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Research Article

Interspecific hybridization with African marigold (*Tagetes erecta*) can improve flower-related performance in French marigold (*T. patula*)

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Abstract

The present research was intended for interspecific hybridization between two male sterile African marigold lines and six self-lines of French marigolds. The results show that as indicated by the full seed number per capitulum, the pollen amount of French marigold pollen influenced the authentic compatibilities reckoned for the 10 cross combinations configured to some amount. Based on the field performance of these interspecific hybrids, it is known that the parental French marigold's single-petaled and silvery flower type may well be improved to a heavy-petaled type while the flower colour remains the same or changes to a lighter version with the same hue. Furthermore, the progeny of certain crossings exceeded the male parents in terms of growth, leaf, and flower-related features, particularly plant height, crown breadth, flower number per plant, and ligulate flower quantity. The most remarkable finding was that, compared to the parental French marigold, most of the hybrid combinations' progeny could blossom 0-11 days earlier. Finally, we identified two excellent hybrid combinations that may be used as a reference for future breeding and commercialization of new marigold varieties.

Keywords: heterosis; interspecific hybridization; phenotypic evaluation; *Tagetes erecta; T. patula*

Introduction

The African marigold (*Tagetes erecta*) is an annual plant in the Marigold genus of the Asteraceae family (Panwar *et al*., 2013; Zhang *et al*., 2020). It is a plant native to Mexico and South America (Sreekala and Raghava, 2003; Cicevan *et al*., 2015) and is commonly cultivated as an ornamental plant worldwide (Vasudevan, *et al*., 1997; Panwar *et al*., 2013; Cicevan *et al*., 2022). It may be used for more than landscaping only; it is widely grown for loose flowers used for religious offerings and making garlands in India (Panwar *et al*., 2018; Kumar *et al*., 2019; Patel *et al*., 2019). It is an important medicinal plant with a diverse

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pharmacological spectrum and medicinally important phytoconstituents (Ai *et al.*, 2016; Shahzadi *et al.*, 2016; Santhosh *et al.*, 2018; Sing *et al.*, 2020). It can be used to extract pigments (Zhang *et al.*, 2018; Wei *et al.*, 2021). Furthermore, several reports have indicated that it has a variety of applications, including its strong resistance to the heavy metal cadmium stress (Liu *et al.*, 2019), and its root secretions have a broad-spectrum inhibitory effect on soil nematodes (Wu *et al.*, 2019; Wang *et al.*, 2020). *T. patula*, popularly known as French marigold, is a sister species of African marigold. It may thrive in a variety of soil and climate conditions (Pan, 2014; Ai *et al.*, 2015; He *et al.*, 2006). Because of its short maintenance time, abundant blossoms, lengthy flowering period, inconspicuous residual flowers, and ease of cultivation and care, it is one of the most significant annual herbaceous flowers used as bedding plants (Cicevan *et al.*, 2016).

Plant size, flower diameter, and days leading to flowering are all different between African and French marigolds (Zhang *et al.*, 2016). African marigold, for example, is an upright plant with a large capitulum ranging from 7-12 cm in diameter and flowering 80-90 days after sowing, whereas French marigold is a clump-forming plant with comparatively modest flowers ranging from 3-6 cm in diameter and flowering 30-55 days after sowing. An F_1 hybrid seed-production strategy has been employed in marigold cultivars for commercial breeding. In African marigold breeding (Yoshikazu *et al.*, 2019), male sterile lines are used as maternal parents (Yao, 1998; Tian *et al.*, 2007; Wu and Tang, 2013; He *et al.*, 2016), and they are crossed with inbred lines (Zhang *et al.*, 2011). The varieties of French marigolds in the market are inbred varieties, and they are bred with selfing.

The chromosomal levels of the two species' ploidy may be attributed to the two distinct breeding strategies. The African marigold has a chromosomal number of $2n=2x=24$, but the French marigold has a chromosome number of $2n=4x=48$ (Towner, 1961; Zhang, 2006; Zhang *et al.*, 2011; Whankaew *et al.*, 2014). Triploid marigolds are a variety that are crossed by the two species. There is less information about the interspecific breeding of *T. erecta* and *T. patula*. Previous research has revealed that triploid marigolds blossom sooner than their parents and have larger flowers, longer flowering periods, and a higher proportion of ligulate flowers (Watchara, *et al.*, 2008; Zhang *et al.*, 2019). Similarly, the progeny of these triploid hybrids demonstrated considerable hyperparental benefits in terms of floral diameter, resistance, and growth potential (Li *et al.*, 2005). When interspecific hybrids incorporating the benefits of African and French marigolds are developed, it is clear that attractive qualities in marigold will be considerably improved. Furthermore, because of their triploid character, these hybrids might effectively avoid germplasm loss.

