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.

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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
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 fully use all the 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 has also 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 F1 progenies obtained from natural crossing between Timeline and Chinese chrysanthemum germplasm resources and F1 progenies. After 30 d, the surviving seedlings, which had been pinched, were transplanted to 81-cell trays. Parents and F1 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 × 17 cm and the plant spacing was 35 cm × 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 F1 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 F1 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 ≥50% of the total flower buds were half-opened and fully pigmented, and the wilting flower date was defined as when ≥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 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 

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 F1 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 × 17 cm and the plant spacing was 35 cm × 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 F1 progenies were propagated by cuttings, and field management and planting time were the same as those in 2014.

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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 among the candidates (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, and colors of crossing parents were different, and comprehensive characteristics 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 F1 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 F1 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 F1 progenies were calculated. Moreover, 367 F1 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 most, 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 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 |
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 day-length 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      | Female parent  | 18                 | Germany Brandkamp Company | 125    | Pink group        | Daisy-eyed double              |
| B                   | Branchkiss     | Female parent  | 15                 | Breeding performed ourselves | 133    | Pink group        | Semidouble                  |
| C                   | A20            | Female parent  | 1                  | Breeding performed ourselves | 108    | Pink group        | Daisy-eyed double              |
| D                   | Branchkiss     | Female parent  | 24                 | Breeding performed ourselves | 114    | Red group         | Semidouble                  |
| E                   | Brancandy      | Female parent  | 17                 | Germany Brandkamp Company | 122    | Pink group        | Daisy-eyed double              |
| F                   | Branchkiss     | Female parent  | 4                  | Breeding performed ourselves | 116    | Yellow group      | Semidouble                  |
| G                   | A38            | Male parent    | 31                 | Breeding performed ourselves | 125    | Pink group        | Daisy-eyed double              |
| H                   | Branchkiss     | Male parent    | 17                 | Germany Brandkamp Company | 122    | Red group         | Semidouble                  |
| I                   | Calcar clock   | Male parent    | 27                 | Breeding performed ourselves | 111    | Orange group      | Single                       |
| J                   | A42            | Male parent    | 25                 | Breeding performed ourselves | 470    | Pink group        | Daisy-eyed double              |
| K                   | A49            | Male parent    | 122                | Germany Brandkamp Company | 122    | Pink group        | Daisy-eyed double              |
| L                   | A7             | Male parent    | 66                 | Germany Brandkamp Company | 122    | Pink group        | Daisy-eyed double              |
| M                   | A24            | Male parent    | 193                | Germany Brandkamp Company | 122    | Pink group        | Daisy-eyed double              |
| N                   | Female parent  | 1                | 106                | Germany Brandkamp Company | 122    | Pink group        | Daisy-eyed double              |
| O                   | Male parent    | 1                | 118                | Germany Brandkamp Company | 122    | Pink group        | Daisy-eyed double              |
| P                   | Female parent  | 1                | 122                | Germany Brandkamp Company | 122    | Pink group        | Daisy-eyed double              |
| Q                   | Male parent    | 1                | 122                | Germany Brandkamp Company | 122    | Pink group        | Daisy-eyed double              |

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 | Seeding 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 (r) | 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 | Proportion (%) |
|-------|--------------|---------------|----------------|----------------------------------------|------------------------------|-------------------------------|-------------------------------|---------------------------------------|----------------|
| 1     | r ≥ 0.851    | 52            | 14.2           | 0                                      | 36                          | 11                            | 5                             | 0                                     |                |
| 2     | 0.822 ≤ r < 0.851 | 147 | 40.1           | 1                                      | 13                          | 76                            | 57                            | 0                                     |                |
| 3     | r < 0.822    | 168           | 45.8           | 1                                      | 22                          | 109                           | 36                            | 0                                     |                |
| Total |               | 367           |                | 2                                      | 71                          | 196                           | 98                            | 0                                     |                |

