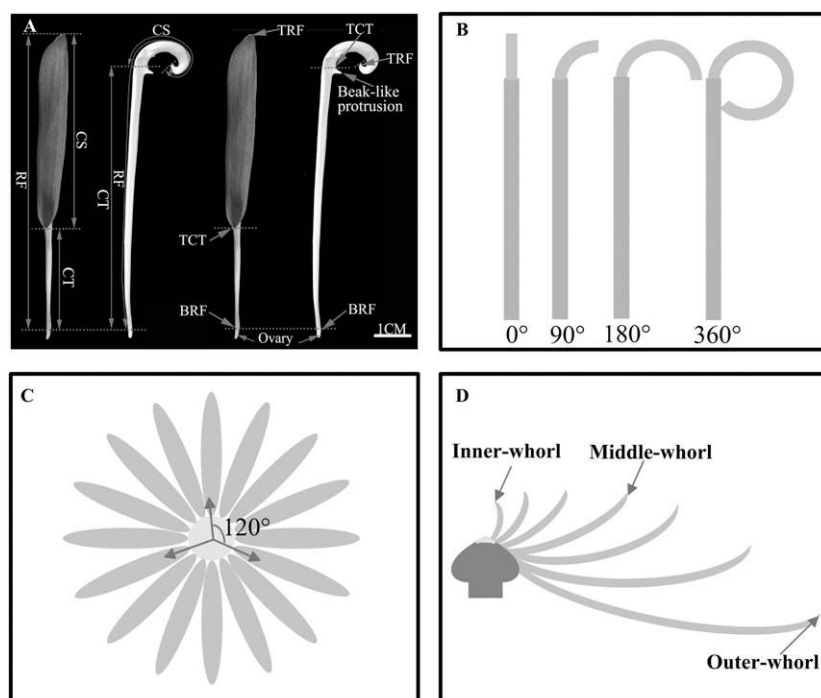


Fig. 2. Measured morphological traits of the ray floret and the capitulum. (A) Measured position of ray floret. (B) Bending angle of corolla splitting. (C) Capitulum transection. (D) Capitulum section. RF = ray floret; CS = corolla splitting; CT = corolla tube; TCT = tip of corolla tube; BRF = bottom of ray floret; TRF = tip of ray floret. Scale bar = 1 cm.

Yoshioka et al., 2005; Zhang et al., 2016, 2018; Zhong and Zhang, 1987). Quantitative traits also can provide the foundation for evaluating germplasm, and variation in quantitative traits can facilitate classification (Zhao et al., 2013). Luo et al. (2016) used probability grading to analyze 13 known quantitative characters of 400 Chinese large flower chrysanthemum cultivars, resulting in a new grading standard for complex quantitative characters of chrysanthemum. However, such a method has not been used to date for the classification of ray florets.

In this current study, we redefined some new traits in Chinese large-flowered chrysanthemum that describe the whole shape of the ray floret, developed a method for measuring of CTMD and other morphological variation in ray florets, and established a new classification system for ray floret shape. The overall goal was to create a standard for the identification and classification of Chinese large-flowered chrysanthemum cultivars. This research also may provide a useful reference for the classification of other ornamental plants.

Materials and Methods

Plant material. A total of 299 ray florets from 151 Chinese large-flowered chrysanthemum cultivars, grown in the Nursery of the Beijing Forestry University, Beijing, China, were selected from 2013 to 2015, from a collection of 880 cultivars that were morphological stable over a 5-year period (Supplemental Table 1). Robust cutting slips with four to five leaves (6–7 cm in length) were cut from mother plants in May and then inserted in plugs (turf: vermiculite = 3:1, v/v). Plantlets with strong roots were transferred to pots (21 cm diameter) after 25 d and then placed a raised land. During this period of growth, standard protocols for water and fertilizer application and disease and insect pest control were adopted. The daily mean temperature 20 ± 2 °C, the mean humidity was 65%, and the soil pH was 6.00–7.90. When the stem was 25 cm in length, the daylength was 13.50 h, and the minimum air temperature was 15 ± 2 °C. The chrysanthemums blossomed in October.

Character selection and measuring methods. Chrysanthemum flower development was divided into five stages: the flower bud is open while the ray florets are not yet visible (S1); the flower bud is open, and the tips of the ray florets are visible (S2); the outer ray florets are horizontally elongated (S3); the inner ray florets begin to elongate (S4); and disc florets are fully opened and the ray floret color begins to fade (S5) (Preece and Wilcox, 1966). The measurements described to follow were mainly made in the S4 stage. We measured some new traits that could be used to describe the whole shape of the ray floret (Table 1; Fig. 2A and B), using three plants that were selected randomly from each cultivar. A ray floret located every 120° in the outer whorl (or middle-whorl) was selected (Fig. 2C and D), and the average of the three ray florets was calculated as the representative of the outer whorl (or middle-whorl). The entire whorl of the chrysanthemum was measured if it had only one or two whorls.

Data analysis method. The cv (cv% = mean value/SD) of morphological characters was calculated, and the Shannon-Wiener diversity index (H') (Zhang et al., 2014) of morphological characters was calculated to analyze variation in qualitative characters. The Kolmogorov-Smirnov test (K-S test) was used to verify whether the quantitative traits were normally distributed and might therefore facilitate the classification. The values of normally distributed quantitative characters could be divided into five grades using four dividing points: $(\bar{X} - 1.2818 \times S)$, $(\bar{X} - 0.5246 \times S)$, $(\bar{X} + 0.5246 \times S)$, and $(\bar{X} + 1.2818 \times S)$ (\bar{X} = mean value, S = standard deviation). The probability of the grades 1 to 5 was 10%, 20%, 40%, 20%, and 10%, respectively (Liu, 1996). Linear regression was carried out, and coefficient of determination (R^2) values were used to assess the relationships between two traits. The Fisher discriminant function (Fisher, 1940) was used to test and

Table 1. Morphological characters of ray florets selected in the current study and methods for their assessment. T1, T4, T5-T12 are based on the Ministry of Agriculture of the People's Republic of China (2002) and Zhang et al. (2014). T2 and T3 are based on Dejong and Drennan (1984), where the corolla tube merged degree (CTMD) was described as the percentage of corolla split in the ray floret.

No.	Traits	Abbreviation	Measuring and assessing methods
T1	Ray floret length (cm)	RFL	The maximal length from the bottom of ray floret (BRF) to the tip of ray floret (TRF) (Fig. 2A)
T2	Corolla tube length (cm)	CTL	The length from the tip of corolla tube (TCT) to the bottom of ray floret (BRF) (Fig. 2A)
T3	Corolla tube merged degree	CTMD	Ratio of corolla tube length and ray florets length (CTL/RFL or CTL-RFL ratio)
T4	Ray floret width (cm)	RFW	The maximal width of corolla split (in the flat and spoon types) or perimeter of corolla tube (in the tubular type)
T5	Bending angle of corolla splitting (°)	BACS	(0) No corolla splitting or 0 (1) < 0 (2) 0<≤180 (3) 180<≤360 (4) >360 (Fig. 2B)
T6	Bending posture of corolla splitting	BPCS	(0) No corolla splitting (1) Straight (2) Evaginable (3) Twisty (4) Incurvate (5) Else
T7	Bending posture of ray floret	BPRF	(1) Straight (2) Evaginable (3) Twisty (4) Incurvate (5) Else
T8	Curly state of edge	CSE	(0) No curl (1) Inward curl (2) Outward curl
T9	Closed or not on the tip of ray floret	CNTRF	(0) Open (1) Closed
T10	Beak-like protrusion on the tip of corolla tube	BPTCT	(0) No (1) Yes
T11	Shape on the tip of ray floret	STRF	(1) Normal, including cuspidal, round, and jagged (2) Unguiculate (3) Lacerated (4) Asymmetric quinquepartite
T12	Aristate on the abaxial surface of ray floret	AASRF	(0) No (1) Yes

verify the classification of the CTMD. Pearson's correlation analysis (Pearson, 1986) was carried out to analyze the relationships among different characters. Cluster analysis (Q-mode cluster analysis) of each CTMD was carried out to analyze the relationships among the sample studied, and the cluster analysis was performed by using within groups' linkage and a Euclidean distance index. The software SPSS Statistics 20.0 (IBM Corp., Armonk, NY) was used to carry out the analysis.

