RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
55 (5) 1754-1767, 2023
http://doi.org/10.54910/sabrao2023.55.5.27
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

APETALOID AND PETALOID FEMALE PERFORMANCE ON HORTICULTURAL CHARACTERISTICS OF F_1 AMERICAN MARIGOLD (*TAGETES ERECTA* L.) HYBRIDS

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SUMMARY

This research's objectives focused on using two male-sterile systems, apetaloid and petaloid types, as female parents for the F_1 marigold cut flower hybrid development. These female lines' creation came from the same original line, the gynomonoecious line, with five backcrosses to obtain two female lines, FY1502 and FY1502AP. Their crossing with 10 male lines progressed during the summer from April to August 2016. Then, growing the progenies of 20 crosses and four commercial varieties as checks for characterization and evaluation continued in a lattice design with two replications. Planting them in six blocks contained 10 plants per treatment. The data recorded on eight horticultural characteristics include flower diameter, number of petals per flower, calyx length, peduncle length, flower weight, the number of days from sowing to first flowering, plant height, and plant bush diameter, as well as, the morphology of flowers. The results showed that progenies from two female apetaloid and petaloid with the same male line gave similar outcomes; all progenies produced male sterile double-flowers with golden-yellow flowers, except progenies of MY1501 and MY1502, which created yellow flowers. FY1502 gave the same superior F_1 progenies as FY1502AP in the diameter of the flower. Based on the results, both females could give good characteristics on flower diameter, calyx length, plant height and bush diameter, and early flowering. However, the heterobeltiosis of the F_1 progenies of a few vegetative and reproductive characteristics differed. The FY1502AP gave better progenies in peduncle length heterobeltiosis than those of FY1502 and slightly better for plant bush diameter, whereas FY1502 gave slightly better in flower weight heterobeltiosis than those of FY1502AP. The findings of this study indicate that this apetaloid type could benefit Thailand's marigold seed production program.

Keywords: American marigolds (*Tagetes erecta* L.), F_1 progeny, male sterility, petaloid, apetaloid, horticultural characteristics

Key findings: Two forms of male sterile marigolds, apetaloid and petaloid, could give similar F_1 hybrids. The apetaloid line has an advantage over the petaloid one due to its stability in genetic control. Presently, the petaloid male sterility technique in Thailand is prevalent for hybrid marigold production. The results from this study show that marigold seed production in Thailand could employ this apetaloid type in the seed production program.

Communicating Editor: Dr. Prakit Somta

Citation: Sukwiwat K, Kumchai J, Bundithya W, Potapohn N (2023). Apetaloid and petaloid female performance on horticultural characteristics of F_1 American marigold (*Tagetes erecta* L.) hybrids. *SABRAO J. Breed. Genet.* 55(5): 1754-1767. http://doi.org/10.54910/sabrao2023.55.5.27.

Manuscript received: April 13, 2023; Accepted: August 21, 2023.

INTRODUCTION

American marigolds (*Tagetes erecta* L.), a member of the family Asteraceae, are native species of the Americas, primarily in Mexico and Central America (Tang *et al.,* 2020). The commercial flower is in double form, typically male-sterile, with a high economic value (He *et al*., 2016). For cut flowers, double-type, round-shaped flowers with long stalks are the varieties preferred by consumers because of desirable traits, such as, a longer blooming period, more compact and attractive flowers, and extra shelf life (Giri *et al*., 2019; Bisht *et al.,* 2021). The American marigold has a chromosomal number of 2n=2x=24 (Whankaew *et al.,* 2014). American marigolds have a typical head flower consisting of two morphologically distinct types, with ray (sterile) florets in the periphery and disk (fertile) florets in the center (Zhang *et al*., 2020). Marigold flowers are popular for garland use and decorating arrangements for religious festivals, weddings, and special occasions. The demand for marigold seeds worldwide is around 10,000 kg annually (Anonymous, 2021). Marigold seeds' extensive production has persisted in the northern region, especially in Chiang Mai. These seeds are exported to India, Vietnam, and China (East-West Seed, 2017).

Developing hybrid varieties of marigolds has been for improving ornamental characteristics. Many researchers have studied marigold breeding and selection (Chaudhary *et al.,* 2015; Nilima *et al*., 2017; Lohar *et al*., 2018), but information on improving hybrid varieties is rare. Heterosis has a wide usage among crops for yield and quality advancement. In marigolds, the heterosis expression in the F_1 combination appears as compact and robust growth, double flowers and vibrant color of flowers, profuse flowering, and better adaptation to the environment (Singh and Mirsa, 2017). Most information on heterosis in marigolds focused on ornamental purposes and biochemical extraction (Singh and Vishwakarma*,* 2021).

In seed production, male sterility has proven to highly benefit producing hybrid seeds, which are often superior in quality and yield compared with their parents (Meena and Visarada, 2021). Two types of females in the seed production program of marigolds exist: apetaloid and petaloid. The apetaloid, flower, and capitulum have degenerated rays and disc florets. Petals are purely absent in these flowers. Apetaloid females have better reliability for producing F_1 hybrid seeds commercially in the USA, UK, Holland, and France (Sindhuja *et al.*, 2018). Therefore, commercially apetalous male sterility is popular as it is stable and easily identified. A single recessive, nuclear gene controls male sterility (genic male sterility, GMS) in apetaloid types (Huo *et al.,* 2016).