Seed manufacturers throughout the world have developed a plethora of commercial African and French marigold cultivars. However, only several series/varieties of interspecific hybrids have been issued, including the 'Zenith' series (6 colors), 'Sunburst' (1 color), 'Konstance' (1 color), 'Huadu' (5 colors), and 'Caiyun' series (4 colors). In addition, only Li *et al.* (2005), He *et al.* (2007), Namita *et al.* (2009) and Zhang *et al.* (2019) have reported on interspecific breeding hybrids between *T. erecta* and *T. patula*. In their research works, they found two favorable hybrid combinations.

For flower beds in landscaping, herbaceous cultivars that are low, compact, have a large number of flowers with continuous bloom and good resistance have been highly recommended. The foregoing needs can be better met by a suitable triploid marigold with superparental features derived through interspecific crosses between the African marigold and French marigold. As a result, we sought to construct 10 crosses utilizing two male sterile lines of African marigold as the female parent and six inbred varieties of French marigold as the male parents in this study. By examining the performance of triploid marigold combinations, the two best parental combinations were selected. This research is critical to meet the diversity requirements of herbaceous plant species in landscaping.

Materials and Methods

Plant materials

The female parents were two male sterile lines of African marigold, 384 and 686, while the male parents were six inbred varieties derived from three French marigold series ('Hot pack', 'Safari', and 'Disco'), specifically 805, 806, 807, 808, 809, and 810. For each parent, Table 1 displays the full phenotypic and origin information.

Seedling nurturing and management

The experiment was conducted in the greenhouse of the Beijing Academy of Forestry and Landscape Architecture. Approximately 200 and 400 seeds of the materials were first sown within 200-hole plugs with a 4:1:1 (V:V:V) substrate of Jeffy peat, vermiculite, and perlite on January 23 and February 20, 2019, respectively. A water-soluble fertilizer with nitrogen, phosphorous, and potassium ratios of 14:0:14 and 20:10:20 was sprayed alternately at 50 to 100 mg/L after seedling emergence.

Approximately one month later, both seedlings were transplanted into larger containers. For the paternal material, we used 12-hole plugs with dimensions of 12 cm × 12 cm, with the substrate supplemented with a controlled-release fertilizer (14-14-14 for N, P, and K). Plastic pots with a diameter of 13 cm × 13 cm were chosen for the maternal parents, and each pot was supplemented with 2-3 g of fertilizer. Watering was done in the traditional way by alternating wet and dry conditions. Throughout the vegetative stage, a 100 mg/L water-soluble compound fertilizer (N:P:K = 20:10:20) was sprayed two to three times. The roots of the plants should be watered after pollination to avoid soaking the blooms. In addition, depending on the flower type, fertile plants with hermaphroditic flowers were eliminated, leaving only the sterile plants as the female for hybridization.

Pollination hybridization and seed collection

For pollination, male sterile lines 686 and 384 (African marigold) each retained 97 and 114 plants, respectively. A total of ten cross combinations were developed. French marigold pollen was collected every day from 10:30 a.m. to 15:00 p.m. using a modified keyboard vacuum cleaner, following which pollen was transported to Petri plates. Pollination began on April 30, with 4-20 inflorescences pollinating each combination. After opening 2 to 3 rounds on the inflorescence's periphery, pollination could be started. The stigma remains gamma-shaped and pollinable at this time. To apply the pollen to the stigma uniformly, homemade tiny brushes were employed, followed by bagging and hanging a label with the hybrid combination and date. Each inflorescence was pollinated three times and once every other day.

The seeds were harvested and separately detailed in gauze bags for drying approximately 20 to 25 days later. Full seed number refers to the number of seeds that contain testa and embryo and developed from ovules. The seed setting rate was calculated by the formula full seed number/flower number of one capitulum × 100%. Finally, the seeds were kept at 4 °C for storage.

Phenotypic observation and measurement for interspecies hybrids

To evaluate morphological changes, interspecific hybrids of marigolds were sown in that year. On July 17th, we sown 10 interspecific hybrids and 6 paternals, and on August 16th, we transplanted them into 32-hole plugs. The same administration and upkeep procedures apply as before.