Results

Classification of the CTMD of ray florets. First, the K-S tests illustrated that both RFL and CTL fit the normal distribution (Table 2). The values of RFL, CTL, and CTL/RFL (CTMD) were then divided into five grades using probability grading (Table 3). To elucidate the relationship between CTL and RFL, we used a linear regression model. The RFL was used as the abscissa, the CTL was used as the ordinate, and the CTL/RFL value was used as the dividing point. The five grades of CTL/RFL were combined step-by-step until the best combination scheme was found based on the coefficient of determination (R^2). Based on this method, we found that separating the first ($R^2 = 0.75$) and second grade of CTL/RFL ($R^2 = 0.78$) into different groups was more effective than combining the two ($R^2 = 0.20$). The R^2 value for combining the third and fourth grade of CTL/RFL was greater than that for combining the second and third grade. We concluded that separating the first and second grade of CTL/RFL into different groups was more suitable; however, the R^2 value for combining the third and fourth grade of CTL/RFL was less than that for combining the third, fourth, and fifth grades, indicating that the scheme of combining the third, fourth, and fifth grades was more suitable (Table 4). Based on the aforementioned result, we divided the five grades into three groups: 1) flat group, $0 \leq \text{CTL/RFL} \leq 0.20$; 2) spoon group, $0.20 < \text{CTL/RFL} \leq 0.60$; and 3)

Table 2. The variation and Kolmogorov-Smirnov (K-S) normal test of the ray floret length and corolla tube length in the outer whorl and middle whorl. When the trait of P value of K-S test is greater than 0.05, it follows a normal distribution and can be used for probability grading.

Traits	Mean	SD	Intravarietal cv %	Intervarietal cv %	P value of K-S test
RFL in the outer whorl	10.20	3.44	6.67	33.68	0.46
CTL in the outer whorl	7.49	4.96	11.62	66.31	0.16
RFL in the middle whorl	8.11	2.49	9.71	30.70	0.22
CTL in the middle whorl	4.34	3.15	18.95	72.56	0.06

cv = coefficient of variation; RFL = ray floret length; CTL = corolla tube length.

Table 3. The five grades of ray floret length (RFL), corolla tube length (CTL), and the ratio of corolla tube length and ray floret length (CTL/RFL) based on probability grading.

Traits	Grade				
	1	2	3	4	5
RFL	≤5.80	5.80–8.40	8.40–12.00	12.00–14.60	>14.60
CTL	≤1.12	1.12–4.88	4.88–10.09	10.09–13.85	>13.85
CTL/RFL	≤0.20	0.20–0.60	0.60–0.85	0.85–0.95	>0.95

Table 4. The R^2 value after combining the different five grades of corolla tube length/ray floret length (CTL/RFL).

Grade	Grade range of CTL/RFL	R^2
The first grade (except 0)	$0 < \leq 0.20$	0.76
The first grade	$0 \leq \leq 0.20$	0.23
The second grade	$0.20 < \leq 0.60$	0.78
Combine first and second grade	$0 < \leq 0.60$	0.20
Combine second and third grade	$0.20 < \leq 0.85$	0.71
Combine third and fourth grade	$0.60 < \leq 0.95$	0.90
Combine third, fourth and fifth grade	$0.60 < \leq 1.00$	0.91
Combine second, third, fourth, and fifth grade	$0.20 < \leq 1.00$	0.82

R^2 = coefficient of determination.

tubular group, where CTL/RFL is $0.60 < \text{CTL/RFL} \leq 1.00$ (Fig. 3). Based on the aforementioned result, we established a new classification standard for the CTMD.

To verify the results, discriminant analysis was used to establish the discriminant model of the CTMD (Supplemental Table 2). When we used the outer ray florets as the training samples, and the middle ray florets as the validation samples, >76% of ray florets could be correctly classified (Supplemental Table 3, taxonomic group a). Moreover, when we used the middle ray florets as the training samples, and the outer ray florets as the validation samples, >86% of ray florets

could be correctly classified (Supplemental Table 3, taxonomic group b). We therefore concluded that the new classification standard for CTMD was effective.

Classification of the flat group. According to the study of Luo et al. (2016), the ray floret width (RFL) was divided into five grades: narrow (0–0.23 cm), medium I (0.23–0.65 cm), medium II (0.65–1.20 cm), medium III (1.20–1.60 cm), and wide (RFL > 1.60 cm) and then applied to the Q-mode cluster. The same computing method was used for the spoon and tubular type. In the flat type, seven morphological characters of flowering were significantly different between cultivars

(Supplemental Table 4), and the Q-mode cluster analysis showed that the flat type was divided into nine groups (Supplemental Fig. 1; Table 5). In group 5, the abaxial side of the ray floret of all cultivars was aristate, whereas all cultivars in group 8 had a prominent laceration on the tip of the ray floret. In groups 1 and 2, the bending posture of corolla splitting (BPCS) was incurvate, but the RFW values were >1.60 cm and 0.65–1.20 cm, respectively. In groups 6 and 7, the BPCS was straight, but the RFW values were 1.20–1.60 cm and 0.65–1.2 cm, respectively. In groups 3, 4, and 9, the BPCS was evaginable or twisty, and the RFW values were from 0 to 0.65 cm, 0.65–1.20 cm, and >1.20 cm, respectively.

The flat type was classified into three types: straight, curved, and atypical types, based on the traits of shape on the tip of ray floret (STRF), aristate on the abaxial surface of ray floret (AASRF), and BPCS. When the characteristic of STRF was chenille-like, or being AASRF, they were defined as the atypical type, including aristate and chenille-like forms. When the BPCS was straight, it was assigned to the straight type, and when it was curved, it belonged to the curved type, including incurvate, evaginable, and twisty forms. Finally, the RFW was used as another classification criteria, including narrow (0–0.65 cm), medium (0.65–1.20 cm), and wide (>1.20 cm) (Fig. 4).

Classification of the spoon group. In the spoon type, eight flower morphological characters were significantly different between cultivars (Supplemental Table 4) and the Q-mode cluster analysis showed that the spoon type was divided into nine groups (Supplemental Fig. 2; Table 6). In groups 3 and 8, the main defining features included being aristate on the abaxial surface of the ray floret, whereas in groups 3 and 8, the BPCS was straight and incurvate, respectively. In group 9, all cultivars were unguiculate on the tip of the ray floret. In groups 1, 2, and 7, the BPCS was incurvate, and the BPCS was from 0 to 180°, 0–180°, and >180°, respectively. Moreover, a beak-like protrusion was present on the tip of the corolla tube in groups 2 and 7. In group 4, the BPCS was straight and in groups 5 and 6, the BPCS was evaginable or had twisty corollas, and a beak-like protrusion also was found on the tip of corolla tube in group 6.

