Cross Breeding New Cultivars of Early-flowering Multiflora Chrysanthemum Based on Mathematical Analysis

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Abstract. Conventional crossbreeding remains an effective technique for chrysanthemum (*Chrysanthemum × morifolium* Ramat.) breeding. However, there are always many problems when breeding chrysanthemum because of its complex genetic background, such as difficulty matching parents, selecting superior hybrid progenies, quantitatively describing certain target traits, and evaluating breeding results. A recent mathematical analysis method is an effective method for evaluating plant breeding progress. In this study, we used 505 multiflora chrysanthemum germplasm resources as test materials; we divided the flowering time into five groups using a grading analysis method, including extremely early group (genotypes that flowered when daylength was longer than 13.5 hours), early group (genotypes that flowered when daylength was 13.5–12.0 hours), medium group (genotypes that flowered when daylength was 12.0–11.0 hours), late group (genotypes that flowered when daylength was 11.0–10.0 hours), and extremely late group (genotypes that flowered when daylength was shorter than 10.0 hours). Moreover, the breeding objective was to breed early-flowering genotypes. Using 15 phenotypic characters as evaluation factors, 37 excellent genotypes, including four early-flowering genotypes, were screened out from the aforementioned resources according to an analytic hierarchy process (AHP) and weighting of the gray relational grade. We selected one early-flowering genotype and eight medium-flowering genotypes from these 37 genotypes and matched six hybridized combinations based on the genetic distance between genotypes calculated by the Q cluster analysis method. We used a comprehensive evaluation method combining AHP and the gray relational analysis (GRA) method for the evaluation of 367 progenies. Moreover, we screened out 52 superior hybrids, including 36 early-flowering hybrids. The results of this study demonstrate that the mathematical analysis method is an immensely effective method to breed new cultivars of early-flowering multiflora chrysanthemum. This study also provides an effective method to define and improve the flowering time of other cultivated plants.

Because of its high ornamental value and wide range of applications, multiflora chrysanthemum (*Chrysanthemum × morifolium* Ramat.) plays an important role in the global flower market (Anderson, 2006; Augustinova et al., 2016; Teixeira da Silva et al., 2013). However, most multiflora chrysanthemum

cultivars are short-day plants, which initiate flowering when the daylength is shorter than 12 h (Cockshull, 1985). Thus, they usually flower from late October to November. To meet market demands, growers have been using techniques to regulate (such as shading in summer) and advance flowering (Ochiai et al., 2015). These techniques are rather costly and laborious, causing the ornamental quality to decline. Therefore, advancing flowering is an important breeding objective for multiflora chrysanthemum.

Flowering time, an important adaptive trait that strongly influences planting area and productivity and the market price of cultivated plants, is affected by both endogenous and environmental factors. An accurate definition of flowering time will be vital for breeding research and the practical production of ornamental plants. The number of

days between the transplanting date and the initial flowering date has been recorded as initial flowering time in previous studies (Zhang et al., 2011, 2013). However, to date, flowering time has only been qualitatively described and not quantitatively analyzed. In *The guidelines for the conduct of tests for distinctness, homogeneity and stability—Chrysanthemum*, published and implemented by The International Union for the Protection of New Varieties of Plants (UPOV), flowering time is divided into three groups, including early group, medium group, and late group (UPOV, 2010). However, because of the lack of a numerical index to define flowering time groups, the evaluation criteria are always different among breeding programs. The establishment of a numerical grading index of flowering time is the basis for the normalized and standard description of multiflora chrysanthemum germplasm resources.

Chrysanthemum cultivars are genetically highly heterozygous, so conventional crossbreeding between parental cultivars with contrasting target traits is still the most effective way to breed new cultivars (Teixeira da Silva et al., 2013). The key to success is parent-pair selection; therefore, the genetic difference between parents is an important factor. Parental genetic difference and hybrid performance are positively related (Ajmone Marsan et al., 1998; Hung et al., 2012). Compared with molecular markers and other methods, morphological markers are easier to observe and obtain. In chrysanthemum, previous studies that used the cluster analysis method based on morphological markers were considerably restricted to studying cultivar classification (Zhang et al., 2014a) rather than evaluating the genetic difference between parents (Su et al., 2017). Moreover, breeding new cultivars is a sophisticated technique. Intraspecific and interspecific hybridization has been used to improve the plant type (Anderson and Ascher, 2016; Chen et al., 1995), stress resistance (Cheng et al., 2011), flower color (Anderson et al., 2014), and even flowering time of chrysanthemum. Although a series of new cultivars with different flowering times have been obtained (Anderson and Ascher, 2016), the number of early-flowering multiflora chrysanthemum cultivars is still limited (Lim et al., 2012, 2014). In particular, other ornamental traits of some cultivars tend to perform poorly during introduction and cultivation. Further studies are needed to breed superior early-flowering multiflora chrysanthemums that are suitable for different geographical cultivation conditions.