Before transplanting, the soil was modified with grass charcoal fertilized with a controlled-release fertilizer (N:P:K = 14:13:13) as a base fertilizer and disinfected with chlorothalonil wettable powder. The soil was then tilled at a depth of 15-25 cm using a rotary tiller before being scraped to create a 2 m wide bed. The potassium dihydrogen phosphate fertilizer was mostly applied on September 20 and was well watered thereafter. On September 4th, all of the plants were transplanted to the field, and 20 plants per material were planted.

The germination rate of each combination was investigated at the seedling stage. The majority of the phenotypic research measurements were performed during the seedling and blooming phases. Plant height, plant spread, four leaf-related traits (leaf length, leaf width, leaf blade length and width), and eight flower-related traits (flower type, flower initiation, flower colour, flower size, quantity of flowers per plant, quantity of ligulate florets, outer ligulate floret length and width) were all investigated during the flowering stage. The measurement was performed according to the DUS guideline of marigold (Liu *et al*., 2012; UPOV: TG 246/1). The RHS color chart was used to determine flower colour. A total of twelve plants were chosen at random from each hybrid combination for the measurement.

Data analysis

The SPSS 17.0 program was used to perform analysis of variance (ANOVA) for distinct features of each cross combination, as well as multiple comparisons using the S-N-K technique ($P \leq 0.05$). The mean and heterosis over male-parent (MP) values were calculated by using the formula $100 \times (F_1 - MP)/MP$.

Results

Variation in the seed setting rate and germination rate of hybrids of African-French marigolds

The pollen amount of different males was found to vary substantially. Only the 'Safari' (807 and 808) and 'Disco' (809 and 810) series possess the highest pollen amount, according to our previous study (Table 1), while the 'Hot Pack' (805 and 806) series have the lowest or nearly negligible pollen amount. As a result, the 'Safari' and 'Disco' series were allowed to be allocated more hybrid combinations by satisfying the hybridization criteria in a basic or effective manner. Pollen abundance was linked to flower type, with single flowers being the most plentiful, followed by anemone and crested flowers.

Table 1. Characterization of the main morphological traits of African and French marigolds

Species	*T. erecta* ♀		*T. patula* ♂					
Variety	/	/	Hot pak		Safari		Disco	
Accession	686	384	805	806	807	808	809	810
Pollen amount	/	/	*	*	***	***	*****	*****
Flower type	Apetalous		Crested		Anemone		Single	
Flower color	Yellow		Gold	Orange	Yellow	Orange	Yellow	Orange
RHS Color Chart	5A	4A	14A	24A	9A	23A	13A	25A
Flower initiation (days)	95	90	47	44	49	49	51	47
Source	Beijing Academy of Forestry & Landscape Architecture		Guangzhou HaoMei horticulture Co.Ltd		Xiamen Aiken horticulture Co.Ltd			

Note: The symbols *****, ***, and * denote high, medium, and low pollen levels, respectively.

There were 10 hybrid combinations (Table 2) configured by crossing the six male parents with two female parents, and each of them had 4 to 20 pollinated inflorescences. As shown in Table 2, there were differences in the extreme values of the average number of full seeds in a single inflorescence among the crosses, with the highest in combinations 686×810 and 686×808, both with 103 seeds, and the lowest in 686×805, with 28 seeds.

Table 2. Seed setting rate and germination rate of F_1 hybrids of African-French marigolds

Cross	Capitulum number	Full seeds number/Capitulum		Total seed number		Seed setting rate (%)		Germination rate (%)
		Extreme	Average	Extreme	Average	Extreme	Average	
686×810	11	74~132	103 a	202~262	224 a	33~64	46 a	73
686×809	10	36~157	91 a	175~248	219 a	16~68	42 a	57
686×808	16	52~135	103 a	169~256	214 a	21~65	49 a	79
686×807	20	41~149	96 a	177~257	211 a	22~73	45 a	85
686×806	4	59~107	89 a	199~232	212 a	29~54	42 a	76
686×805	6	14~46	28 b	187~220	206 a	6~21	13 b	62
384×810	8	25~126	81 a	167~278	219 a	15~58	37 a	63
384×809	6	28~139	94 a	203~280	248 a	14~53	38 a	48
384×808	7	56~95	74 a	161~308	248 a	19~56	32 a	52
384×807	9	36~115	79 a	162~335	248 a	11~51	33 a	57

Note: The S-N-K test for differences in means has a significance threshold of 0.05.