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 combination 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...
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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 | Natural crossing between Timeline with Chinese chrysanthemum | 152 | 299 | between timeline | 164 |
| Pixie Time | 136 | 106 | with Chinese | 153 | 300 | with Chinese | 150 |
| Midnight Time | 152 | 107 | Chinese chrysanthemum | 165 | 301 | Chinese 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 |
| Brancloud | 130 | 114 | 160 | 309 | 153 |
| Branhil | 132 | 115 | 160 | 311 | 156 |
| 183* BH-42 | Artificial crossing between Bran Multiflora line with the other germplasm | 121 | 116 | 157 | 317 | 155 |
| 183* BH-46 | | 158 | 298 | | 165 | 312 | 157 |
| 183* Q-12 | | 164 | 301 | | 159 | 313 | 150 |
| 183* Q-18 | | 150 | 316 | | 155 |
| 183* Q-5 | | 150 | 317 | | 153 |
| 183* Q-83 | | 160 | 318 | | 165 |
| 183* Q-85 | | 160 | 319 | | 163 |
| 388* Q-15 | | 155 | 320 | | 163 |
| 388* Q-219 | | 154 | 321 | | 162 |
| 388* Q-284 | | 163 | 322 | | 160 |
| 388* Q-298 | | 160 | 323 | | 157 |
| 388* Q-317 | | 150 | 324 | | 156 |
| 388* Q-4 | | 147 | 325 | | 162 |
| 388* Q-48 | | 160 | 326 | | 152 |
| 388* Q-72 | | 157 | 327 | | 165 |
| 388* Q-76 | | 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 | 154 | 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 | 159 | 370 | 158 |
| d* 388-3 | 126 | 178 | 157 | 371 | 157 |
| d* 388-5 | 132 | 180 | 155 | 372 | 157 |
| d* 388-8 | 129 | 181 | 160 | 373 | 158 |
| E*R-4 | 125 | 182 | 155 | 374 | 158 |
| E*R-5 | 138 | 183 | 134 | 376 | 155 |
| ENBH-11 | 138 | 184 | 155 | 380 | 162 |
| ENBH-14 | 134 | 185 | 155 | 381 | 165 |
| ENBH-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 | Genotype no. | Source |
|--------------|--------|--------------|--------|
| EN*BH-5      | 125    | 187          | 155    |
| K*183-8      | 103    | 189          | 160    |
| Natural crossing between timeline with chinese chrysanthemum | 126 | 190 | 161 |
| 1            | 126    | 190          | 161    |
| 2            | 160    | 191          | 146    |
| 3            | 150    | 192          | 155    |
| 4            | 144    | 193          | 155    |
| 5            | 152    | 194          | 153    |
| 6            | 152    | 195          | 155    |
| 7            | 155    | 196          | 155    |
| 8            | 165    | 197          | 155    |
| 9            | 148    | 198          | 164    |
| 10           | 152    | 199          | 154    |
| 11           | 152    | 200          | 157    |
| 12           | 152    | 201          | 152    |
| 13           | 150    | 203          | 155    |
| 14           | 151    | 204          | 155    |
| 15           | 165    | 205          | 147    |
| 16           | 150    | 206          | 157    |
| 17           | 149    | 207          | 155    |
| 18           | 165    | 208          | 154    |
| 19           | 155    | 209          | 160    |
| 20           | 152    | 210          | 155    |
| 21           | 161    | 211          | 155    |
| 22           | 152    | 212          | 154    |
| 23           | 155    | 214          | 153    |
| 24           | 152    | 215          | 155    |
| 25           | 151    | 217          | 155    |
| 26           | 142    | 218          | 155    |
| 27           | 162    | 219          | 154    |
| 28           | 155    | 220          | 154    |
| 29           | 154    | 221          | 155    |
| 30           | 163    | 222          | 155    |
| 31           | 155    | 223          | 158    |
| 32           | 151    | 224          | 149    |
| 33           | 160    | 225          | 148    |
| 34           | 152    | 226          | 155    |
| 35           | 152    | 227          | 153    |
| 36           | 153    | 228          | 155    |
| 37           | 154    | 230          | 153    |
| 38           | 155    | 231          | 153    |
| 39           | 153    | 232          | 149    |
| 40           | 152    | 233          | 163    |
| 41           | 153    | 234          | 149    |
| 42           | 150    | 235          | 155    |
| 43           | 150    | 236          | 154    |
| 44           | 151    | 237          | 155    |
| 45           | 164    | 238          | 160    |
| 46           | 165    | 239          | 154    |
| 47           | 155    | 240          | 165    |
| 48           | 160    | 243          | 162    |
| 49           | 155    | 244          | 149    |
| 50           | 155    | 245          | 149    |
| 51           | 149    | 246          | 152    |
| 52           | 161    | 247          | 161    |
| 53           | 161    | 248          | 154    |
| 54           | 161    | 249          | 157    |
| 55           | 154    | 250          | 153    |
| 56           | 155    | 251          | 149    |
| 57           | 152    | 253          | 159    |
| 58           | 153    | 254          | 152    |
| 59           | 152    | 255          | 157    |
| 60           | 155    | 256          | 157    |
| 61           | 157    | 257          | 153    |
| 62           | 152    | 258          | 157    |
| 63           | 151    | 259          | 158    |
| 64           | 151    | 261          | 162    |
| 65           | 154    | 263          | 155    |
| 66           | 150    | 264          | 153    |
| 67           | 150    | 265          | 152    |
| 68           | 155    | 266          | 160    |
| 69           | 152    | 267          | 152    |
| 70           | 152    | 268          | 152    |