Thus, the spoon type is classified into three types based on traits of STRF, AASRF, and BPCS: straight, curved, and atypical types. When the characteristic of STRF was unguiculate, or being AASRF, they were defined as the atypical type, including unguiculate and aristate form. When the BPCS was straight, it belonged to the straight type, whereas when the BPCS was curved, incurvate, evaginable, and twisty forms, it was classified as the curved type. Finally, the incurvate form was further divided into bent (0–180°) and curly (>180°) types, based on bending angle of corolla splitting (BACS). Beak-like protrusion on the tip of corolla tube (BPTCT) also was considered

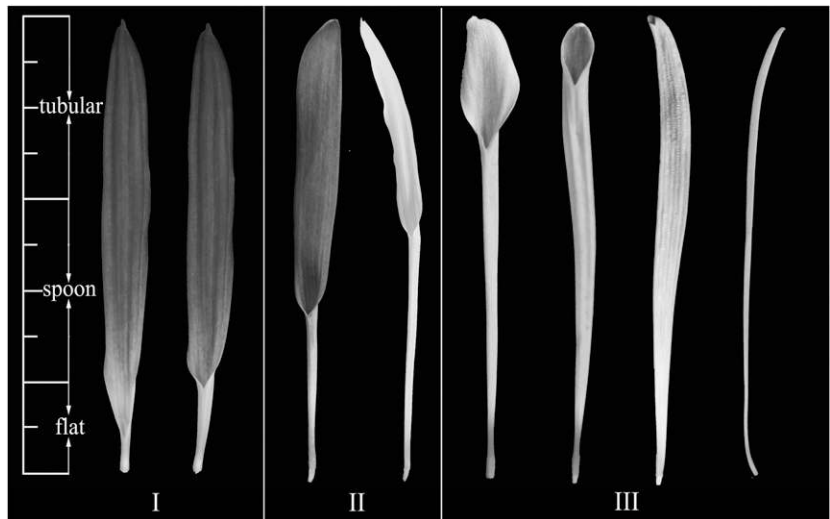


Fig. 3. Different shapes of the corolla tube merged degree. (I) Flat type: $0 \leq \text{CTL/RFL} \leq 0.20$. (II) Spoon type: $0.2 < \text{CTL/RFL} \leq 0.60$. (III) Tubular type: $0.60 < \text{CTL/RFL} \leq 1.0$. CTL = corolla tube length; RFL = ray floret length.

Table 5. Main features of nine groups of flat type based on the Q-cluster analysis.

Group	RFW (cm)	BACS (°)	BPRF	BPCS	CSE	STRF	AASRF
1	≥ 1.60	$0 < \leq 360$	Incurvate	Incurvate	Inward curl or no curl	Normal	No
2	$0.65 \leq \leq 1.20$	$0 < \leq 360$	Incurvate	Incurvate	Inward curl or no curl	Normal	No
3	$0.65 \leq \leq 1.20$	≤ 0	Twisty	Twisty or evaginable	Outward curl	Normal	No
4	$1.20 \leq \leq 1.60$	0	Twisty	Twisty	Outward curl	Normal	No
5	$1.20 \leq \leq 1.60$	$0 < \leq 180$	Incurvate	Curl inward	Inward curl	Normal	Yes
6	$1.20 \leq \leq 1.60$	0	Straight	Straight	Outward curl or inward curl	Normal	No
7	$0.65 \leq \leq 1.20$	0	Straight	Straight	No curl	Normal	No
8	$0.23 \leq \leq 0.65$	0	Straight	Straight	Inward curl	Lacerated	No
9	$0 \leq \leq 0.23$	0	Twisty	Twisty	No curl	Normal	No

RFW = ray floret width; BACS = bending angle of corolla splitting; BPRF = bending posture of ray floret; BPCS = bending posture of corolla splitting; CSE = curly state of edge; STRF = shape on the tip of ray floret; AASRF = aristate on the abaxial surface of the ray floret.

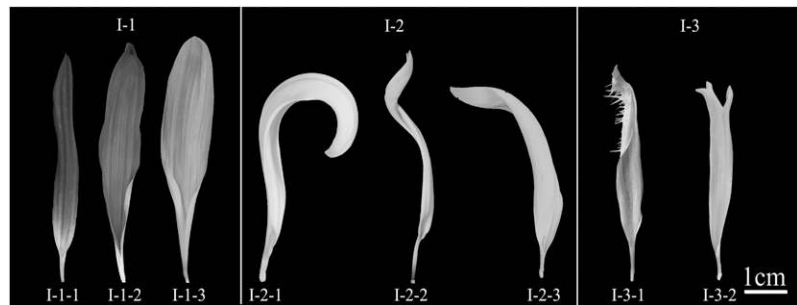


Fig. 4. Different types of flat groups. (I-1) Straight: narrow (I-1-1), medium (I-1-2), and wide (I-1-3). (I-2) Curved: incurvate (I-2-1), twisty (I-2-2), and evaginable (I-2-3). (I-3) Atypical: aristate (I-3-1), chenille-like (I-3-2). Scale bar = 1 cm.

as a next classification criteria in the curved type (Fig. 5).

Classification of the tubular group. In the tubular type, nine flower morphological characters were significantly different among cultivars (Supplemental Table 4), and the Q-mode cluster analysis showed that the tubular types were divided into 12 groups (Supplemental Fig. 3; Table 7). The tips of the ray florets of all cultivars in groups 7 and 12 were mainly asymmetric and quinquepartite,

but the RFW values were <0.65 cm and 0.65–1.20 cm, respectively. All cultivars in group 10 had a prominent laceration on the tip of the ray floret. Most cultivars in groups 2 and 3 were unguiculate on the tip of the ray floret, whereas there was a beak-like protrusion on the tip of corolla tube in group 3. In group 6, the ray florets of all cultivars were closed on the tip and were aristate on the abaxial surface. Cultivars in group 8 were mainly aristate on the abaxial surface

of the ray floret. In groups 1, 4, and 11, the BPCS was incurvate, whereas the RFW values were 0–0.65 cm, 0.23–1.20 cm, and >1.2 cm, respectively. In group 4, all cultivars were closed on the tip of the ray floret, whereas in groups 2, 3, and 5, the BPCS was mainly incurvate. In group 2, the RFW values were 0.23–0.65 cm, and the BACS values were >180°. In group 3, the RFW was 0.23–1.20 cm, and the BACS was >180°. In group 5, the cultivars of RFW values were 0.65–1.20 cm, and the BACS ranged from 0 to 180°, whereas in group 9, the BPCS was evaginable and twisty.

Thus, the tubular type was further classified into three types based on the traits STRF,

ANSRF, and BPCS: straight, curved, and atypical type. When the characteristic of STRF was unguiculate, or being aristate on the abaxial side of ray floret, it was defined as the atypical type, including aristate, unguiculate, star-like, and chenille-like forms. Straight or curved BPCS defined the other two types, and the latter included incurvate, twisty, and evaginable forms. Finally, closed or not on the tip of ray floret was considered in the classification of the straight type, and the incurvate form was further divided into bent (0 to 180°) and curly (>180°) forms based on BACS. BPTCT was considered in another classification criteria for the curved type, as was RFW, which included narrow

(0–0.65 cm), medium (0.65–1.20 cm), and wide (>1.20 cm) forms (Fig. 6).

Discussion

Description of the CTMD. The depth of the corolla split and the length of the corolla are key characters in defining the petal type of tobacco (*Nicotiana L.*) flowers, which are sympetalous (Anderson, 1939), and the same applies for the ray florets of chrysanthemum. However, the petal type of chrysanthemum has not been clearly defined to date, and the genetic analysis of petal type is quite distinct (Crook, 1942; Dejong and Drennan, 1984; Xu et al., 2000). Most notably, the description of petal type in their study was only the differences of flat, spoon, and tubular types. In fact, as mentioned in the Introduction, it was one of the most important factors of petal type, but not the only one. Therefore, it is necessary to have a new idea to describe the differences of flat, spoon, and tubular types. In this study, we propose here the idea of “corolla tube merged degree” or CTMD, which was used to describe the differences. In addition, the concept of “corolla tube” was mentioned in other composite families (Binns et al., 2002; Drennan et al., 1986). The traits of CTL and CTMD are based on Dejong and Drennan (1984), where the CTMD was described as the percentage of corolla split in the ray floret, although the authors determined the different types of ray floret based on subjective definitions. Compared with corolla split length in the ray floret, the CTL is easier to measure and calculate in Chinese large-flowered chrysanthemum. In addition, we found that the CTMD was a quantitative trait rather than a qualitative trait. Consequently, we unified a measurement standard of CTMD (CTL/RFL value, corolla tube length/ray floret length).