The extreme values of each inflorescence seed setting rate differed significantly among the combinations. The average seed setting rate per capitulum was 49% in 686×808, followed by 46% in 686×810, and 45% in 686×807; the lowest was 13% in 686×805, which was substantially different from the other nine combinations. Furthermore, with the exception of the combination 686×805, the average seed setting rate per capitulum of female parent 686 was higher than that of parent 384, but the total number of seeds of the two parents was the opposite. The germination rates of the hybrids ranged from 48% to 85%, with five of them germinating at less than 60%. Combination 686×807 had the highest germination rate of up to 85%, while combination 384×809 had the lowest at 48%.

Variation in vegetative traits of the hybrids of African-French marigolds

Table 3 shows that the combination 384×810 had the maximum plant height of 28.75 cm among the 10 combinations, followed by 384×807 and 686×808 with 27.83 cm and 26.17 cm, respectively. The plant spread of the 10 combinations ranged from 24.17 cm to 40.67 cm, with the cross 384×810 showing the maximum width of 40.67 cm but being no different from the combinations 686×810, 384×809, and 384×808, and 686×806 being the lowest width.

With a leaf length of 12.78 cm, the cross 686×810 had the longest leaf of all the hybrids, followed by the crosses 384×810 and 384×808, which had leaf lengths of 12.65 cm and 12.05 cm, respectively. While the leaf width of 686×810 was the highest at 8.87 cm and that of cross 384×807 was the shortest at 6.59 cm, the two combinations were considerably different from the other eight. The lengths of the terminal leaflet of these crosses varied from 3.94 to 4.87 cm, and the widths of the terminal leaflet varied from 1.21 to 1.72 cm, with the cross of 686×810 and 686×806 being equivalent on this characteristic.

Table 3. Phenotypic variation in the field among the progeny of 10 cross combinations of African-French marigolds

Cross combination	Plant height (cm)	Plant spread (cm)	Leaf-related traits				Flower-related traits						
			Leaf length (cm)	Leaf width (cm)	Terminal leaflet length (cm)	Terminal leaflet width (cm)	Flower initiation (days)	Flower colour	Flower diameter (cm)	Quantity of flowers per plant (n)	Quantity of ligulate florets (n)	Outer ligulate floret length (cm)	Outer ligulate floret width (cm)
686×810	24.75 bc	36.67 de	12.78 c	8.87 d	4.82 b	1.58 bc	44	21A	6.92 c	11 abc	82 b	3.15 c	2.34 b
686×809	23.33 b	31.71 bc	11.05 ab	7.52 abc	4.39 ab	1.37 ab	47	13A	6.68 c	9 ab	86 b	3.17 c	2.33 b
686×808	26.17 bcd	31.83 bc	10.58 ab	7.55 abc	3.96 a	1.21 a	49	17A	6.49 c	11 abc	80 b	2.91 b	2.03 a
686×807	23.29 b	30.67 bc	10.06 a	6.91 ab	3.94 a	1.27 ab	44	7A	6.46 c	10 abc	87 b	3.11 c	2.19 ab
686×806	14.50 a	24.17 a	10.43 ab	7.21 abc	4.39 ab	1.58 bc	40	21A	4.91 a	10 abc	26 a	2.42 a	1.95 a
686×805	16.50 a	28.58 b	10.73 ab	7.17 abc	4.61 b	1.72 c	42	14A	5.43 b	13 c	28 a	2.47 a	1.98 a
384×810	28.75 d	40.67 e	12.65 c	8.21 cd	4.74 b	1.49 abc	40	21A	6.92 c	12 bc	80 b	3.13 c	2.07 a
384×809	24.58 bc	37.75 de	10.17 a	7.37 abc	4.78 b	1.61 bc	40	9A	6.63 c	10 abc	100 b	3.03 bc	2.01 a
384×808	26.00 bcd	39.08 e	12.05 bc	7.86 bc	4.87 b	1.54 b	47	17A	6.68 c	8 a	85 b	2.89 b	1.99 a
384×807	27.83 cd	33.75 cd	10.11 a	6.59 a	4.22 ab	1.48 c	47	7A	7.53 d	12 bc	103 b	3.21 c	2.01 a

Note: The S-N-K test for differences in means has a significance threshold of 0.05.

Variation in floral-related traits of the hybrids of African-French marigolds

Flowering initiation was tracked among each cross. Table 3 indicates that all crosses took 40 to 49 days to blossom from sowing, with the combination 686×806, 384×809, and 384×810 flowering the fastest, all less than 40 days, and the cross 686×808 had the latest blossom, taking 49 days.