In contrast, cytoplasmic male sterility (CMS) controls male sterility in the petaloid flower (Xiong *et al*., 2019). In most cases, a double flower results from transforming the anthers into petals. Therefore, the double flower characteristic can illustrate male sterility (Ashok and Velmurugan, 2020). The petaloid female is unstable at high temperatures, and the male sterile lines may become partially fertile due to the petaloid and hermaphrodite flower being present on the same plant, enabling seed setting when self or cross-pollination occurs (Tejaswini *et al*., 2016). This phenomenon became known as a gynodioecious-gynomonoecious sexual system (Soriguer *et al*., 2013). The first report of the gynomonoecious sex form came from Bharathi *et al.* (2014), who observed this in 28 genotypes of American marigolds collected from different sources, and the gynomonoecious sex form emerged in the open pollination cultivar, "Siracole." They described that the gynomonoecious plants showed high variability in the overall proportion of female flowers; in addition, the proportion of female flowers in each plant varied widely on the age of the plant. They suggested that selecting the

gynomonoecious line, which produces high female and low hermaphrodite flowers, would benefit in developing marigold varieties. Tejaswi and Gadre (2016) introduced petaloid male sterile "IIHRMGYP-1" for breeding programs. The genotype can continue by vegetative propagation through tip cuttings. It is a costly production to maintain maternal parents. In Thailand, the petaloid female system is predominant for hybrid marigold production; first propagated from seed, they are hand-pollinated under plastic greenhouses. With petaloid females becoming partially fertile in relatively high temperatures, rouging is necessary to remove partly male sterile plants to avoid contamination in seed production, which is costly for labor.

This study employed two types of male-sterile systems, petaloid and apetaloid, developed from the petaloid one and backcrossed for five generations in the breeding program to compare the outcome of these two different forms. This research's objectives focused on using two male-sterile systems, apetaloid and petaloid types, as female parents for the F_1 hybrid development of cut flowers.

MATERIALS AND METHODS

The marigold breeding programs at Ameriseed Company, Chiang Mai, Thailand, provided the plant materials. They were products of hybridization and selection from 2007 to 2015, with male-sterile plants identified, stabilized, and characterized. The source listing of the two male sterile lines and 10 inbred lines appears in Table 1. The seed propagation helped maintain these within the station. In this study, petaloid female FY1502 (Figure 1A) is a derivative from a "local variety," a gynomonoecious male-sterile plant. When this local variety was selfed, progenies underwent segregation into gynomonoecious male-sterile and petaloid male-sterile plants. Then, repeated selfing of the gynomonoecious male-sterile plants continued for five generations. Maintaining the FY1502 ensued by crossing with a gynomonoecious line (Figure 1B, Figure 2A).

Table 1. Source of two female and 10 male parents of American marigolds.

Stem	Code	Original	Source of material	No. of generation	Parent
1	FY1502AP	F_1 Climax yellow-1	Burpee seed, USA	Backcrossing (BC_5)	Female
2	FY1502	OP, local varieties	Ameriseed Company, Thailand	Sib- mating-5	Female
3	MY 1501	OP, local varieties-yellow-1	Ameriseed Company, Thailand	S_5	Male
4	MY 1502	OP, Crackerjack mixed- yellow-1	Bodger seed, USA	S_5	Male
5	MG 1503	OP, Crackerjack mixed-gold-1	Bodger seed, USA	S_5	Male
6	MG 1504	OP, Crackerjack mixed-gold-2	Bodger seed, USA	S_5	Male
7	MG 1505	Crossed, Crackerjack mixed-gold-1/local varieties-yellow-1	Ameriseed Company, Thailand	S_5	Male
8	MG 1506	Crossed, Climax gold/Crackerjack mixed-gold-1	Ameriseed Company, Thailand	S_5	Male
9	MG 1507	Crossed, Climax yellow/Sierra gold-2	Ameriseed Company, Thailand	S_5	Male
10	MG 1508	Crossed, Inca I gold/Crackerjack mixed-yellow-1	Ameriseed Company, Thailand	S_5	Male
11	MG 1509	Crossed, Marvel gold/Crackerjack mixed-gold-2	Ameriseed Company, Thailand	S_5	Male
12	MG 1510	Crossed, Gold coin/Crackerjack' mixed-gold-2	Ameriseed Company, Thailand	S_5	Male