The chrysanthemum flower type. The chrysanthemum flower type is based on the shape, number, relative length, and orientation of florets (Crook, 1942; Culbert, 1957; Kawata, 1978; Mulford, 1937; Viehmeyer, 1959). Ray floret shape is the most complex, difficult to identify, and the main factor affecting capitulum shape. According to the ray floret shape, the flower type of chrysanthemum was classified into many different

Table 6. Main features of nine groups of spoon type based on the Q-cluster analysis.

Group	RFW (cm)	BACS (°)	BPRF	BPCS	CSE	STRF	AASRF	BPTCT
1	≥0.23	0<<≤180	Incurvate	Incurvate	Inward curl	Normal	No	No
2	0.23≤<≤1.60	0<<≤180	Straight	Incurvate	No curl or inward curl	Normal	No	Yes or no
3	0.23≤<≤1.20	0	Straight	Straight	Inward curl	Normal	Yes	No
4	0.65≤<≤1.20	0	Straight	Straight	All	Normal	No	No
5	≥0.65	≤0	All	Evaginable or twisty	Outward curl	Normal	No	No
6	0.23≤<≤1.20	0	All	Twisty or evaginable	Outward curl	Normal	No	Yes
7	0.23≤<≤1.60	≥180	Incurvate	Incurvate	Inward curl	Normal	No	Yes
8	0.23≤<≤1.20	0<<≤360	Straight	Incurvate	Inward curl	Normal	Yes	No
9	0.23≤<≤0.65	180≤<≤360	Straight	Incurvate	No curl	Unguiculate	No	Yes

RFW = ray floret width; BACS = bending angle of corolla splitting; BPRF = bending posture of ray floret; BPCS = bending posture of corolla splitting; CSE = curly state of edge; STRF = shape on the tip of ray floret; AASRF = aristate on the abaxial surface of the ray floret; BPTCT = beak-like protrusion on the tip of the corolla tube.

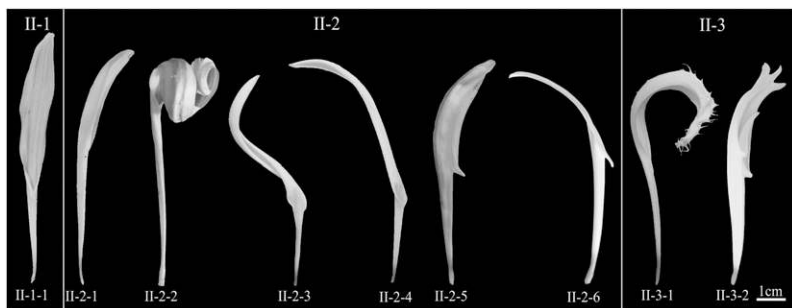


Fig. 5. Different types of spoon groups. (II-1) Straight (II-1-1). (II-2) Curved: incurvate and bent (II-2-1), incurvate and curly (II-2-2), twisty (II-2-3), evaginable (II-2-4), incurvate and beak-like protrusion (II-2-5), evaginable and beak-like protrusion (II-2-6). (II-3) Atypical: aristate (II-3-1), unguiculate (II-3-2). Scale bar = 1 cm.

Table 7. Main features of 12 groups of tubular type based on the Q-cluster analysis.

Group	RFW (cm)	BACS (°)	BPRF	BPCS	CSE	STRF	AASRF	BPTCT	CNTRF
1	0.65≤<≤1.20	0	Straight	Straight, evaginable	No curl	Normal	No	No	Open
2	0.23≤<≤0.65	180≤<≤360	Straight	Incurvate	No curl	Normal, unguiculate	No	No	Open
3	0.65≤<≤1.20	180≤<≤720	Straight	Incurvate	No curl	Normal, unguiculate	No	Yes	Open
4	0.65≤<≤1.20	No	Straight, incurvate	No corolla splitting	No curl	Normal	No	No	Closed
5	0.65≤<≤1.20	0<<≤180	Incurvate	Incurvate	No curl	Normal	No	No	Open
6	0≤<≤0.65	No	Straight	No corolla splitting	No curl	Normal	Yes	No	Closed
7	0≤<≤0.65	0	Incurvate	Else	Outward curl	Asymmetric quinquepartite	No	No	Open
8	0.65≤<≤1.20	0<<≤360	Straight	Incurvate	No curl	Normal	Yes	No	Open
9	0.65≤<≤1.20	≤0	Straight	Twisty, evaginable	Outward curl	Normal	No	No	Open
10	0.65≤<≤1.20	0	Straight	Straight	No curl	Lacerated	No	No	Open
11	≥1.20	0	Incurvate	No corolla splitting	No curl	Normal	No	No	Open
12	0.65≤<≤1.20	0	Straight	Straight	Outward curl	Asymmetric quinquepartite	No	No	Open

RFW = ray floret width; BACS = bending angle of corolla splitting; BPRF = bending posture of ray floret; BPCS = bending posture of corolla splitting; CSE = curly state of edge; STRF = shape on the tip of ray floret; AASRF = aristate on the abaxial surface of the ray floret; BPTCT = beak-like protrusion on the tip of the corolla tube; CNTRF = closed or not on the tip of the ray floret.

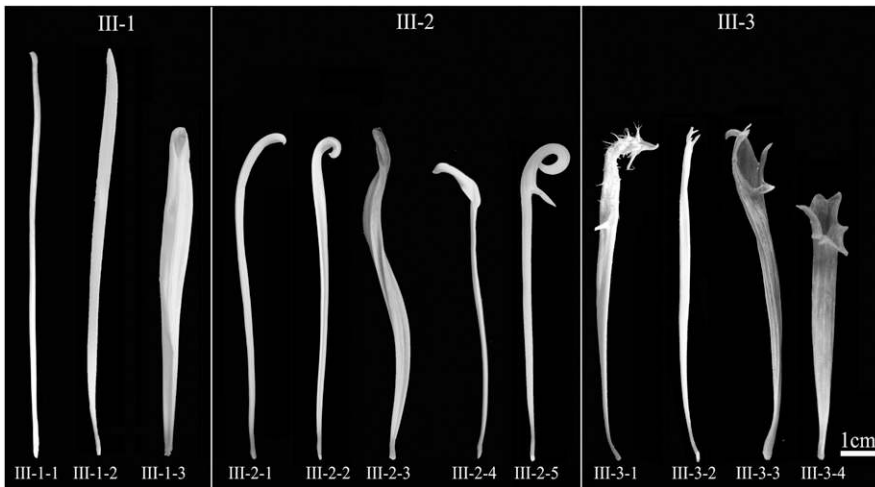


Fig. 6. Different types of the tubular group. (III-1) Straight: narrow and closed (III-1-1), medium and closed (III-1-2), wide and open (III-1-3). (III-2) Curved: incurvate and bent (III-2-1), incurvate and curly (III-2-2), twisty (III-2-3), evaginable (III-2-4), incurvate and beak-like protrusion (III-2-5). (III-3) Atypical: aristate (III-3-1), chenille-like (III-3-2), unguiculate (III-3-3), star-like (III-3-4). Scale bar = 1 cm.