We concentrated on comparing the genetic variation of flower-related features in the progeny of the African and French crossings with the male parents since the female as a male sterile line did not have a ligulate inflorescence and was not attractive. In comparison to their corresponding paternals, the African-French marigolds showed some variance in flower type. The F_1 hyrbids that use paternal parents with single flowers, 810 and 809, all have double flowers (Figure 1A-C, 1D-F). As illustrated in Figure 1G-I and 1J-L, this also occurred in the progeny of crossings of the two parents, 807 and 808, which both have double flowers. However, female 686 was crossed with two crested-type males (805 and 806), and their F_1 hybrids maintained the paternal-specific crested type (Figure 1M and 1N). As a result, when African marigold was employed as the female parent, it was able to considerably improve the single and anemone flower type of French marigold.

We also observed that compared to the male parent, the flower color of the Afro-French marigold hybrids remained the same or became lighter. As demonstrated in Table 3 and Figure 1, parental 810 had a floral color of 25A (Figure 1A), whereas the progeny of its cross both had floral colors of 21A (Figure 1B and 1C), which were orange in hue. Paternal 809 itself has a flower color of 13A (Figure 1D), and its progeny have flower colors of 13A (Figure 1E) and 9A (Figure 1F), both of which are yellow. The 808 and 807 flower colors were orange (23A, Figure 1G) and yellow (9A, Figure 1J), respectively, and their progenies had the same orange (Figure 1H and 1L) and yellow (Figure 1K and 1L) flowers, although the latter were lighter in color, 17A (Figure 1H) and 7A (Figure 1K and 1L), respectively. The same lightening occurred in the progenies of the crosses between males 806 (24A) and 805 (14A) and female 686, with the former having 21A orange flowers and the latter having 14A yellow flowers.