(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                | 152          | 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    | 13.5    | 12.0     | 11.0    | 10.0    |
| Daylength    | 13.5    | 12.0     | 11.0    | 10.0    |
| Daylength    | 13.5    | 12.0     | 11.0    | 10.0    |
| Daylength    | 13.5    | 12.0     | 11.0    | 10.0    |
| Daylength    | 13.5    | 12.0     | 11.0    | 10.0    |

Supplemental Table 3. The judgment matrix and its consistency check: A−Cij.

| A  | C1   | C2   | C3   | Wt (wi) |
|----|------|------|------|---------|
| C1 | 1/7  | 1/6  | 1    | 0.078   |
| C2 | 1/6  | 1    | 3    | 0.171   |
| C3 | 1    | 1/3  | 1    | 0.750   |

λmax = 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−Pij.

| C1 | P1   | P2   | Wt (wi) |
|----|------|------|---------|
| P1 | 1    | 7    | 0.875   |
| P2 | 1/7  | 1    | 0.125   |

λmax = 2.00, CI = 0, CR = 0 < 0.1.

Supplemental Table 5. The judgment matrix and its consistency check: C2−Pij.

| C2 | P3   | P4   | P5   | P6   | P7   | P8   | P9   | Wt (wi) |
|----|------|------|------|------|------|------|------|---------|
| P3 | 1/2  | 1/4  | 6    | 2    | 2    | 0.110|      |         |
| P4 | 1/2  | 1    | 1/4  | 4    | 1/3  | 1/8  | 3    | 0.0654  |
| P5 | 4    | 4    | 1    | 5    | 1/2  | 2    | 0.529|         |
| P6 | 1/6  | 1/4  | 6    | 4    |     | 1/5  | 2    | 0.043   |
| P7 | 1/2  | 1/8  | 1    | 2    | 1    | 1/9  | 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   |

λmax = 7.79, CI = 0.131, RI = 1.32, CR = 0.0994 < 0.1.

Supplemental Table 6. The judgment matrix and its consistency check: C3−Pij.

| C3 | P10  | P11  | P12  | P13  | P14  | P15  | Wt (wi) |
|----|------|------|------|------|------|------|---------|
| P10| 1    | 4    | 6    | 6    | 1/3  | 1/4  | 0.162   |
| P11| 1    | 1/4  | 1    | 1    | 1/6  | 1/5  | 0.0694  |
| P12| 1/6  | 1/3  | 1/3  | 1    | 1/7  | 1/7  | 0.0414  |
| P13| 1/6  | 1    | 1    | 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   |

λmax = 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            | Dense            | 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         |