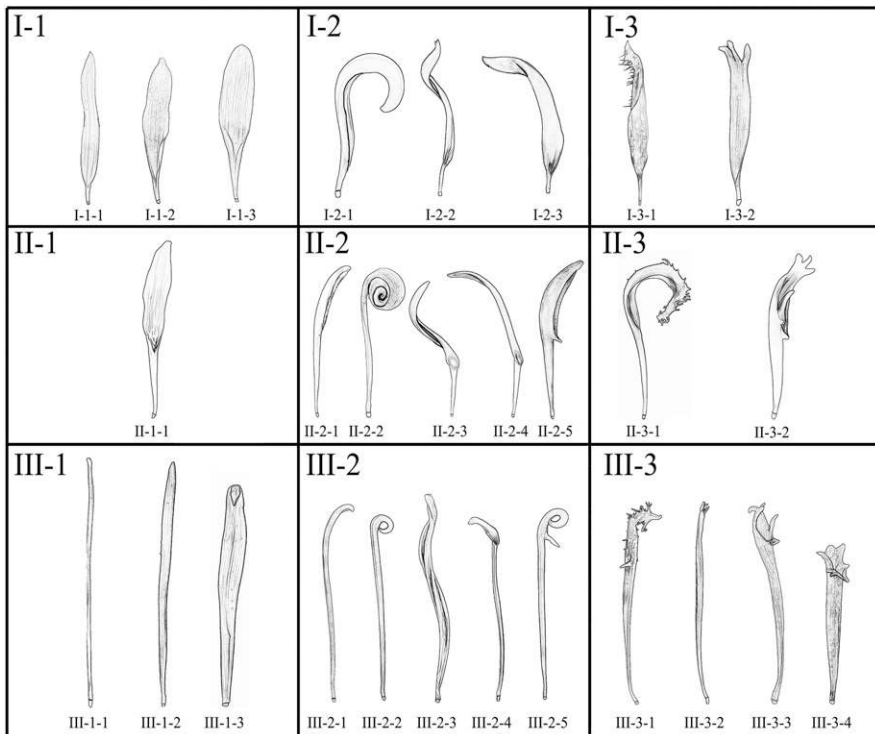


Fig. 7. Different types of ray florets. (I-1) Flat-straight types, (I-2) flat-curve types, (I-3) flat-atypical types. (II-1) Spoon-straight types, (II-2) spoon-curve types, (II-3) spoon-atypical types. (III-1) Tubular-straight types, (III-2) tubular-curve types, (III-3) tubular-atypical types.

types, such as lotus-like (flat type, where the capitulum has the appearance of a lotus flower), sparrow-tongue-like (spoon type), filiform form (tubular, with the appearance of long-hairs), dragon-claw-like (tubular or spoon type, the tips of ray floret look like a claw), and aristate (aristate on the abaxial side of ray floret) (Ackerson, 1957; Anderson, 2006; Zhang et al., 2014). Other floral traits are relatively easy to classify and identify. For example, the number of ray florets also was

used as another classification criterion and mainly affects flower doubleness in chrysanthemum, where there is a single (one to two whorls of ray florets, visible disk florets), semidouble, double, and superdouble or pom-pom form (no disk florets visible) (Crook, 1942; Meek, 1968). Other floral traits are ray floret orientation and relative length, which may vary from straight to incurved or reflexed, or different lengths between the outer and center whorls. The flower types include an

incurved form (petal tips curved inward), reflexed form (opposite of incurved), decorative form (outer ray florets longer than the center florets, disk florets hidden), and spider form (ray florets are long and quilled, hooked, and drooping) (Ackerson, 1957; Anderson, 2006). Until now, most of the classification studies of flower type have been based on direct observation of morphological characters but lack uniform measurement and classification criteria. In addition, an anemone type, belonging to the disc floret group, is characterized by hermaphroditic central disc florets that are elongated and colored (Anderson, 2006; Chen et al., 2009). The differences in disc florets are easier to distinguish than the variation in ray florets, so for the purpose of this study we focused on ray florets.

Different combinations of ray florets can form a variety of flower types, ranging from simple to complex. Interestingly, petal shape and flower shape in some cultivars are very stable but in other cultivars are susceptible to variation. In our study, we selected 151 samples from 880 cultivars that were morphologically stable in our nursery for 5 years. For sensitive cultivars, many factors, such as soil conditions, cultivation environment, temperatures, and growth stage, may affect variation. Some studies have shown that flower characteristics are affected by temperatures (Carvalho et al., 2005; Willits and Bailey, 2000); however, over several years of cultivation and observation, we found that the cultivation environment also can affect flower type. For example, when given adequate water and fertilizer, the number of ray florets is relatively high and flower type looks plump.

Establishment of a ray floret classification system. Flower shape is an important target for breeding and ornamental plant improvement, and in China, flower head shape, as well as ray floret shape in large-flowered chrysanthemum, has been subject to selection to meet different consumer demands. The classification of ray floret type and capitulum type are important features in determining whether a cultivated line represents a new breed. Moreover, it also provides a reference of selecting and breeding new varieties of other ornamental plants. In this study, the 151 samples were selected from 880 cultivars that were morphological stable in our nursery for 5 years. These 880 cultivars were collected from all parts of China and covered all the flower head types, petal types, and colors according to the traditional chrysanthemum classification criterion (Zhang and Dai, 2013). Collectively, the 151 varieties included all flower head and petal types and so the three groups and nine types that make up the classification system in this study have a wide range of applications. This system also provides a reference for the classification of ray florets in other chrysanthemums, such as those small-flower and cut flower cultivars. Notably, this system was developed based on multivariate statistical analysis methods, which provided a more objective and

accurate approach than previous classification studies based on direct observation of morphological characters.

There have been some reports of floral organ regulation in the Compositae. For example, some studies have shown that *CYC-like* genes play an important role in the symmetry of ray floret in members of the Compositae (Chapman et al., 2012; Juntheikki-Palovaara et al., 2014; Liu et al., 2016; Mizzotti et al., 2015). Ray floret architecture in chrysanthemum may range from a flat type (lateral symmetry) to tubular type (radial symmetry), and we found that there are many intermediate states between these extremes, suggesting that this is quantitative character. As mentioned previously, ray floret shape also includes other variations. This may be the reason that the genetic regulation model of floral organ development in higher plants cannot fully explain the development of the capitulum and different types of ray florets (Huang et al., 2016), a factor that limits novel flower shapes in chrysanthemum breeding.

In conclusion, in this study we demonstrated the use of CTMD and other important morphological variations of ray florets in Chinese large-flowered chrysanthemum to establish a new classification system. 1) The CTMD is divided into three groups based on CTL/RFL value: flat, spoon, and tubular. 2) According to appearing atypical variations on the tip or abaxial surface of ray floret or not, the three groups were divided into normal and atypical types, respectively, and the normal type was further divided into two types based on BPCS, including the straight and curved forms. 3) In conclusion, the ray floret was classified into nine types, including flat-straight, flat-curved, flat-atypical, spoon-straight, spoon-curve, spoon-atypical, tubular-straight, tubular-curve, and tubular-atypical (Fig. 7). The nine types were further classified based on their different characteristics. This ray floret classification system is helpful for the classification of capitulum shape and has great significance for the identification and breeding of chrysanthemum cultivars.

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Supplemental Table 1. 151 Chinese large-flower chrysanthemum cultivars in the outer whorl.