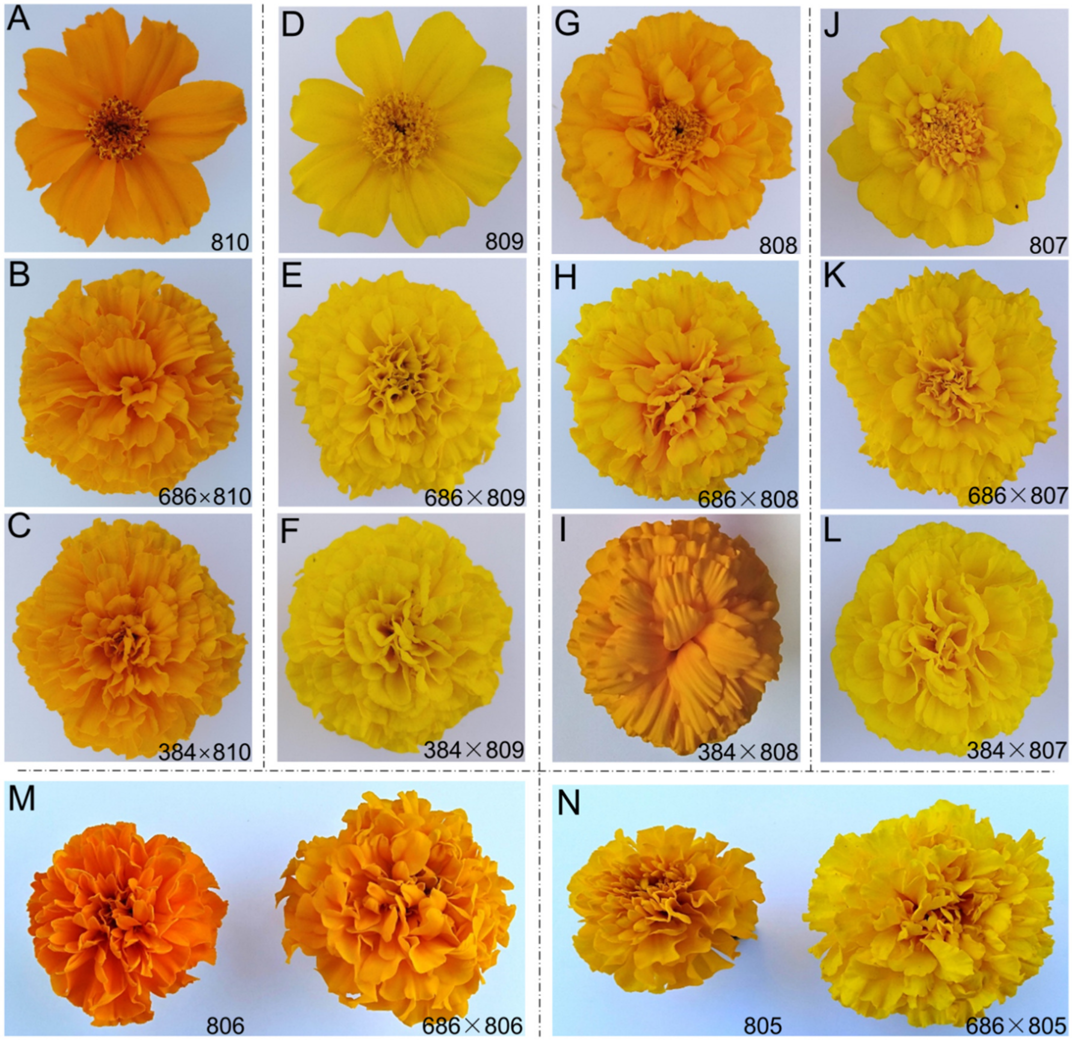

Figure 1. Genetic variation in the floral traits of Afro-French marigold hybrids and their corresponding paternals

We further examined which other flower-related features varied in each interspecies cross offspring. The progeny of combination 384×807 had the largest flower diameter of 7.53 cm among the 10 cross combinations, which was substantially different from the others. The quantity of flowers per plant ranged from 8 to 13, with the combination 686×805 producing the most blooms (13), but was not substantially different from the other combinations except 686×809 (9) and 384×808 (8). The number of ligulate florets in each cross ranged from 80 to 103, with combination 384×807 having the most (103), followed by 384×809 (100), 686×805 (28) and 686×806 (26) having the fewest. The progeny of the combination 384×807 had the longest ligulate floret, measuring 3.21 cm, which was substantially different from 686×805, 686×806, 686×808, and 384×808, but not from other combinations. The outer ligulate floret width of progenies varied from 1.95 cm to 2.34 cm, with combination 686×810 being the widest, followed by combinations 686×809 and 686×807 with 2.33 cm and 2.19 cm, respectively.

Comprehensive assessment of African-French marigold hybrids

On the basis of the foregoing, we may realize that superior phenotypic traits differ among the progenies of African-French marigold hybrids. Hybrids generated by the crossing of 686×810, for example, had the largest terminal leaflet length and width and outer ligulate floret width; the cross of 384×810 had the largest plant height and spread but flowered first; the cross of 384×807 had the largest flower diameter, the largest number of ligulate florets, and the longest outer ligulate floret; and the cross of 686×805 had the highest quantity of flowers per plant and the widest terminal leaflet. Finally, we established that the F_1 hybrids of both combinations 686×807 and 686×808 had better comprehensive performance, as shown in Figure 2A and 2B, on the basis of paternal pollen amount, maternal fertility, seed germination rate, and in-field cultivation performance.

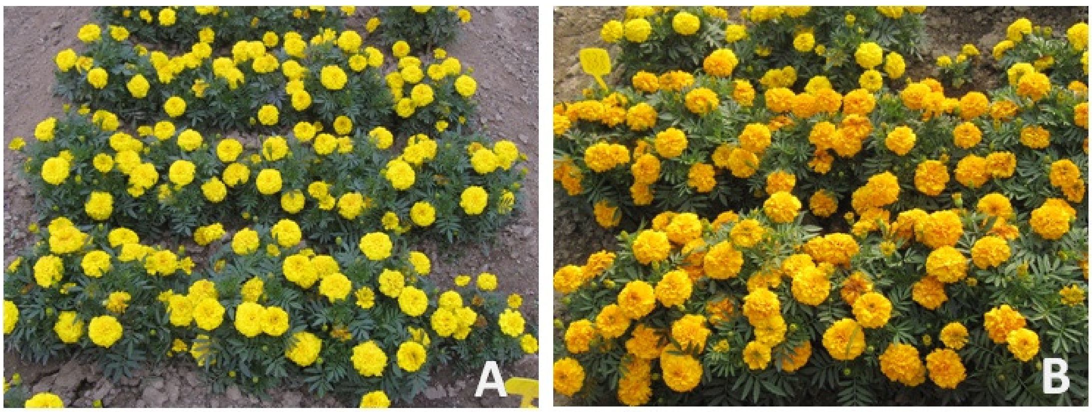

Figure 2. Field performance of two hybrid combinations of African-French marigolds (A: 686×807; B: 686×808)

Heterosis over the male parent (HM%) in morphological traits of African-French marigold hybrids

In comparison to the parents, we assessed the heterosis advantage of 12 traits exhibited by the offspring of 10 combinations (Table 4). Plant height and crown width, two growth-related traits, displayed positive heterosis over the male parents in five and seven out of the ten crosses, respectively. Leaflet width and compound leaf length were observed to have positive HM% in four and five progenies of the cross combinations, respectively, while leaflet length and compound leaf width were observed in nine and ten cross combinations, respectively.