Comprehensive evaluation of hybrids is an important part of breeding. The primary breeding objective should be considered when selecting superior hybrids of ornamental plants, and conversely, comprehensive traits including flower-related traits, leaf-related traits, vegetative-related traits, and stress resistance should also be considered. It is imperative to establish a scientific, reasonable, and feasible comprehensive evaluation system. The GRA method is a comprehensive

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evaluation approach for the gray system. It is characterized by less data and can avoid inconsistency between results of quantitative and qualitative analyses (Deng, 1989). This method has been widely used in various fields of science, including the evaluation of new crop cultivars. However, it has a strong subjectivity in the determination of the weight of traits. AHP is a systematic and hierarchical analysis method for combining qualitative and quantitative analysis methods and can decompose complex problems into multiple layers and multiple factors (Saaty, 1990). It can easily calculate the weight of each trait but cannot make full use of the original information. A comprehensive evaluation system combining the AHP and GRA methods would be practical and comprehensive, allowing these methods to complement each other. This system has been widely used in industry, environment, architecture, and many other fields (Huang and Wang, 2014; Liang et al., 2015; Xu et al., 2011). It also has been used to evaluate a variety of resources and select new cultivars in many crops, and results have shown that it is an effective method for the comprehensive evaluation of germplasm resources (Ma et al., 2012; Xiong et al., 2015). This comprehensive evaluation system has less application in chrysanthemum breeding. Considering plant type as the main breeding objective, Wang et al. (2012) first used this method and selected new cultivars with excellent comprehensive characters quickly and effectively. Different main breeding objectives and a different judgment matrix constructed in AHP result in different weights of each index. A study using this method to select early-flowering multiflora chrysanthemum has not been reported.

In this study, we used several mathematical analysis methods to establish the numerical grading index of flowering time, comprehensively evaluate germplasm resources, select and match crossing parents, and screen out superior hybrids. The objectives of this study were to provide an effective method for multiflora chrysanthemum breeding by conventional crossbreeding and to provide a reference method for breeding programs of other ornamental plants.

Materials and Methods

Plant materials. A total of 505 multiflora chrysanthemum germplasm resources were used in this study and were maintained at the chrysanthemum germplasm nursery, Beijing Forestry University, China (Supplemental Table 1). These included eight cultivars of Timeline introduced from the Netherlands, 430 F_1 progenies obtained from natural crossing between Timeline and Chinese chrysanthemum, four cultivars of the Bran Multiflora line introduced from Germany (Brandkamp Company), and 63 F_1 progenies obtained from artificial crossing between Bran Multiflora and the other germplasms.

Sexual hybridization and cultivation. An artificial hybridization test was conducted during Fall 2013. Before the disc flowers of

female parents lost powder, the disc flowers were removed for emasculation, and the ray flowers were docked to expose the stigma; then, the capitulum was covered with a paper bag. Pollen collected from freshly opened flowers of male parents was transferred to “Y” type stigma of the emasculated flower of female parents with a brush, and the pollinated flowers were re-enclosed in a paper bag. Pollination was carried out once a day, and each capitulum was pollinated three to five times continuously for a week. After ≈ 60 d of natural growth, the capitula were collected, and the seeds were screened out; then, they were kept in a dry and ventilated place.

All experiments were completed in solar greenhouses of the chrysanthemum breeding nursery of Beijing Forestry University. In Feb. 2014, seeds were sown in 128-cell trays. After ≈ 30 d, the surviving seedlings, which had been pinched, were transplanted to 81-cell trays. Parents and F_1 progenies were propagated by cutting in April, and rooted cuttings were transplanted to pots on 6 June. The size of the pots was 19 cm $\times$ 17 cm and the plant spacing was 35 cm $\times$ 35 cm in the planting bed. Standard commercial practices were used to manage the plants, which flowered naturally in the fall. In 2015 and 2016, the parents and F_1 progenies were propagated by cuttings, and field management and planting time were the same as those in 2014.

Data collection. Continuous tests for 15 phenotypic traits (Table 1) of germplasm resources (2012–13) and F_1 progenies (2014–16) were performed. At least three randomly selected plants per genotype were measured, and average values were used in statistical analysis. According to Zhang et al. (2011), the initial flowering date was defined as when $\approx 50\%$ of the total flower buds were half-opened and fully pigmented, and the wilting flower date was defined as when $\approx 10\%$ of the total flowers appeared wilted. The color of the inner side of the ray floret was measured according to Hong et al. (2012).

Grading analysis method of flowering time. From 2012 to 2016, the daylength of key time nodes in the Beijing area was almost the same (Supplemental Table 2). It indicated that the daylengths on the same date in different years were almost the same. Most chrysanthemum cultivars maintain vegetative growth when the daylength is longer than 13.5 h and initiate flowering when daylength is shorter than 12.0 h (Cockshull, 1985). In this study, we used 13.5, 12.0, 11.0, and 10.0 h as points of division to perform the grading analysis of flowering time.

Comprehensive evaluation method of multiflora chrysanthemum germplasm resources and F_1 progenies. A comprehensive evaluation method combining AHP and GRA was used to evaluate germplasm and screen out superior early-flowering hybrids from F_1 progenies.

A multihierarchy analytic model was constructed based on the relationship between

15 traits (Table 2). Target layer (A): superior early-flowering multiflora chrysanthemum genotypes; constrained layer (C): three main factors that influence the ornamental value of multiflora chrysanthemum, including flowering time-related traits, flower head-related traits, and vegetative-related traits; and index layer (P): including 15 specific evaluation indexes.

The judgment matrix was structured, and the greatest characteristic root and characteristic vector were calculated (Supplemental Tables 3–6). The characteristic vector of each judgment matrix was the weight of the lower index relative to that of the upper index.

The “reference cultivar” X_0 was constructed based on the breeding objective and the actual observed value. Then, taking X_0 as the reference sequence and observed genotypes as the compare sequence, gray relational coefficients were calculated. Thus, the weight value (represented by r) of each genotype was calculated by including the weight value of 15 traits that were calculated by AHP in the equation (Wang et al., 2012).