No.	Name	No.	Name	No.	Name	No.	Name
1	'Taizhenhanxiao'	39	'Yuhudie'	77	'Jiangfengyuhuo'	115	'Nijinshizi'
2	'Diebaochunfeng'	40	'Yaotaiyufeng'	78	'Zilongxianzhao'	116	'Xiaguang'
3	'Tongfajiaorong'	41	'Qianshouguanyin'	79	'Yuxianyinzen'	117	'Yizhibaoxiao'
4	'Biyugoupan'	42	'Baiweixian'	80	'Gushuiliuxia'	118	'Fenjianrong'
5	'Xuridongsheng'	43	'Fenweixian'	81	'Hehua'	119	'Nijinhongguan'
6	'Xiuhupo'	44	'Renmiantaohua'	82	'Jingetiema'	120	'Jinhongzhanpao'
7	'Jinlongxianzhao'	45	'Bihaiyinlong'	83	'Fenxianmingzhu'	121	'Yizhiduxiu'
8	'Yongshoumo'	46	'Jinxiaguan'	84	'Huanshuimingzhu'	122	'Hongyidaxia'
9	'Yulingguan'	47	'Lvchaoyun'	85	'Cunguhanxiao'	123	'Zixiaogong'
10	'Nanchaofendai'	48	'Lvping'	86	'Morong'	124	'Xishanhongri'
11	'Changongguise'	49	'Ziyusongzhen'	87	'Hubeixieyang'	125	'Xuezhongxiao'
12	'Taobaochunfeng'	50	'Qiansiwanlv'	88	'Qilinjiao'	126	'Chunfengxiyu'
13	'Qingshuilian'	51	'Tianxiayipin'	89	'Lvjiangnan'	127	'Huxu'
14	'Chunshuilvbo'	52	'Jinpaoyuanshuai'	90	'Tianhexima'	128	'Xiantanqiushui'
15	'Fengjuanhongqi'	53	'Baimaoju'	91	'Zhin'	129	'Riluojinshan'
16	'Zihongtuogui'	54	'Taiyechihe'	92	'Qiushuimingxia'	130	'Oufentuogui'
17	'Jinbeidahong'	55	'Huangguanqiu'	93	'Bailongzhao'	131	'Yudie'
18	'Xiaoye'	56	'Jinkuixiangyang'	94	'Lvkongque'	132	'Xichuangxiyu'
19	'Fengguanxiapai'	57	'Tiangezhuiyu'	95	'Chilongzhao'	133	'Xuezhongmeigui'
20	'Shuiyunxiang'	58	'Yuezhonggui'	96	'Fengxuezhulou'	134	'Hupoqiu'
21	'Yingfengdanchen'	59	'Bailuhengjiang'	97	'Huanghe'	135	'Kuihuatuogui'
22	'Jinboyongcui'	60	'Jinquenazhi'	98	'Jinxie'	136	'Tangyuqiuyun'
23	'Qionгдаosanyou'	61	'Huihexianzhu'	99	'Menglongpao'	137	'Ziyuanyanghe'
24	'Changshengle'	62	'Huanshuijingui'	100	'Hongyun'	138	'Hujulongpan'
25	'Zirui Gong'	63	'Taoranzui'	101	'Jinmajiong feng'	139	'Mulanhuanzhuang'
26	'Dianjiangchun'	64	'Zhushahongshuang'	102	'Yushizidai'	140	'Songlinfeibao'
27	'Xixiangdaiyue'	65	'Baimaoji'	103	'Annigongzhu'	141	'Huazhuangfen'
28	'Huangxiangli'	66	'Nenzhuyusun'	104	'Tangyuzhiguang'	142	'Fentuogui'
29	'Jinlongxianxuezhao'	67	'Fengmaolinjiao'	105	'Hualouyufeng'	143	'Xiangyunchunyu'
30	'Maguxianrui'	68	'Fenxuanqiu'	106	'Hukoutengyu'	144	'Dahuangmaoju'
31	'Wucaifeng'	69	'Nijinqiehua'	107	'Fenzishiguan'	145	'Jinhongjiaohui'
32	'Jinhuxiao'	70	'Huangkuilong'	108	'Taohuachunshui'	146	'Hongwenyun'
33	'Saijinhua'	71	'Fenkuilong'	109	'Chaoqunshang'	147	'Hangongdai'
34	'Fengqingyuebai'	72	'Lihua'	110	'Zilong'	148	'Cuilongzhao'
35	'Qianchifeiliu'	73	'Xiangshanchufeng'	111	'Zilongnaohai'	149	'Hongriyinghui'
36	'Shibafenghuan'	74	'Huangjianrong'	112	'Shushanqingtao'	150	'Qingrenmeng'
37	'Tangyushouweng'	75	'Jinlingguan'	113	'Bailinqing'	151	'Dongfengwanli'
38	'Xinghuachunyu'	76	'Fenghuangyi'	114	'Hantanyingyue'		
152	'Taizhenhanxiao'	189	'Yuhudie'	226	'Jiangfengyuhuo'	263	'Nijinshizi'
153	'Diebaochunfeng'	190	'Yaotaiyufeng'	227	'Zilongxianzhao'	264	'Xiaguang'
154	'Tongfajiaorong'	191	'Qianshouguanyin'	228	'Yuxianyinzen'	265	'Jinjibaioxiao'
155	'Biyugoupan'	192	'Baiweixian'	229	'Gushuiliuxia'	266	'Fenjianrong'
156	'Xuridongsheng'	193	'Renmiantaohua'	230	'Hehua'	267	'Nijinhongguan'
157	'Xiuhupo'	194	'Bihaiyinlong'	231	'Jingetiema'	268	'Jinhongzhanpao'
158	'Jinlongxianzhao'	195	'Jinxiaguan'	232	'Fenxianmingzhu'	269	'Yizhiduxiu'
159	'Yongshoumo'	196	'Lvchaoyun'	233	'Cunguhanxiao'	270	'Hongyidaxia'
160	'Xuri'	197	'Lvping'	234	'Yinpantuogui'	271	'Zixiaogong'
161	'Yulingguan'	198	'Ziyusongzhen'	235	'Hubeixieyang'	272	'Xishanhongri'
162	'Nanchaofendai'	199	'Qiansiwanlv'	236	'Qilinjiao'	273	'Xuezhongxiao'
163	'Taobaochunfeng'	200	'Tianxiayipin'	237	'Lvjiangnan'	274	'Chunfengxiyu'
164	'Qingshuilian'	201	'Jinpaoyuanshuai'	238	'Tianhexima'	275	'Huxu'
165	'Chunshuilvbo'	202	'Baimaoju'	239	'Zhin'	276	'Xiantanqiushui'
166	'Fengjuanhongqi'	203	'Taiyechihe'	240	'Qiushuimingxia'	277	'Riluojinshan'
167	'Zihongtuogui'	204	'Huangguanqiu'	241	'Bailongzhao'	278	'Oufentuogui'
168	'Jinbeidahong'	205	'Jinkuixiangyang'	242	'Lvkongque'	279	'Yudie'
169	'Xiaoye'	206	'Tiangezhuiyu'	243	'Chilongzhao'	280	'Xichuangxiyu'
170	'Fengguanxiapai'	207	'Yuezhonggui'	244	'Fengxuezhulou'	281	'Hupoqiu'
171	'Shuiyunxiang'	208	'Bailuhengjiang'	245	'Huanghe'	282	'Kuihuatuogui'
172	'Yingfengdanchen'	209	'Jinquenazhi'	246	'Jinxie'	283	'Tangyuqiuyun'
173	'Jinboyongcui'	210	'Huihexianzhu'	247	'Menglongpao'	284	'Ziyuanyanghe'
174	'Qionгдаosanyou'	211	'Huanshuijingui'	248	'Hongyun'	285	'Hujulongpan'
175	'Changshengle'	212	'Taoranzui'	249	'Jinmajiong feng'	286	'Mulanhuanzhuang'
176	'Zirui Gong'	213	'Zhushahongshuang'	250	'Yushizidai'	287	'Songlinfeibao'
177	'Dianjiangchun'	214	'Baimaoji'	251	'Annigongzhu'	288	'Jinhongyuanshuai'
178	'Xixiangdaiyue'	215	'Nenzhuyusun'	252	'Tangyuzhiguang'	289	'Huazhuangfen'
179	'Huangxiangli'	216	'Fengmaolinjiao'	253	'Hualouyufeng'	290	'Fentuogui'
180	'Jinlongxianxuezhao'	217	'Fenxuanqiu'	254	'Hukoutengyu'	291	'Xiangyunchunyu'
181	'Wucaifeng'	218	'Nijinqiehua'	255	'Fenzishiguan'	292	'Dahuangmaoju'
182	'Jinhuxiao'	219	'Huangkuilong'	256	'Taohuachunshui'	293	'Jinhongjiaohui'
183	'Saijinhua'	220	'Fenkuilong'	257	'Chaoqunshang'	294	'Hongwenyun'
184	'Fengqingyuebai'	221	'Lihua'	258	'Zilong'	295	'Hangongdai'
185	'Qianchifeiliu'	222	'Xiangshanchufeng'	259	'Zilongnaohai'	296	'Cuilongzhao'
186	'Shibafenghuan'	223	'Huangjianrong'	260	'Shushanqingtao'	297	'Hongriyinghui'
187	'Tangyushouweng'	224	'Jinlingguan'	261	'Bailinqing'	298	'Qingrenmeng'
188	'Xinghuachunyu'	225	'Fenghuangyi'	262	'Hantanyingyue'	299	'Dongfengwanli'

Supplemental Table 2. Classification function coefficients.