Table 4. Heterosis over the male-parent (HM%) analysis of 12 traits in the progeny of 10 hybrid combinations of African-French marigolds

Cross combination	Plant height (cm)	Plant spread (cm)	Leaf-related traits				Flower-related traits					
			Leaf length (cm)	Leaf width (cm)	Terminal leaflet length (cm)	Terminal leaflet width (cm)	Flower initiation (d)	Flower diameter (cm)	Quantity of flowers per plant (n)	Quantity of ligulate florets (n)	Outer ligulate floret length (cm)	Outer ligulate floret width (cm)
686×810	9.39 bcd	8.78 abc	10.31 bc	17.93 a	37.50 b	36.49 d	-6.4	6.10 c	12.75 ab	1373.88 c	8.81 ab	15.77 ab
686×809	-1.11 abc	-1.56 abc	-3.11 ab	5.12 a	21.95 b	-5.14 abc	-7.8	4.34 bc	27.93 b	998.07 b	24.29 cd	15.85 ab
686×808	-14.36 ab	-14.55 a	-8.28 a	5.12 a	2.26 a	-4.09 abc	0.0	-9.22 a	3.17 ab	200.38 a	-2.22 a	3.71 ab
686×807	-17.90 a	-6.49 ab	-2.58 ab	5.33 a	-5.32 a	-22.43 a	-10.2	-4.93 ab	13.74 ab	156.33 a	13.14 cd	26.13 b
686×806	13.21 cd	2.05 abc	13.98 bc	19.04 a	18.88 b	-14.76 ab	-9.1	8.47 c	-25.12 a	42.57 a	28.34 c	25.31 b
686×805	4.92 abc	5.92 abc	17.78 c	26.16 b	24.95 b	-6.73 abc	-10.6	13.33 d	-26.04 a	34.79 a	24.04 cd	21.79 ab
384×810	29.25 d	20.03 c	9.74 bc	10.00 a	35.63 b	31.92 d	-14.9	6.42 c	21.90 b	1364.82 c	8.20 ab	2.30 ab
384×809	4.79 abc	17.58 bc	-11.14 a	3.17 a	32.11 b	10.49 bcd	-21.6	3.52 bc	32.23 b	1176.04 bc	19.35 cd	0.68 a
384×808	-14.17 ab	4.79 abc	3.84 ab	8.82 a	25.34 b	21.78 cd	-4.1	-6.57 a	-26.48 a	229.83 a	-3.22 a	1.90 ab
384×807	-1.73 abc	2.46 abc	-2.24 ab	0.14 a	0.57 a	-9.94 ab	-4.1	10.84 cd	26.82 b	198.90 a	17.39 cd	15.16 ab

Note: The S-N-K test for differences in means has a significance threshold of 0.05.

Furthermore, flower-related traits such as flower diameter, number of flowers per plant in seven, outer ligulate floret length in eight combinations, and outer ligulate floret width and quantity of ligulate florets in all ten combinations with positive dominance showed superparental hybrid dominance in more offspring of each cross combination. Combination 686×810 had the greatest advantage with 1373.88%, followed by 384×810, 384×809, and 686×809, which were considerably different from other crosses in terms of the quantity of ligulate florets. After crossing, the hybrid of these four combinations, which had single flower paternal parents 809 and 810, developed double flowers, substantially improving the floral type of the paternal parents. The combination 686×808 exhibited no evident benefit for the flower initiation period, while the other nine combinations had a negative advantage, indicating that they all bloomed 0 to 11 days earlier than their paternal parents.

Discussion

To increase hybridization success, distant crossings strive to use plant material that is closely related to parents. For example, the genus Aster is believed to be the most closely related to the genus Chrysanthemum, and natural intergeneric hybrids between cultivated chrysanthemums and the genus Aster are often found (Sun *et al*.,2010). Interspecific crossings between African and French marigolds were established with no prefertilization hurdles in previous research (He, 2010; Zhang *et al*., 2019), but cross affinity was dependent on parental genotypes (Li *et al*., 2005; He *et al*., 2016). *T. patula* L. is an allotetraploid species (2n = 48) that probably originated by hybridization between the diploids *T. erecta* L. and *T. tenuifolia* Cav. or species closely related to them (Towner, 1961). On the other hand, Bao *et al*. (2016) used the male sterile line BY of French marigold as the parent and crossed it with African marigold, finding no affinity between the two species, implying that the sterile line of French marigold is not suitable for breeding interspecific crosses between French and African marigold.