Cluster analysis method. Using PASW Statistics (version 20.0) software, a depiction of R cluster analysis of the selected 15 phenotypic traits and Q cluster analysis of the superior genotypes was constructed using a between-groups linkage method based on squared Euclidean distance.

Results

Grading analysis of flowering time of multiflora chrysanthemum germplasm resources. Given that most chrysanthemum cultivars remain in vegetative growth when the daylength is longer than 13.5 h and start reproductive growth when the daylength is shorter than 12.0 h (Cockshull, 1985), the daylength was classified as long day (when daylength was longer than 13.5 h), short day (when daylength was 13.5–10.0 h), and extremely short day (when daylength was shorter than 10.0 h). Then, the flowering time of the multiflora chrysanthemum was classified into five continuously distributed grading ranges: extremely early group (genotypes that flowered when daylength was longer than 13.5 h), early group (genotypes that flowered when daylength was 13.5–12.0 h), medium group (genotypes that flowered when daylength was 12.0–11.0 h), late group (genotypes that flowered when daylength was 11.0–10.0 h), and extremely late group (genotypes that flowered when daylength was shorter than 10.0 h). Moreover, these five flowering time groups corresponded to the flowering time and flowering date (Table 3).

Comprehensive evaluation of breeding value of multiflora chrysanthemum germplasm resources. The comprehensive evaluation method combining AHP and GRA has been used for the evaluation of germplasm resources (Table 4). The weight value of the index layer (P) relative to the target layer (A) was calculated based on Supplemental Tables 3–6. The results showed that, in the three constrained layers, the weight value of flowering

Table 1. Fifteen phenotypic traits measured in this study, their measuring methods, and evaluation for qualitative traits.

Code	Trait	Measuring method and evaluation for qualitative traits
P1	Flowering time	Number of days between transplanting time and initial flowering time (days)
P2	Flowering duration	Number of days between initial flowering time and flower wilting time (days)
P3	Flower head diameter	Measure diameter at widest point of terminal flower head (cm)
P4	Peduncle length	Measure length from the bottom of the phyllary to the first true leaf
P5	Flower head type	Visual assessment; single (1), semidouble (2), daisy-eyed double (3), double (4), without ray florets (5)
P6	Predominant type of ray floret	Visual assessment; flat type (1), spoon type (2), tubular type (3)
P7	Number of colors of ray floret inner side	Visual assessment; one (1), two (2), more than two (3)
P8	Main color of ray floret inner side	Brown group (1), orange group (2), pink group (3), purple group (4), red group (5), white group (6), yellow group (7), yellow-green group (8), dark red group (9)
P9	Second color of ray floret inner side	The same as main color of inner side of ray floret
P10	Plant height	Measure height from the bottom of the stem to the top of the plant (cm)
P11	Crown diameter	Measure diameter at the widest point from the top face (cm)
P12	Leaf length	Measure length of leaf on the middle third of the stem
P13	Leaf width	Measure width of leaf on the middle third of the stem
P14	Branching density	Visual assessment; dense (1), medium (2), sparse (3)
P15	Flower density	Visual assessment; dense (1), medium (2), sparse (3)

Table 2. The layered structure model of 15 evaluation indexes in multiflora chrysanthemum.

Target layer (A)	Constrained layer (C)	Index layer (P)
Superior early-flowering multiflora chrysanthemum genotypes	C1 flowering time-related traits	P1, P2
	C2 flower head-related traits	P3, P4, P5, P6, P7, P8, P9
	C3 vegetative-related traits	P10, P11, P12, P13, P14, P15

time-related traits was the largest (0.750), followed by that of flower head-related traits (0.171), and the smallest was that of vegetative-related traits (0.078). In the 15 selected indexes, the weight value of the flowering time was the largest (0.657), followed by that of the flowering duration (0.0938). The order of the other traits can be seen in Table 4.

Based on the grading index of the flowering time in Table 3, we evaluated the flowering time of 505 germplasm resources. The results showed that there were no extremely early-flowering genotypes and only nine early-flowering genotypes, which accounted for 1.8% of the germplasm. There were 41 medium-flowering genotypes, which accounted for 8.1%. The number of late-flowering genotypes was the greatest, at 375, and accounted for 74.3%. Moreover, there were 80 extremely late-flowering genotypes, which accounted for 15.8% (Table 5). Therefore, the flowering time of multiflora chrysanthemum germplasm resources was late, and the number of early-flowering genotypes was small. Based on these results, breeding early-flowering cultivar was taken as a breeding objective in this study.

Subsequently, these germplasm resources were ordered according to the weight value (represented by r) and were divided into three grades (Table 5). There were 37 genotypes in

grade one ($r \geq 0.826$). Except for four genotypes that belonged to the early group, there were 30 medium-flowering genotypes and three late-flowering genotypes. However, the flowering duration of these genotypes was longer, and the comprehensive characters were excellent. They are important germplasms for improving flowering time in the future. There were 216 genotypes in grade two ($0.771 \leq r < 0.826$). Most genotypes were late flowering with a shorter flowering duration. Therefore, these genotypes had a certain defect. There were 252 genotypes in grade three ($r < 0.771$). Among these, most were late flowering with poor comprehensive characters. These results indicated that a few germplasm resources could conform to the breeding objective.

Sexual hybridization to create new early-flowering germplasms of multiflora chrysanthemum. The result of R cluster analysis of 15 selected traits showed that only the characters leaf length and leaf width were correlated, whereas the other 13 traits were independent, indicating the rationality of selection of these traits (as shown in Fig. 1A). The result of Q cluster analysis between 37 superior germplasm resources, which belonged to grade one in Table 5 based on Euclidean distance, is shown in Fig. 1B. The horizontal axis represents the cluster level of each group and the vertical axis

represents 37 genotypes (numbered from 1 to 37) ranking from the biggest to the smallest according to the weight value.