Traits	Classification function coefficients		
	Flat	Spoon	Tubular
Ray florets in the outer whorl			
RFL	4.04	2.31	0.58
CTL	-3.81	-1.84	0.47
(Constant)	-18.23	-7.37	-6.58
Ray florets in the middle whorl			
RFL	3.05	1.80	0.27
CTL	-3.27	-1.03	1.97
(Constant)	-12.71	-6.60	-8.88

RFL = ray floret length; CTL = corolla tube length.

Supplemental Table 3. Identifiable fitness of different corolla tube type in different floret whorl. Taxonomic group a: 94.60% of original grouped cases correctly classified, and 93.90% of cross-validated grouped cases correctly classified. Taxonomic group b: 90.10% of original grouped cases correctly classified, and 89.40% of cross-validated grouped cases correctly classified.

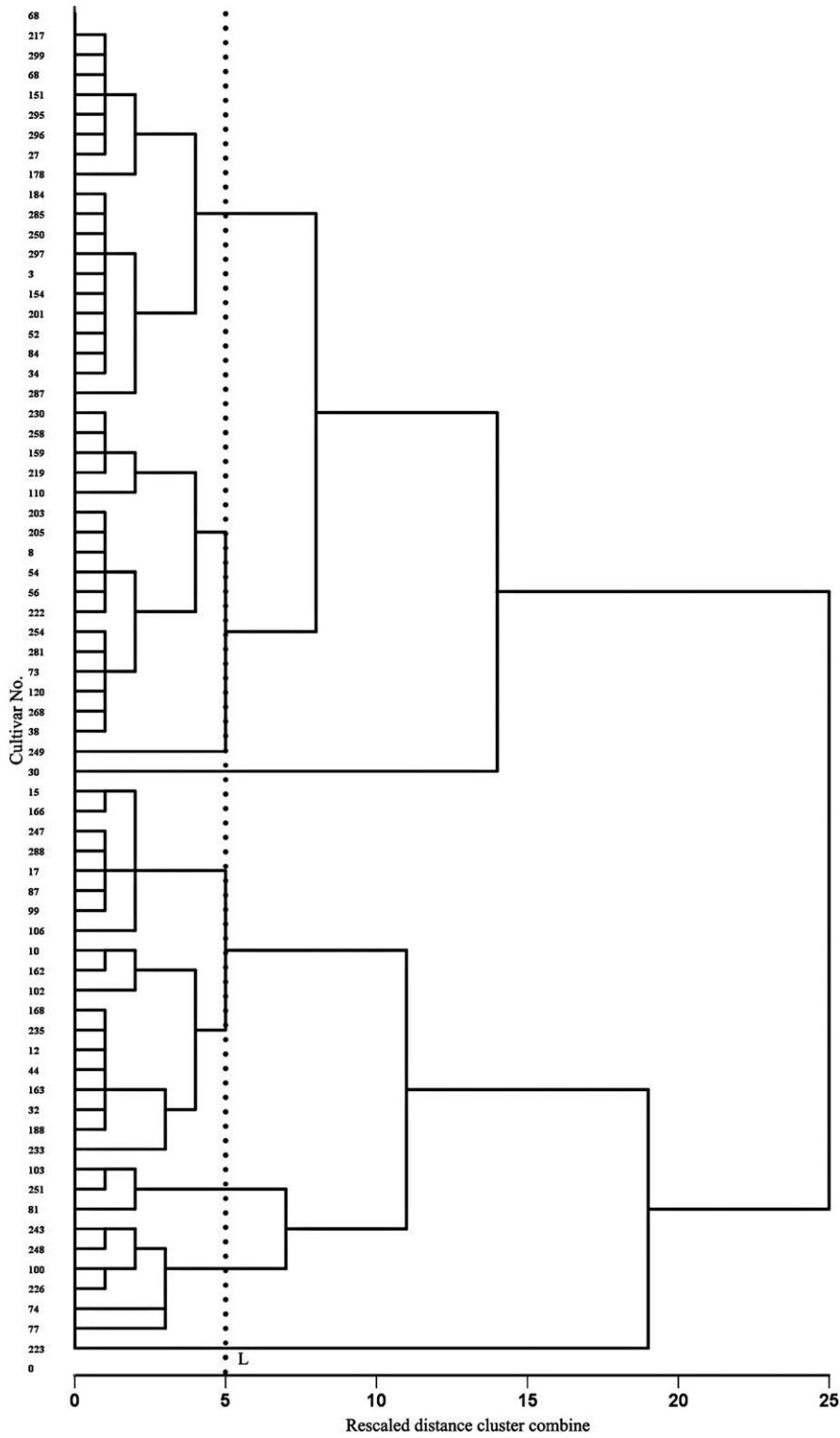
Taxonomic group	Sample classification	Source		Distribution number			Identifiable fitness (%)
				Flat	Spoon	Tubular	
a	Training samples	Ray florets in the outer whorl	Flat	24	6	0	80.00
			Spoon	2	14	0	87.50
			Tubular	0	8	97	92.38
	Validation samples	Ray florets in the middle whorl	Flat	26	11	0	76.48
			Spoon	6	29	0	89.40
			Tubular	0	18	58	82.94
b	Training samples	Ray florets in the middle whorl	Flat	33	4	0	89.19
			Spoon	3	32	0	91.43
			Tubular	0	2	74	97.37
	Validation samples	Ray florets in the outer whorl	Flat	26	4	0	86.67
			Spoon	0	15	1	93.75
			Tubular	0	1	104	99.05

Supplemental Table 4. Descriptive statistics of morphological characters of flat, spoon and tubular types.