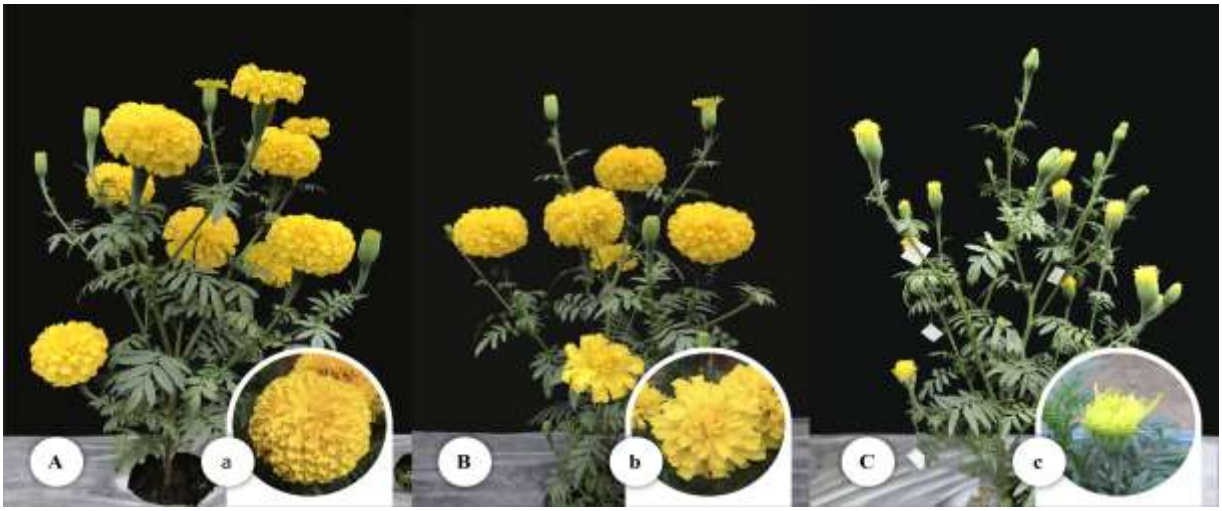

Figure 1. Two male-sterile lines petaloid male sterile (A), maintainer line, gynomonoecious (B), and apetaloid male sterile (C); (a) closed-up flower of a petaloid male sterile, (b) closed-up flower of a gynomonoecious sterile, and (c) closed-up flower of an apetaloid male sterile.

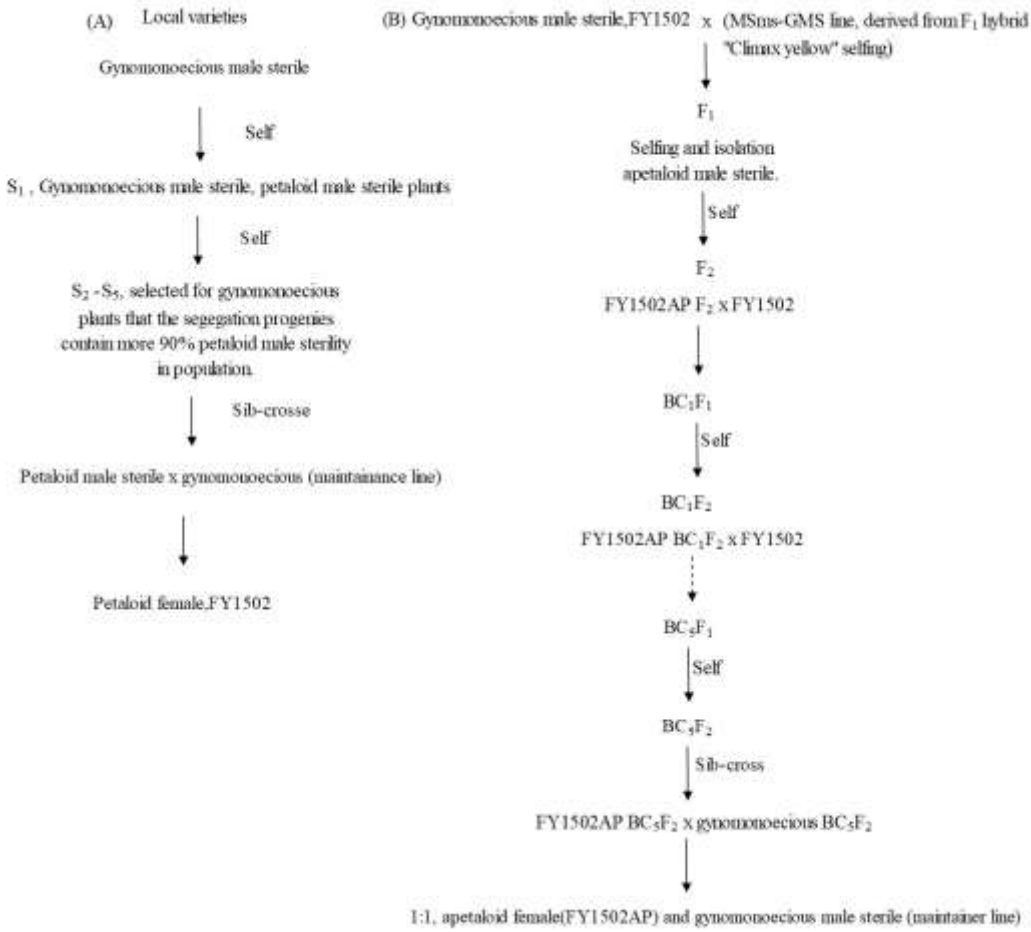

Figure 2. Diagram of petaloid (A) and apetaloid (B) female improvement.