A number of researchers have conducted investigations and evaluated the morphological genetic traits of their hybrids to produce novel interspecific hybrids between African and French marigolds. The F_1 offspring of two crosses between sterile lines of African marigold and French marigold, according to Li *et al*. (2005), displayed superparental traits in terms of growth potential, flower head diameter, and resistance potential. He *et al*. (2007) also found that the triploid of African and French marigolds had outstanding phenotypes and substantial distant hybrid benefits, particularly in terms of large flower head diameter, plentiful blooms, extended flowering time, early initial blooming, and resistance. After examining the genetic interactions and ability to combine flower-related features in interspecific hybrids of African and French marigolds, Namita *et al*. (2011) further selected several parents that should be involved in interspecific hybridization programs by producing triploids that have longer flowering durations. Pan (2014) also found that interspecific hybrids of African and French marigolds not only had a stronger heterosis advantage in terms of the quantity of flowers per plant than intraspecific hybrids of African marigolds but also a superparental advantage in terms of inflorescence diameter and number of ligulate layers. Our earlier research (Zhang *et al*., 2019) similarly demonstrated that the progeny of both crossings exhibited favourable heterosis in flower head diameter and quantity of ligulate florets, as well as plant height and plant spread.

In this study, we used additional crossing combinations, and the findings verified that the hybrid progeny of 10 cross-breeding combinations had a positive heterozygous advantage in the quantity of ligulate florets, compound leaf width, and outer ligulate floret width, with the superiority in the quantity of ligulate florets ranging from 10.02% to 1347.1%. Additionally, the flower type of the progenies was improved from single to double; within that trait of flower initiation, only one combination had no advantage, while the remaining nine combinations had a negative advantage. It takes 40 to 49 days on average for the combinations to flower, and the progenies flower approximately 0 to 11 days earlier than their male parents. All of the research suggests that Afro-French marigold hybrids may successfully combine the best qualities of both to create new marigold strains for future commercial application.

Given the widespread heterosis benefit of F_1 interspecific crosses between African and French marigolds in features such as floral petal ability and early blooming, they nevertheless have some drawbacks, the most serious of which is the low seed setting rate (He *et al*., 2007). According to a previous study, the seed germination rates of hybrids between the two species were as low as 38.67% (Bao *et al*., 2016) and as high as 65% (Zhang *et al*., 2019), and in this research, they ranged from 48% to 85% with just one combination, 686×807, exceeding 80%. As a result, if the two outstanding interspecific hybrid combinations of marigold tested are immediately employed for hybrid seed production, it may be challenging to meet the demand for commercial production.

Previously, Chen *et al*. (1981) reported that the pollen mother cells in 'Papaya crush' ($2n=2x= 24$), a French marigold variety, could not normally undergo mitosis to generate n gametes. This may have resulted from the low frequency of quadrivalent formation and subsequent meiotic abnormalities, such as univalent formation, lagging and unequal chromosome distribution. They also discovered that 50.84% of pollen mother cells of the African marigold variety Bolero ($2n=4x=48$) were multivalent, implying that multivalents were largely generated by intragenomal pairing and little by intergenomal pairing. If confirmed, these findings may match the fact that African and French marigold female gametophytes also produce defective female gametes due to meiotic chromosomal abnormalities. This might explain why, after calculating the number of chromosomes in the F_1 generation of African-French marigold crosses, Li *et al*. (2005) assessed that the hybrids generated were not all 36 ($2n=3x=36$) but 33-36. Therefore, further study should aim to identify marigold hybrid parents that can generate male and female gametes regularly and combine well.

Conclusions

It is necessary to assess hybrid parental combinations before breeding commercial marigold cultivars. In this study, we examined how the decorative features of the hybrid offspring differed between two African marigold females and six French marigold males. Several flower-related variables, such as blossom diameter, number of flowers per plant, and floral type, demonstrated a superparental hybrid advantage in the cross offspring, according to the findings. Additionally, we believe that employing African marigolds as females would enhance the current variation in French marigolds' limited flowering period and single flowering type. We also proposed that more investigation be conducted to identify hybrid parents that can generate male and female gametes on a regular basis and combine properly.

Authors' Contributions

Conceptualization, Z.W. and S.Z.; Formal analysis, H.Z., R.C. and L.L.; investigation, H.X., L.S. and Z.L.; methodology, H.Z. and L.L.; software L.L.; writing-original draft, H.Z. and Z.W.; writing-reviewing editing, Z.W. and S. Z. All authors have read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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