One early-flowering (A20) and eight medium-flowering genotypes (as shown in Supplemental Fig. 1) were selected as crossing parents from 37 superior genotypes, and six hybridized combinations were matched using them (Table 6) according to the following principles: genetic distance was farther between crossing parents, sources of crossing parents were different, flowering times of the crossing parents were similar, female parent was double-flowered, male parent had a large number of pollen grains, colors of crossing parents were different, and comprehensive characters were excellent.

A total of 105 flower heads were pollinated and 961 seeds were collected in Fall 2013. The mean number of seed per flower head of combination A was 18.8, which was the highest; those of combinations B and C were 8.8 and 12.1, respectively; and those of combinations D, E and F were lower. These seeds were seeded in Spring 2014 and 406 seeds germinated. Low seed germination rates were observed for the six hybridized combinations and 367 F_1 progenies flowered in Fall 2014. The seedling rate of combination A (84.0%) was lower than that of the other five combinations (Table 7).

Based on the grading index of flowering time in Table 3, we evaluated the flowering time of 367 F_1 progenies (Table 8). There were two extremely early-flowering hybrids (accounted for 0.5%, Supplemental Table 7) and 71 early-flowering hybrids (accounted for 19.4%). Medium- and late-flowering hybrids accounted for 53.4% and 26.7%, respectively. Moreover, there were no extremely late-flowering hybrids. Compared with the germplasm resources, we obtained extremely early-flowering genotypes and the number of early-flowering genotypes was markedly increased.

Based on the weight value of every index in Table 4, the weight value (represented by r) of the F_1 progenies were calculated. Moreover, 367 F_1 progenies were divided into three grades (Table 8). There were 52 hybrids in grade one ($r \geq 0.851$). Among them, 36 hybrids belonged to the early-flowering group. These 36 hybrids with good comprehensive characters (red color, long flowering duration, double flowered and big crown diameter) accorded with the demands of the market and could be potential new cultivars. There were 147 hybrids in grade two ($0.822 \leq r < 0.851$). Most of them were medium flowering and a few were early or late flowering. The main flower color was red, pink, or purple and most hybrids had specific defects. There were 168 hybrids in grade three ($r < 0.822$). Among these, most were medium flowering with poor comprehensive characters. Finally, 36 early-flowering hybrids (Supplemental Table 8) with good comprehensive characters were screened out. A comprehensive analysis of the aforementioned data showed that the expected breeding objective was achieved.

Table 3. Grading criteria for flowering time of multiflora chrysanthemum.

Grade	1	2	3	4	5
Daylength (h·d ⁻¹)	≥13.5	13.5–12.0	12.0–11.0	11.0–10.0	<10.0
Daylength group	Long day	Short day	Short day	Short day	Extremely short day
Flowering time group	Extremely early group	Early group	Medium group	Late group	Extremely late group
Grading range of flowering date	Before Aug. 23	Aug. 24 to Sept. 26	Sept. 27 to Oct. 20	Oct. 21 to Nov. 15	After Nov. 15
Grading range of flowering time (d)	≤75	76–110	111–134	135–160	>160

Table 4. Weight value of the evaluation indexes.

Layer C contributes to layer A		Index layer P	Layer P contributes to layer C	Layer P contributes to layer A	Order
C1	0.750	P1	0.875	0.657	1
		P2	0.125	0.0938	2
C2	0.171	P3	0.110	0.0189	7
		P4	0.0654	0.0112	9
		P5	0.259	0.0444	4
		P6	0.0430	0.0074	11
		P7	0.0627	0.0107	10
		P8	0.421	0.0722	3
		P9	0.0385	0.0066	12
C3	0.078	P10	0.162	0.0126	8
		P11	0.0694	0.0054	13
		P12	0.0414	0.0032	14
		P13	0.0269	0.0021	15
		P14	0.414	0.0324	5
		P15	0.287	0.0224	6

Table 5. Results of comprehensive evaluation and grade division in multiflora chrysanthemum germplasm.

Grade	Wt value (<i>r</i>)	No. genotypes	Proportion (%)	No. extremely early-flowering genotypes	No. early-flowering genotypes	No. medium-flowering genotypes	No. late-flowering genotypes	No. extremely late-flowering genotypes
1	$r \geq 0.826$	37	7.33	0	4	30	3	0
2	$0.771 \leq r < 0.826$	216	42.8	0	2	7	176	31
3	$r < 0.771$	252	49.9	0	3	4	196	49
Total	Number of genotypes	505		0	9	41	375	80
	Proportion (%)			0.00	1.8	8.1	74.3	15.8

Fig. 1. Depiction of cluster analysis of 37 superior genotypes screened from germplasm resources: (A) R cluster analysis diagram of 15 characters; (B) Q cluster analysis diagram of 37 genotypes.

Discussion

Grading analysis of flowering time. The establishment of a scientific and rigorous numerical grading index is the basis for further research of the target trait. Luo et al.