Apetaloid (Figure 1C) came from the cross between FY1502 × MSms-GMS line (derived from F_1 hybrid "Climax yellow" selfing). F_1 of this cross was selfing, and F_2 generation showing apetaloid forms became a choice to backcross with FY1502, with five repetitions. These two female sterile lines, FY1502 and FY1502AP, were the specimens used in this study (Figure 2B). Sowing the seeds of all the parents in nursery beds transpired in January 2015. Transplanting of these seedlings continued a month after seeding. Crop management followed standard practices. The covering of flower buds of male-sterile plants before anthesis contamination prevented undesired pollination. Bagging the male parent also avoided pollination and contamination from others to ensure their progenies. Pollination occurred between the hours of 9:00 AM and 1:00 PM. These two male sterile lines, FY1502 and FY1502AP, gained crossing with 10 male lines. After pollination, the flower's bagging and labeling followed. Each female flower attained pollination for three days to ensure good seeds. Seeds reached maturity in 25–30 days after pollination, afterward, harvested, dried, and stored.

Experimental design and field evaluation

The progenies of 20 crosses and four commercial varieties as checks, grown for characterization and evaluation, continued during the summer from April to August 2016. A lattice design with two replications planted into six blocks had 10 plants per treatment (Karladee, 2002). Seeds sown in a plug tray with 200 holes contained a sowing media of peat moss. After germination, applying liquid fertilizer 15-0-0 at 15 g per 20 l of water occurred twice a week. Thirty-day-old seedlings' transplanting in a field consisted of 1 m × 3 m plots with two rows per plot with spacing of 30 cm × 30 cm. Two tons of cow manure, in addition to 31.25 kg/ha of fertilizer (15-15-15), achieved mixture into the soil twice at the beginning and 20 days after transplanting. After that, applying 15–20 g of fertilizers (0-52-34 and 13-0-46) per 20 l of water progressed at the flower bud stage. Other applications included insecticides (Cypermethrin, Methomyl, and Carbosulfan) and a fungicide (Captan). The soil type was sandy loam, and the average temperature (April to August 2016) was 33 °C to 28 °C. The rainfall in 2016 was 1256.4 mm. The light intensity (April to August) was 17.0 to 14.57 MJ/m2/day.

The morphology of the flowers' recording started from the plants at the first flowering stage to the complete flowering phase, with five plants randomly selected from each replication. Data recording comprised the flower color and form and the presence of fertility flowers. The flower color intensity measurement of the American marigold flowers included observing the petal color of freshly opened florets using the Royal Horticultural Society (RHS) color chart (2015) (Figure 3). The data for the eight quantitatively horticultural characteristics consisted of flower diameter (cm), number of petals per flower (petals), calyx length (cm), peduncle length (cm), flower weight (g), number of days from sowing to first flowering (days), plant height (cm), and plant bush diameter (cm).

Data analysis

Mid-parent (MP) heterosis calculation used the formulas MP (%) = (F_1 -MP)/MP × 100 and, with heterobeltiosis (HB), the formulas, HB (%) = (F_1 - BP)/BP × 100, (BP = better parent) (Lou *et al*., 2010). Duncan's Multiple Range Test (DMRT) measured the mean values of each trait to determine the significant differences and $p \leq 0.01$ among genotypes for the mean values for each of the horticultural characteristics (Kempthorne, 1957).

RESULTS

Flower morphology

The flower morphology of 12 parental lines and 20 progenies' list is in Table 2. Color variation observation used the RHS color chart. Compared with the female lines, the flower color of the progenies remained constant or altered depending on the male color. These