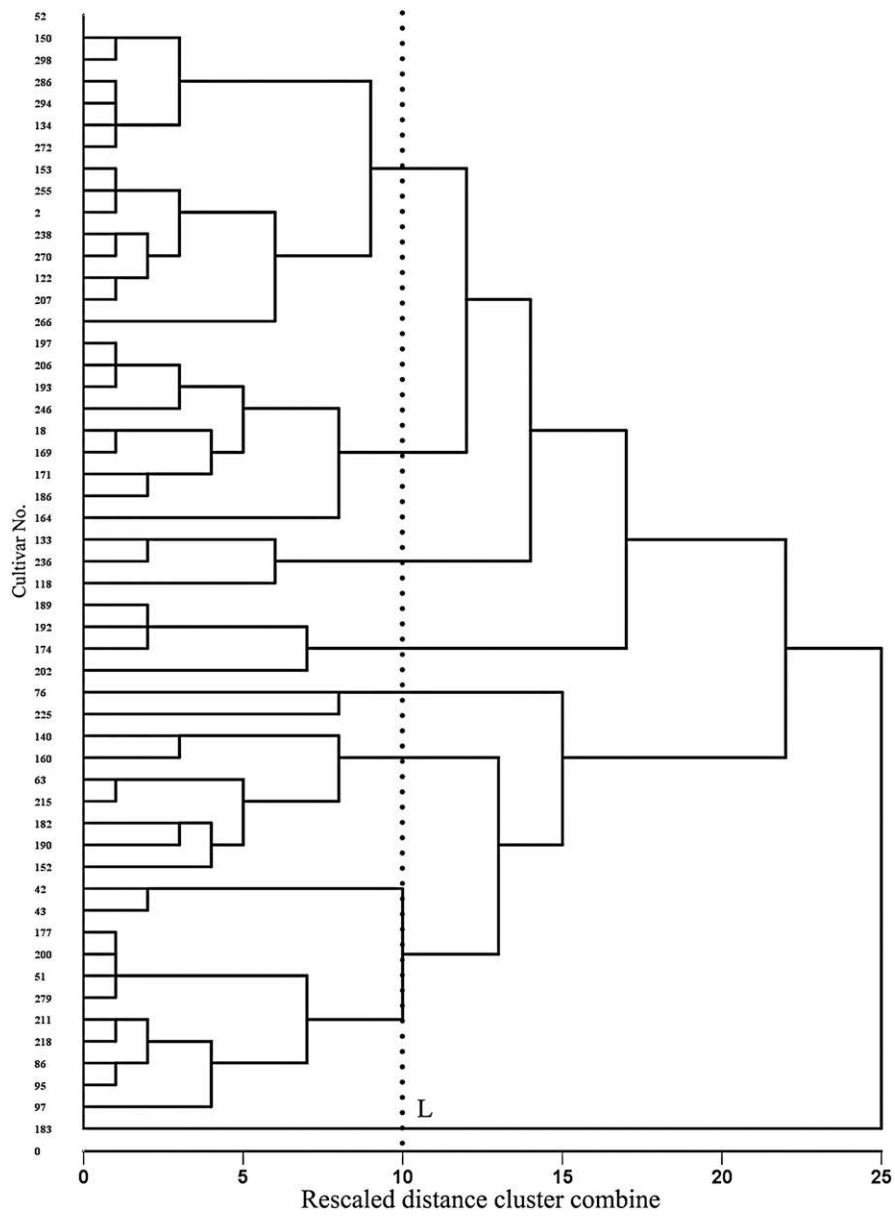
Traits	Flat type			Spoon type			Tubular type		
	Mean	SD	H'	Mean	SD	H'	Mean	SD	H'
RFW (cm)	1.32	0.55	1.08*	1.01	0.50	1.19*	0.74	0.42	1.18*
BACS (°)	101.68	105.92	1.24*	135.48	176.25	1.03*	88.70	141.13	1.10*
BPRF	2.00	1.33	1.12*	2.84	1.42	1.07*	32.85	47.45	0.93*
BPCS	3.16	1.23	1.12*	0.73	0.81	1.14*	0.68	1.22	1.27*
CSE	1.11	0.72	1.07*	0.02	0.18	1.05*	1.91	1.71	0.51*
STRF	0.07	0.37	0.08*	0.16	0.37	0.10*	0.26	0.61	0.37*
AASRF	0.02	0.14	0.08*	0.86	0.35	0.40*	0.10	0.44	0.42*
BPTCT	—	—	—	0.10	0.31	0.36*	0.15	0.35	0.51*
CNTRF	—	—	—	—	—	—	0.21	0.41	0.20*

*Indicates significant difference at 0.05 P level.

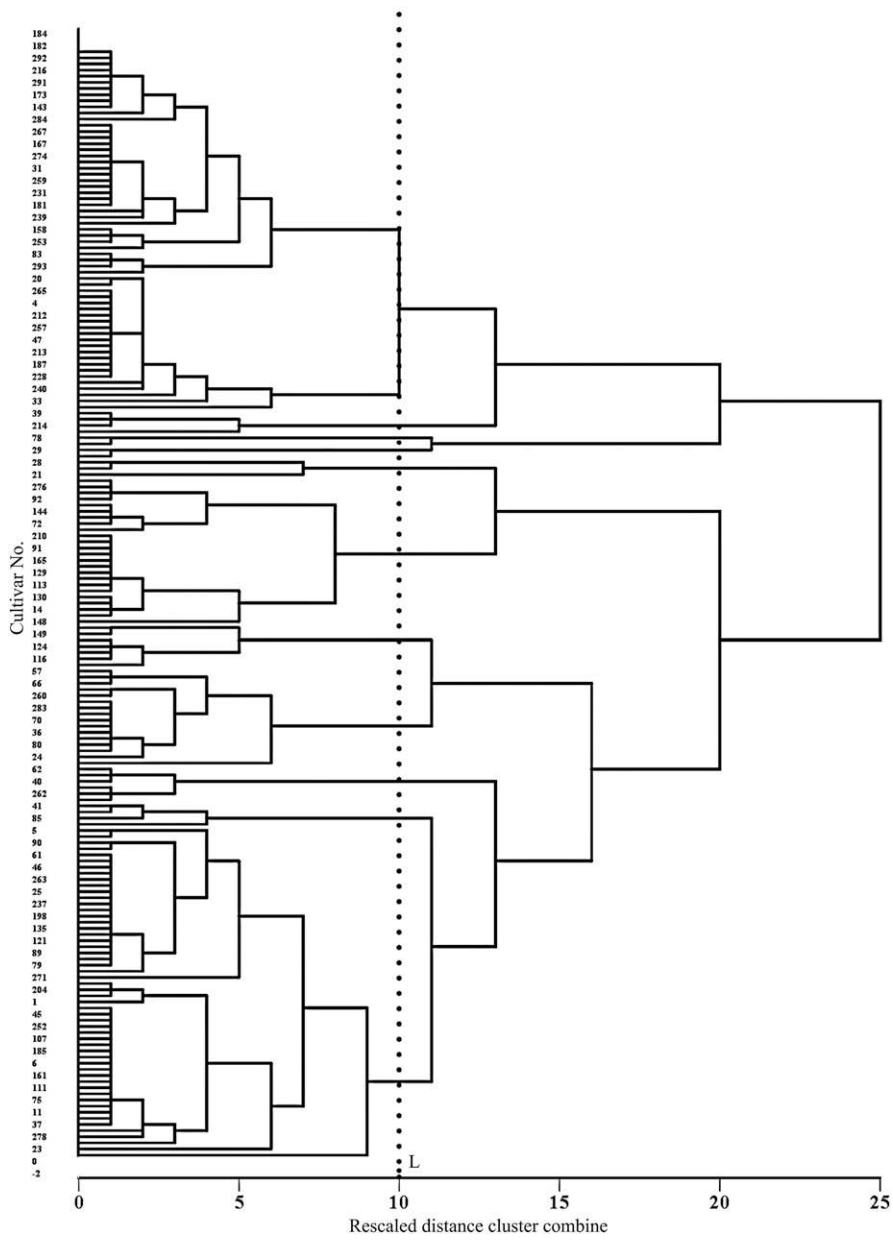
RFW = ray floret width; BACS = bending angle of corolla splitting; BPRF = bending posture of ray floret; BPCS = bending posture of corolla splitting; CSE = curly state of edge; STRF = shape on the tip of ray floret; AASRF = aristate on the abaxial surface of the ray floret; BPTCT = beak-like protrusion on the tip of the corolla tube; CNTRF = closed or not on the tip of the ray floret.



Supplemental Fig. 1. Dendrogram of Q cluster analysis for the flat type.



Supplemental Fig. 2. Dendrogram of Q cluster analysis for the spoon type.



Supplemental Fig. 3. Dendrogram of Q cluster analysis for the tubular type.