(2016) have established a grading index of 19 quantitative traits of Chinese traditional chrysanthemum using the probability grading method. However, the flowering time was not analyzed, and the method did not apply to the grading analysis of quantitative trait that

skew distribution. According to Zhang et al. (2014b), 82 ornamental crabapple cultivars were divided into five major flowering groups with 5 d (5 d is a “*Hou*” in Chinese) as the level differential. There were only 22 d between the earliest and latest flowering cultivars of ornamental crabapple but there were 61 d of multiflora chrysanthemum in our study. Therefore, this method was also not appropriate for our study. In a chrysanthemum DUS (distinctness, uniformity, and stability) test (UPOV, 2010), the flowering time was divided into three groups, including the early group, medium group, and late group. However, chrysanthemum lacks a numerical index to define the flowering time group in this grading method. Most chrysanthemum cultivars show vegetative growth when the daylength is longer than 13.5 h and start reproductive growth when the daylength is shorter than 12.0 h (Cockshull, 1985). In this study, considering the natural blooming time of multiflora chrysanthemum in the Beijing area as an example, we have established a grading index of flowering time based on the aforementioned character of multiflora chrysanthemum. The flowering time of multiflora chrysanthemum was divided into five groups: extremely early group (genotypes that flowered when daylength was

Table 6. Description list of combinations and the main ornamental characters of parents in sexual hybridization.

Code of combination	Name of parents	Type of parents	Ordered by wt value	Source	Flowering time (d)	Main color of inner side of ray floret	Flower head type
A	Brancandy 317	Female parent	18	Germany Brandkamp Company	125	Pink group	Daisy-eyed double
		Male parent	15	Breeding performed ourselves	133	Pink group	Semidouble
B	Brankiss A49	Female parent	17	Germany Brandkamp Company	122	Pink group	Daisy-eyed double
		Male parent	11	Breeding performed ourselves	118	Red group	Single
C	A20	Female parent	1	Breeding performed ourselves	106	Pink group	Daisy-eyed double
		Male parent	24	Breeding performed ourselves	114	Red group	Semidouble
D	Brankiss A7	Female parent	17	Germany Brandkamp Company	122	Pink group	Daisy-eyed double
		Male parent	4	Breeding performed ourselves	116	Yellow group	Semidouble
E	Brancandy A38	Female parent	18	Germany Brandkamp Company	125	Pink group	Daisy-eyed double
		Male parent	31	Breeding performed ourselves	111	Red group	Semidouble
F	Brankiss Ca*388-45	Female parent	17	Germany Brandkamp Company	122	Pink group	Daisy-eyed double
		Male parent	27	Breeding performed ourselves	111	Orange group	Single

Table 7. Statistical analysis of the results of sexual hybridization in multiflora chrysanthemum.

Code of combination	No. flower heads	No. harvested seeds	Mean no. seeds per flower head	No. seedlings	Seed germination rate (%)	No. surviving seedlings	Seedling rate (%)
A	25	470	18.8	212	45.1	178	84.0
B	20	176	8.8	92	52.3	89	96.7
C	16	193	12.1	71	36.8	69	97.2
D	12	66	5.5	26	39.4	26	100
E	20	51	2.6	3	5.9	3	100
F	12	5	0.4	2	40.0	2	100
Total	105	961	9.2	406	42.2	367	90.4

Table 8. Results of the comprehensive evaluation and grade division in F₁ progenies of multiflora chrysanthemum.

Grade	Wt value (<i>r</i>)	No. genotypes	Proportion (%)	No. extremely early-flowering genotypes	No. early-flowering genotypes	No. medium-flowering genotypes	No. late-flowering genotypes	No. extremely late-flowering genotypes
1	$r \geq 0.851$	52	14.2	0	36	11	5	0
2	$0.822 \leq r < 0.851$	147	40.1	1	13	76	57	0
3	$r < 0.822$	168	45.8	1	22	109	36	0
Total	Number of genotypes	367		2	71	196	98	0
	Proportion (%)			0.5	19.4	53.4	26.7	0.00

longer than 13.5 h), early group (genotypes that flowered when daylength was 13.5–12.0 h), medium group (genotypes that flowered when daylength was 12.0–11.0 h), late group (genotypes that flowered when daylength was 11.0–10.0 h), and extremely late group (genotypes that flowered when daylength was shorter than 10.0 h). The flowering time of multiflora chrysanthemum was further refined, and each group corresponded to the flowering time and flowering date. We quantitatively described our breeding objective, and it not only was convenient for evaluating the breeding results but also provided references for defining the flowering time of multiflora chrysanthemum cultivated in different areas. These results were supplemented the chrysanthemum DUS test and established a foundation to perform a normalized and standard description of chrysanthemum flowering time. Moreover, it provided a method for grading analysis of the other quantitative traits.

Breeding results of early-flowering breeding of multiflora chrysanthemum. The investigation of multiflora chrysanthemum germplasm resources in the Beijing area showed that there were few early-flowering genotypes. In the 505 germplasm resources, there were only nine early-flowering genotypes (accounted for 1.8%). Only four early-flowering genotypes with good comprehensive

characters were selected out through a comprehensive evaluation. One early-flowering and eight medium-flowering genotypes were used as crossing parents to obtain 367 F₁ progenies, including 71 early-flowering hybrids (accounted for 19.4%) and two extremely early-flowering hybrids (Supplemental Table 7). These two extremely early-flowering hybrids can flower under long day conditions, so they may be day-neutral genotypes, but further study is needed. Thirty-six early-flowering hybrids with excellent comprehensive characters were selected through comprehensive evaluation (Supplemental Table 8) and could be developed into new cultivars. Thus, the breeding objective is complete and traditional crossing breeding is an effective method for improving the flowering time of multiflora chrysanthemum.