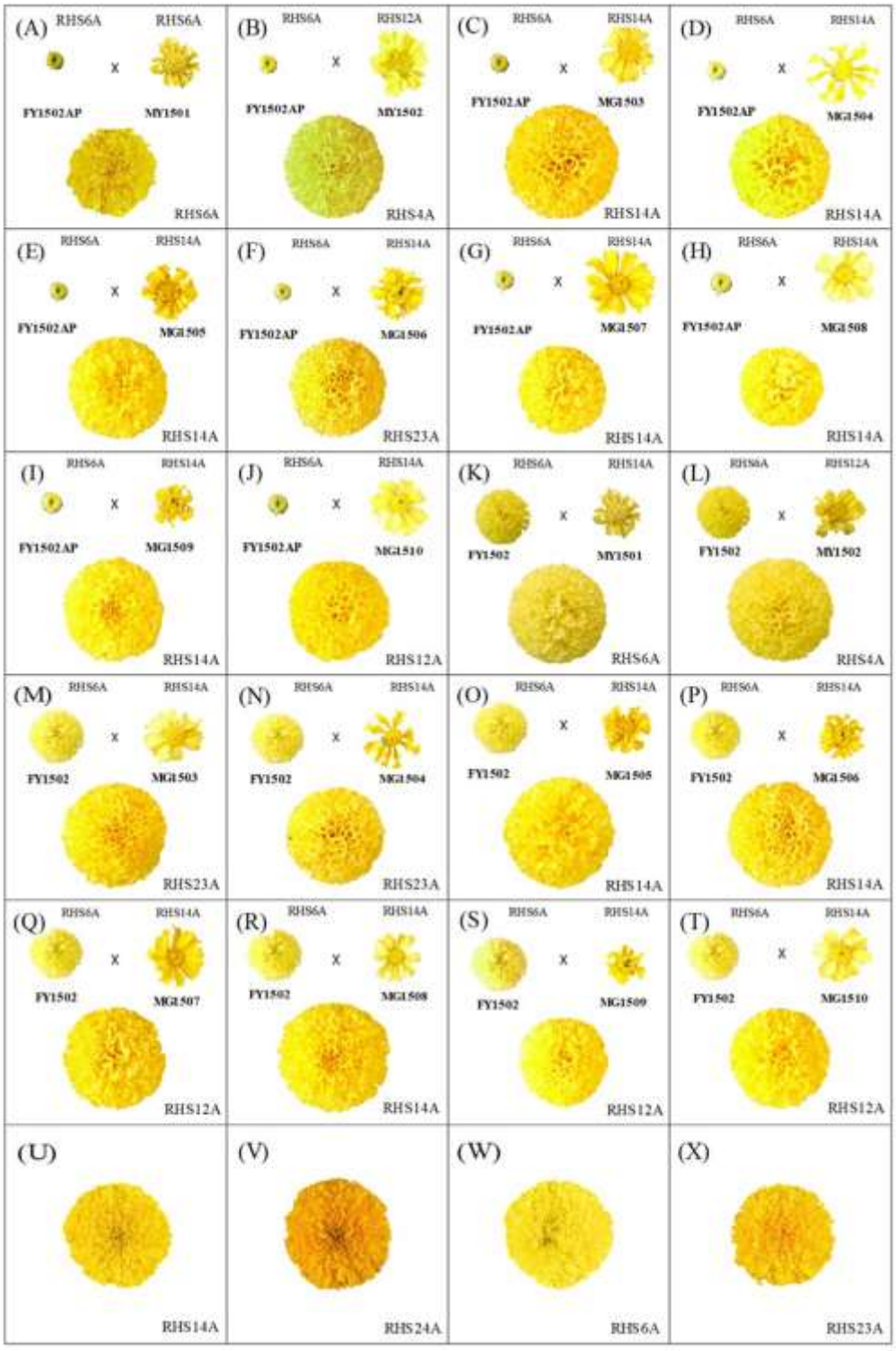

Figure 3. Flowers and the Royal Horticultural Society (RHS) color of parental lines and their progenies, (A)-(J): the flowers of progenies derived from the apetaloid female parent crossed with 10 male parents, (K)-(T): flowers of progenies derived from the petaloid female parent crossed with 10 male parents, and (U)-(X): commercial varieties.

Table 2. Morphology of flower characteristics in parental lines and F_1 progenies of American marigold.

Item	Hybrids	Flower color	RHS color*	Flower form	Presence of fertile flower
F_1 progenies					
1	FY1502AP × MY1501	Yellow	RHS6A	Double	All male sterile
2	FY1502AP × MY1502	Yellow	RHS4A	Double	All male sterile
3	FY1502AP × MG1503	Golden-yellow	RHS14A	Double	All male sterile
4	FY1502AP × MG1504	Golden-yellow	RHS14A	Double	All male sterile
5	FY1502AP × MG1505	Golden-yellow	RHS14A	Double	All male sterile
6	FY1502AP × MG1506	Golden-yellow	RHS23A	Double	All male sterile
7	FY1502AP × MG1507	Golden-yellow	RHS14A	Double	All male sterile
8	FY1502AP × MG1508	Golden-yellow	RHS14A	Double	All male sterile
9	FY1502AP × MG1509	Golden-yellow	RHS14A	Double	All male sterile
10	FY1502AP × MG1510	Golden-yellow	RHS12A	Double	All male sterile
11	FY1502 × MY1501	Yellow	RHS6A	Double	All male sterile
12	FY1502 × MY1502	Yellow	RHS4A	Double	All male sterile
13	FY1502 × MG1503	Golden-yellow	RHS23A	Double	All male sterile
14	FY1502 × MG1504	Golden-yellow	RHS23A	Double	All male sterile
15	FY1502 × MG1505	Golden-yellow	RHS14A	Double	All male sterile
16	FY1502 × MG1506	Golden-yellow	RHS14A	Double	All male sterile
17	FY1502 × MG1507	Golden-yellow	RHS12A	Double	All male sterile
18	FY1502 × MG1508	Golden-yellow	RHS14A	Double	All male sterile
19	FY1502 × MG1509	Golden-yellow	RHS12A	Double	All male sterile
20	FY1502 × MG1510	Golden-yellow	RHS12A	Double	All male sterile
Males					
23	MY 1501	Yellow	RHS6A	Single ray floret	All fertile
24	MY 1502	Yellow	RHS12A	Single ray floret	All fertile
25	MG 1503	Golden yellow	RHS14A	Single ray floret	All fertile
26	MG 1504	Golden yellow	RHS14A	Single ray floret	All fertile
27	MG 1505	Golden yellow	RHS14A	Single ray floret	All fertile
28	MG 1506	Golden yellow	RHS14A	Single ray floret	All fertile
29	MG 1507	Golden yellow	RHS14A	Single ray floret	All fertile
30	MG 1508	Golden yellow	RHS14A	Single ray floret	All fertile
31	MG 1509	Golden yellow	RHS14A	Single ray floret	All fertile
32	MG 1510	Golden yellow	RHS14A	Single ray floret	All fertile
Females					
33	FY1502AP	Yellow	RHS6A	Apetaloid	50% of fertile
34	FY1502	Yellow	RHS6A	Mixture petaloid, gynomonoecious	10% of fertile

Note: * The Royal Horticultural Society (RHS) color chart.

progenies could have two flower color groups: 1) yellow and 2) golden-yellow. The results showed that progenies generated from MY1501 and MY1502 produced yellow flowers in shades 4A and 6A, respectively, but other males, MG1503 through MG1510, produced golden-yellow flowers in shades 12A, 14A, and 23A. Comparing the genetic variations in flower-related characteristics of the progenies of the two female lines, apetaloid and petaloid, and crossing with single-flower male parents, all progenies had male sterile double flowers, as seen in Figures 3(A)-3(J) and 3(K)-3(T), like the commercial variety in Figures 3(U)-3(X).

Mean performance of parents and hybrids

Reproductive characteristics

The reproductive characteristics of flower diameter, number of petals per flower, calyx length, the peduncle length of the F_1 progeny, parental lines, and commercial varieties were significantly different. The flower diameter of

the progenies of all crosses differed from the commercial varieties (Table 3). However, the flower diameter of all progenies was significantly larger than those of the male and all-female parents. The number of petals of the progenies of FY1502 × MG1505 and commercial No. 4 had the maximum number of petals, and they were significantly different from the other progenies, as well as, commercial varieties number 1-3. In addition, the other progenies had more petals than the female parents. The peduncle length of the progenies of FY1502AP showed slightly better stretch than those of some commercial varieties and parents. The calyx length of the progenies of FY1502 × MG1505 differed nonsignificantly from the commercial varieties numbers one, two, and four. However, it was substantially longer than the other progenies, all the male parents and one of the female parents. The flower weights of the progenies of FY1502 × MG1505 were the heaviest and significantly different from others, except for FY1502AP × MG1505. The number of days from sowing to the first flowering varied among progenies, parental lines, and commercial varieties.

Vegetative characteristics

The plant height of the progenies of FY1502 × MG1505 was not significantly different from other progenies. However, it was notably taller than the female parents, most of the male parents, and some commercial varieties. Meanwhile, the progenies of FY1502AP × MG1505 were not substantially different from the other progenies and the commercial collections. Additionally, the plant bush diameter of all crosses differed nonsignificantly from F_1 progenies and market selections. However, it was significant for most male and female parents.

Heterosis and heterobeltiosis

The heterosis and heterobeltiosis percentages of the horticultural characteristics in American marigolds, 20 progenies derived from two female parents and 10 male lines, are available in Table 4. The heterosis and heterobeltiosis in the reproductive characteristics, flower diameter, flower weight, calyx length, and peduncle length were significantly different positively; however, the number of days from sowing to first flowering days was negative. The maximum flower diameter heterosis showed in FY1502AP × MG1505 progeny at 99.55% and 88% heterobeltiosis. FY1502AP showed significant heterosis and heterobeltiosis in the flower diameter of progenies of 10 and eight crosses, respectively. FY1502 provided significant heterosis in 10 and eight crosses. Heterosis and heterobeltiosis results showed positive and negative values in calyx length. Progenies of FY1502AP × MG1510 had significant positive heterosis and heterobeltiosis (34.19%), while FY1502AP × MG1505 had positive heterosis and heterobeltiosis (27.15%). Using FY1502AP as the female parent resulted in positive heterosis and heterobeltiosis in progenies of eight and four crosses, respectively. FY1502 showed positive and significant heterosis in five and six crosses. Peduncle length gave the highest positive, significant heterosis in progenies of FY1502AP × MG1505 with 92.73% heterosis and 68.16% heterobeltiosis. When using FY1502AP as a female parent, positive heterosis and heterobeltiosis also resulted in the peduncle length of progenies of 10 and eight crosses, respectively. However, FY1502 could give positive and significant heterosis of this character in 10 and five crosses, respectively. The weight per flower heterosis and heterobeltiosis in F_1 progenies differ significantly. Progenies of FY1502 × MG1508 showed the highest heterosis and heterobeltiosis at 206.24% and 187.75%, respectively. When using FY1502AP as the female parent, significant positive weight per flower heterosis was evident in progenies of nine and seven crosses. Progenies of FY1502 showed significant positive heterosis in 10 and eight crosses.

Concerning the time to the first flowering, the progenies of FY1502AP × MG1504 gave the highest negative heterosis (-17.71%), and those of FY1502AP × MG1507 and FY1502AP × MG1508, which gave negative significant heterobeltiosis (-19.94%). FY1502AP, as a female parent, showed

Table 3. Horticultural characteristics of F_1 progenies, parental lines, and commercial varieties of American marigolds.