In this study, relatively few extremely early-flowering genotypes were obtained. Breeding to improve the flowering time of multiflora chrysanthemum needs further improvements and increased efforts. Considering combinations A, B, and C, the flowering time of most F₁ progenies was the same as that of their parents. In addition, some variations flowered earlier than their parents. Among these three combinations, the number of early-flowering F₁ progenies in combina-

tion C was the most. Therefore, the breeding result of combination C was the best. Among the parents of these three combinations, only the female parent of combination C belonged to the early group; all others belonged to the medium group. This result showed that it was easier to get early-flowering progenies using parents that flowered earlier. The results were similar to those obtained by other researchers (Fukai et al., 2000). Therefore, these two extremely early-flowering and 36 early-flowering hybrids were elite germplasms for breeding extremely early-flowering multiflora chrysanthemum.

Application of mathematical analysis method in ornamental plant breeding. Proper parent-pair selection is a prerequisite to achieving the breeding objective. Because of its complex genetic background, the parent-pair selection is difficult for chrysanthemum, and the traditional method of selecting and matching parents is grueling and time-consuming (Bestfleisch et al., 2014; Zeng et al., 2014). The correlation between parental genetic distance and hybrid performance using morphological and molecular markers, respectively, was measured in chrysanthemum (Su et al., 2017). However, only waterlogging tolerance-related characters were discussed. In this study, the genetic distances between 37 superior genotypes

were measured by cluster analysis of 15 morphological markers. Moreover, six hybridized combinations were matched based on the cluster results. The method based on genetic distance to select and match parents has been used in the breeding of *Liriodendron* (Yao et al., 2016), chinese cabbage (Kawamura et al., 2016), and other crops. However, there are few studies using this method in chrysanthemum. Using this method, we found that the flowering time of F₁ was promoted. Therefore, the parent-pair selection method that we used was effective.

The hybrid progenies of chrysanthemum are widely separated, so it is a challenge for chrysanthemum breeders to evaluate breeding results accurately. The complexity of ornamental plant breeding lies not only in the need to achieve the main breeding objective but also to consider comprehensive characters of new cultivars. This objective needs to evaluate hybrid progenies comprehensively. The GRA method is a comprehensive evaluation approach for the gray system. However, it has strong subjectivity in the determination of the weight of traits. Using AHP, the weight of every index relative to comprehensive characters can be calculated by structuring the judgment matrix. Moreover, it is more objective and reasonable to determine the weight. A comprehensive evaluation system combining the AHP and GRA methods can allow these methods to complement each other. This combined method has been widely used in many fields (Huang and Wang, 2014; Liang et al., 2015; Xu et al., 2011). It also has been used in the comprehensive evaluation of winter jujube (Ma et al., 2012), tulip (Xiong et al., 2015), and other crops. If the main breeding objectives are different and the judgment matrix constructed in AHP is different, the weights of each index are different. Considering the plant type as the main breeding objective, Wang et al. (2012) first used this method to perform a comprehensive evaluation of multiflora chrysanthemum and selected 43 genotypes with excellent comprehensive characters. In this study, we used flowering time as the main breeding objective by redefining flowering time and selected 36 early-flowering hybrids with excellent comprehensive characters using this method. The results showed that this is an effective and comprehensive evaluation method.

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Supplemental Fig. 1. Individual performance of the selected nine crossing parents: (A) A20; (B) A7; (C) A49; (D) 317; (E) Brankiss; (F) Brancandy; (G) A42; (H) Ca*388-45; (I) A38.

Supplemental Table 1. The source and flowering time of 505 germplasm resources.

Genotype no.	Source	Flowering time (d)	Genotype no.	Source	Flowering time (d)	Genotype no.	Source	Flowering time (d)
Smoothie Time	Timeline	144	104	Natural crossing	158	298	Natural crossing	151
Dragon Time		150	105	between Timeline	152	299	between timeline	164
Pixie Time		136	106	with chinese	153	300	with chinese	150
Midnight Time		152	107	chrysanthemum	165	301	chrysanthemum	153
Jewel Time		147	108		158	302		141
Energy Time		146	109		156	303		160
Sahara Time		152	110		158	304		146
Grace Time		149	111		159	305		142
Brancandy	Bran Multiflora line	125	112		157	307		150
Brankiss		122	113		165	308		148
Branccloud		130	114		160	309		153
Branhill		132	115		160	311		156
183*BH-42	Artificial crossing	121	116		165	312		158
183*BH-46	between Bran	111	117		159	313		150
183*BH-72	Multiflora line	110	118		163	314		151
183*Q-12	with the other	124	119		155	315		155
183*Q-18	germplasm	111	120		159	316		155
183*Q-5		108	121		157	317		133
183*Q-83		127	122		160	318		165
183*Q-85		143	123		162	319		163
388*Q-15		126	124		155	320		163
388*Q-219		135	127		154	321		162
388*Q-284		121	128		163	322		160
388*Q-298		136	129		160	323		157
388*Q-317		110	130		150	324		156
388*Q-4		115	131		147	325		162
388*Q-48		123	132		160	326		152
388*Q-72		141	133		157	327		165
388*Q-76		135	134		150	330		148
A1		130	135		152	332		155
A10		123	136		162	333		157
A11		110	137		152	334		160
A12		126	138		155	335		149
A13		120	139		152	336		155
A15		141	140		150	337		155
A16		133	142		152	338		157
A17		137	144		151	339		145
A2		140	145		158	340		148
A20		106	146		151	341		158
A22		116	147		160	342		160
A25		155	148		155	343		149
A32		133	150		155	344		151
A35		123	151		155	345		160
A36		137	153		167	347		162
A38		111	155		165	348		158
A39		138	156		160	349		147
A42		114	157		163	350		154
A44		117	158		147	352		159
A45		145	159		153	353		158
A46		148	160		152	354		157
A49		118	161		155	355		150
A5		141	162		155	356		155
A54		117	163		148	358		155
A55		134	164		145	359		150
A7		116	165		154	360		152
A8		137	166		157	361		155
A9		124	167		152	362		155
Ca*388-15		136	169		157	363		161
Ca*388-17		109	170		157	364		160
Ca*388-30		137	172		165	365		157
Ca*388-35		151	173		158	366		164
Ca*388-42		142	174		156	367		153
Ca*388-44		110	175		157	368		155
Ca*388-45		111	176		158	369		160
Ca*388-46		155	177		155	370		158
cl*388-3		126	178		157	371		157
cl*388-5		132	180		155	372		157
cl*388-8		129	181		160	373		158
E*R-4		125	182		155	374		158
E*R-5		138	183		134	376		155
EN*BH-11		138	184		155	380		162
EN*BH-14		134	185		155	381		165
EN*BH-27		106	186		155	382		160

(Continued on next page)

Supplemental Table 1. (Continued) The source and flowering time of 505 germplasm resources.

Genotype no.	Source	Flowering time (d)	Genotype no.	Source	Flowering time (d)	Genotype no.	Source	Flowering time (d)
EN*BH-5		125	187		155	383		160
K*183-8		103	189		160	384		153
1	Natural crossing between timeline with chinese chrysanthemum	126	190		161	385		162
2		160	191		146	386		157
3		150	192		155	387		160
4		144	193		155	388		112
5		152	194		153	389		160
6		152	195		155	390		155
7		155	196		155	391		155
8		165	197		155	392		155
9		148	198		164	393		155
10		152	199		154	394		160
11		152	200		157	395		146
12		152	201		152	396		157
13		150	203		155	397		163
14		151	204		155	398		160
15		165	205		147	399		155
16		150	206		157	401		158
17		149	207		155	402		158
18		165	208		154	403		162
19		155	209		160	404		159
20		152	210		155	405		159
21		161	211		155	406		162
22		152	212		154	407		162
23		155	214		153	408		157
24		152	215		160	409		155
25		161	217		155	410		157
27		142	218		155	411		164
29		162	219		154	412		157
30		155	220		154	413		160
31		155	221		155	414		165
32		163	222		155	415		162
33		155	223		158	416		162
34		151	224		149	417		157
35		160	225		148	418		162
37		151	226		155	419		158
38		152	227		153	420		158
39		153	228		160	421		160
40		154	230		160	422		158
41		155	231		153	423		158
42		153	232		149	424		157
43		152	233		163	425		158
44		153	234		149	426		163
45		150	235		155	427		151
46		146	236		154	428		158
47		151	237		155	429		162
48		164	238		160	430		158
49		165	239		154	431		162
50		155	240		165	433		163
51		160	243		162	434		162
52		155	244		149	435		160
53		155	245		149	436		163
54		149	246		152	437		160
55		161	247		161	438		160
56		161	248		154	439		166
57		161	249		157	440		161
58		154	250		153	441		159
59		155	251		149	442		156
60		152	253		159	443		162
61		153	254		152	444		158
62		152	255		157	445		160
63		155	256		157	446		165
64		157	257		153	447		155
65		152	258		157	448		153
66		151	259		158	449		160
67		151	261		162	450		153
69		154	263		155	451		155
70		160	264		153	452		150
71		160	265		152	454		151
72		155	266		160	455		162
73		161	267		152	456		165
74		152	268		152	457		155

(Continued on next page)

Supplemental Table 1. (Continued) The source and flowering time of 505 germplasm resources.

Genotype no.	Source	Flowering time (d)	Genotype no.	Source	Flowering time (d)	Genotype no.	Source	Flowering time (d)
75		159	269		155	458		153
77		163	270		162	459		162
78		158	272		164	461		155
79		156	273		155	463		166
81		155	275		155	466		155
82		160	276		151	467		151
83		155	277		147	468		153
84		164	278		160	470		158
85		164	279		151	471		162
86		153	280		163	472		155
87		155	281		155	473		155
88		165	282		155	474		158
89		155	283		164	475		137
91		158	284		150	476		160
93		166	285		155	477		159
94		151	287		153	478		163
95		158	288		153	479		158
96		152	289		155	480		164
97		152	290		161	481		160
98		150	291		150	482		158
99		147	292		155	492		140
100		155	294		151	512		130
101		160	295		149			
102		144	297		152			

Supplemental Table 2. The daylength of key time nodes in the Beijing area in 2012–16.

Date	Aug. 22	Sept. 26	Oct. 20	Nov. 15
Daylength in 2012 (h·d ⁻¹)	13.5	12.0	11.0	10.0
Daylength in 2013 (h·d ⁻¹)	13.5	12.0	11.0	10.0
Daylength in 2014 (h·d ⁻¹)	13.5	12.0	11.0	10.0
Daylength in 2015 (h·d ⁻¹)	13.5	12.0	11.0	10.0
Daylength in 2016 (h·d ⁻¹)	13.5	12.0	11.0	10.0

Supplemental Table 3. The judgment matrix and its consistency check: A-Ci.

A	C1	C2	C3	Wt (w_i)
C1	1	6	7	0.750
C2	1/6	1	3	0.171
C3	1/7	1/3	1	0.078

$\lambda_{\max} = 3.10$, CI = 0.0500, RI = 0.58, CR = 0.0861 < 0.1. CI = consistency index; RI = random consistency index; CR = consistency ratio.