Item	Hybrids			FD	FP	CL	PL	FW	FF	PH	PB
F_1 progenies											
1	FY1502AP	×	MY1501	7.40^{a-f}	232.0^{e-f}	1.59^{c}	10.87^{b-e}	9.00^{e-k}	71.60^{d-g}	60.80^{b-f}	49.20^{b-e}
2	FY1502AP	×	MY1502	7.70^{a-e}	266.2^{c-g}	1.81^{b-c}	10.34^{b-g}	14.24^{b-e}	75.10^{b-g}	73.50^{a-c}	52.30^{a-d}
3	FY1502AP	×	MG1503	8.60^{a-b}	344.9^{b-d}	1.97^{a-c}	11.02^{a-e}	15.42^{b-d}	70.70^{e-g}	73.70^{a-c}	57.50^{a-c}
4	FY1502AP	×	MG1504	8.00^{a-d}	312.0^{b-f}	1.68^{c}	9.88^{b-g}	7.95^{e-l}	64.70^{g}	57.70^{b-f}	50.20^{a-e}
5	FY1502AP	×	MG1505	7.80^{a-d}	458.9^{a}	2.43^{a}	13.52^{a-b}	19.26^{a-b}	81.20^{a-e}	83.60^{a}	69.10^{a}
6	FY1502AP	×	MG1506	7.70^{a-e}	261.7^{c-g}	1.64^{c}	8.97^{c-i}	11.83^{c-g}	72.70^{d-g}	55.60^{c-g}	41.20^{c-f}
7	FY1502AP	×	MG1507	6.80^{a-g}	243.5^{d-g}	1.58^{c}	11.79^{a-e}	9.03^{e-k}	73.10^{d-g}	60.50^{b-f}	45.20^{b-f}
8	FY1502AP	×	MG1508	6.60^{a-g}	290.5^{b-f}	1.74^{c}	9.21^{c-i}	10.70^{c-j}	69.70^{e-g}	50.30^{e-h}	46.50^{b-f}
9	FY1502AP	×	MG1509	7.90^{a-d}	304.0^{b-f}	1.84^{b-c}	10.77^{b-f}	12.70^{c-g}	75.50^{b-g}	77.30^{a-b}	61.70^{a-b}
10	FY1502AP	×	MG1510	8.00^{a-d}	338.0^{b-e}	1.92^{a-c}	11.00^{a-e}	10.90^{c-i}	68.00^{f-g}	70.50^{a-d}	64.00^{a-b}
11	FY1502	×	MY1501	8.00^{a-d}	249.2^{d-g}	1.69^{c}	11.60^{a-e}	9.98^{c-j}	70.50^{e-g}	68.70^{a-e}	56.50^{a-c}
12	FY1502	×	MY1502	8.50^{a-b}	343.9^{b-e}	2.07^{a-c}	11.72^{a-e}	13.00^{c-g}	73.80^{c-g}	75.10^{a-c}	62.20^{a-b}
13	FY1502	×	MG1503	8.50^{a-b}	297.4^{b-f}	1.53^{c}	11.70^{a-e}	14.20^{b-e}	71.00^{d-g}	72.80^{a-c}	51.20^{a-e}
14	FY1502	×	MG1504	8.30^{a-c}	277.6^{b-f}	1.81^{b-c}	11.19^{a-e}	12.50^{c-g}	64.70^{g}	51.80^{d-h}	50.80^{a-e}
15	FY1502	×	MG1505	9.10^{a}	371.7^{a-c}	2.35^{a-b}	14.54^{a}	22.20^{a}	72.80^{d-g}	71.80^{a-d}	57.60^{a-c}
16	FY1502	×	MG1506	8.50^{a-b}	252.5^{d-g}	1.71^{c}	9.56^{c-h}	12.14^{c-g}	71.50^{d-g}	61.80^{b-f}	48.10^{b-f}
17	FY1502	×	MG1507	7.80^{a-d}	267.5^{c-g}	1.79^{b-c}	12.48^{a-c}	10.90^{c-i}	72.70^{d-g}	71.00^{a-d}	54.10^{a-c}
18	FY1502	×	MG1508	8.80^{a}	267.6^{c-g}	1.80^{b-c}	10.66^{b-f}	16.00^{b-c}	69.70^{e-g}	60.20^{b-f}	49.50^{b-e}
19	FY1502	×	MG1509	6.30^{a-g}	261.8^{c-g}	1.64^{c}	9.67^{c-h}	8.59^{e-l}	74.70^{c-g}	71.50^{a-d}	56.80^{a-c}
20	FY1502	×	MG1510	8.00^{a-d}	260.9^{c-g}	1.66^{c}	10.77^{b-f}	11.60^{c-h}	70.50^{e-g}	66.60^{a-e}	53.10^{a-c}
Males											
21	MY1501			4.59^{f-g}	332.9^{b-e}	1.64^{c}	8.29^{d-j}	4.63^{j-l}	79.00^{a-f}	44.80^{f-h}	41.30^{c-f}
22	MY1502			5.40^{c-g}	267.5^{c-g}	1.73^{c}	6.94^{g-k}	7.08^{g-l}	74.20^{c-g}	44.00^{f-h}	32.60^{e-f}
23	MG1503			8.60^{a-b}	291.4^{b-f}	1.92^{a-c}	10.77^{b-f}	13.20^{c-g}	83.70^{a-d}	75.50^{a-c}	53.60^{a-c}
24	MG1504			5.40^{c-g}	249.3^{d-g}	1.50^{c}	5.33^{j-k}	2.87^{l}	80.80^{a-f}	43.30^{f-h}	29.70^{f}
25	MG1505			4.80^{e-g}	338.2^{b-e}	1.91^{a-c}	5.98^{i-k}	10.20^{c-j}	88.50^{a}	63.10^{b-f}	41.70^{c-f}
26	MG1506			4.70^{f-g}	306.2^{b-f}	1.94^{a-c}	6.93^{g-k}	9.61^{d-j}	81.00^{a-e}	61.00^{b-f}	48.60^{b-e}
27	MG1507			8.20^{a-c}	260.0^{c-g}	1.69^{c}	8.02^{e-k}	13.40^{b-f}	81.30^{a-e}	51.60^{d-h}	50.30^{a-e}
28	MG1508			4.50^{f-g}	304.4^{b-f}	1.89^{a-c}	4.73^{k}	4.92^{i-l}	78.00^{a-f}	35.80^{h}	33.80^{d-f}
29	MG1509			4.20^{g}	333.0^{b-e}	1.68^{c}	6.27^{h-k}	3.14^{k-l}	87.30^{a-b}	37.80^{g-h}	46.80^{b-f}
30	MG1510			5.00^{d-g}	325.8^{b-e}	1.57^{c}	7.12^{f-k}	8.50^{e-l}	86.20^{a-c}	48.80^{e-h}	54.00^{a-c}
Females											
31	FY1502AP			4.20^{g}	206.0^{f-g}	1.76^{c}	8.04^{e-k}	7.64^{f-l}	76.50^{a-g}	63.10^{b-f}	47.30^{b-f}
32	FY1502			5.60^{b-g}	163.9^{g}	1.59^{c}	11.33^{a-e}	5.59^{h-l}	79.00^{a-f}	58.80^{b-f}	45.20^{b-f}
Commercial varieties											
33	Commercial 1			8.40^{a-c}	271.4^{c-g}	1.87^{a-c}	11.88^{a-d}	13.40^{b-f}	72.50^{d-g}	71.20^{a-d}	57.60^{a-c}
34	Commercial 2			8.30^{a-c}	256.5^{d-g}	1.73^{c}	11.20^{a-e}	12.20^{c-g}	70.20^{e-g}	58.70^{b-f}	50.20^{a-e}
35	Commercial 3			8.00^{a-d}	283.3^{b-f}	2.00^{a-c}	10.83^{b-e}	11.90^{c-g}	69.20^{e-g}	65.80^{a-e}	60.30^{a-c}
36	Commercial 4			8.20^{a-c}	382.2^{a-b}	2.00^{a-c}	9.58^{c-h}	12.00^{c-g}	72.10^{d-g}	58.50^{b-f}	46.50^{b-f}
	Mean			7.1	291.0	1.8	9.9	10.9	74.9	61.6	50.5
	F-test[2]			**	**	**	**	**	**	**	**
	C.V. (%)			13.05	10.6	8.9	12.06	18.9	4.46	9.61	10.11
	DMRT (0.01)			2.58	83.8	0.43	3.31	5.75	9.1	16.13	13.92