Supplemental Table 4. The judgment matrix and its consistency check: C1-Pi.

C1	P1	P2	Wt (w_i)
P1	1	7	0.875
P2	1/7	1	0.125

$\lambda_{\max} = 2.00$, CI = 0, CR = 0 < 0.1.

Supplemental Table 5. The judgment matrix and its consistency check: C2-Pi.

C2	P3	P4	P5	P6	P7	P8	P9	Wt (w_i)
P3	1	2	1/4	6	2	2	2	0.110
P4	1/2	1	1/4	4	1/3	1/8	3	0.0654
P5	4	4	1	5	4	1/2	6	0.259
P6	1/6	1/4	1/5	1	1	1/5	2	0.043
P7	1/2	3	1/4	1	1	1/8	1	0.0627
P8	1/2	8	2	5	8	1	9	0.421
P9	1/2	1/3	1/6	1/2	1	1/9	1	0.039

$\lambda_{\max} = 7.79$, CI = 0.131, RI = 1.32, CR = 0.0994 < 0.1.

Supplemental Table 6. The judgment matrix and its consistency check: C3-Pi.

C3	P10	P11	P12	P13	P14	P15	Wt (w_i)
P10	1	4	6	6	1/3	1/4	0.162
P11	1/4	1	3	3	1/6	1/5	0.0694
P12	1/6	1/3	1	3	1/7	1/7	0.0414
P13	1/6	1/3	1/3	1	1/9	1/8	0.0269
P14	3	6	7	9	1	3	0.414
P15	4	5	7	8	1/3	1	0.287

$\lambda_{\max} = 6.52$, CI = 0.105, RI = 1.24, CR = 0.0846 < 0.1.

Supplemental Table 7. Description list of the main ornamental characters of two extremely early-flowering superior hybrids.

Hybrid no.	Flowering time (d)	Flowering duration (d)	Main color of ray floret inner side	Flower head type	Branching density	Flower density	Flower head diam (cm)	Plant ht (cm)
B8	71	19	White group	Daisy-eyed double	Sparse	Sparse	3.6	23.0
D45	71	20	Pink group	Semidouble	Medium	Dense	4.5	38.0

Supplemental Table 8. Description list of the main ornamental characters of 36 early-flowering superior hybrids.

Hybrid no.	Flowering time (d)	Flowering duration (d)	Main color of ray floret inner side	Flower head type	Branching density	Flower density	Flower head diam (cm)	Plant ht (cm)
C22	101	21	Red group	Semidouble	Medium	Medium	3.6	23.0
C42	97	22	Red group	Semidouble	Dense	Dense	4.0	23.5
C36	97	21	Red group	Semidouble	Medium	Dense	5.1	20.0
C7	92	27	White group	Semidouble	Medium	Dense	4.7	38.5
D7	107	23	Red group	Double	Medium	Medium	4.3	30.0
D42	82	15	Pink group	Semidouble	Medium	Sparse	2.9	44.5
C37	95	22	Red group	Semidouble	Sparse	Medium	4.4	40.0
C31	106	23	Red group	Semidouble	Dense	Dense	3.7	19.8
D87	107	17	Purple group	Single	Medium	Medium	4.5	20.0
D81	87	24	Pink group	Semidouble	Sparse	Sparse	4.3	37.0
C52	105	26	Red group	Semidouble	Medium	Medium	4.2	43.5
C50	107	18	Red group	Semidouble	Dense	Medium	4.7	38.1
C55	105	28	Red group	Semidouble	Dense	Dense	5.3	16.0
D26	100	20	Red group	Semidouble	Medium	Medium	4.4	44.5
C13	102	21	Red group	Semidouble	Medium	Dense	5.8	49.3
C18	103	23	Red group	Semidouble	Dense	Dense	4.7	32.5
D76	98	18	Red group	Semidouble	Medium	Dense	3.4	26.0
D91	108	28	Red group	Daisy-eyed double	Medium	Medium	3.9	29.4
C34	99	33	Pink group	Semidouble	Dense	Dense	4.4	43.5
C1	105	21	Red group	Semidouble	Dense	Dense	3.7	20.0
D58	104	19	Red group	Daisy-eyed double	Medium	Dense	4.0	19.0
C20	108	19	Red group	Semidouble	Dense	Medium	4.3	47.0
C60	107	20	Red group	Semidouble	Dense	Dense	5.0	37.0
C35	107	20	Red group	Daisy-eyed double	Sparse	Dense	4.1	53.8
C26	86	20	Pink group	Semidouble	Sparse	Medium	3.8	40.0
C65	108	19	Red group	Semidouble	Medium	Dense	5.2	35.2
C23	106	20	Red group	Semidouble	Medium	Dense	4.0	45.3
C64	98	18	Pink group	Semidouble	Sparse	Dense	4.7	23.5
D63	104	21	Red group	Semidouble	Medium	Medium	5.3	45.0
C17	106	25	Pink group	Daisy-eyed double	Dense	Medium	5.2	25.9
C19	107	24	Purple group	Daisy-eyed double	Sparse	Dense	4.0	36.8
D61	108	16	Red group	Semidouble	Medium	Medium	4.1	28.3
C45	108	18	Red group	Semidouble	Medium	Dense	3.7	44.5
C59	108	15	Red group	Semidouble	Medium	Dense	3.4	37.5
C66	108	18	Red group	Single	Sparse	Medium	4.4	19.0
C49	104	26	Purple group	Semidouble	Sparse	Dense	5.1	50.5