Note[1]: FD = flower diameter (cm), FP = number of petals per flower (petals), CL= calyx length (cm), PL = peduncle length (cm), FW = flower weight (g), FF = number of days from sowing to first flowering (days), PH = plant height (cm), PB = plant bush diameter (cm).
Note[2]: *, ** significant difference at $p \leq 0.05$ and $p \leq 0.01$ levels, respectively.

Table 4. Heterosis and heterobeltiosis percentage of 20 progenies derived from two female parents and 10 male lines.

Item	F_1 progenies	FD		No. FP		CL		PL		FW		FF		PH		PB	
		MP	HB	MP	HB	MP	HB	MP	HB	MP	HB	MP	HB	MP	HB	MP	HB
1	FY1502AP × MY1501	68.02**	60.24**	-13.87	-30.29	-6.16**	-9.35**	33.19**	31.18**	46.82**	17.88**	-7.88*	-9.34	12.73*	-3.56	10.99	3.96
2	FY1502AP × MY1502	59.44**	41.2**	12.46	-0.47	4.63**	2.83**	38.16**	28.67**	93.55**	86.57**	-0.33	-10.76*	37.22**	16.44	30.94**	10.55
3	FY1502AP × MG1503	33.93**	-0.7	38.68	18.36	3.71**	2.59**	17.28**	2.41	47.57**	16.24	-11.7*	-1.8	6.4	-2.32	13.86	7.23
4	FY1502AP × MG1504	66.46**	47.72**	37.08	25.16	24.62**	-4.53**	47.72**	22.82**	51.45**	4.19	-17.71**	-6.49	8.45	-8.51	30.31**	6.07
5	FY1502AP × MG1505	99.55**	88**	68.63	35.66	7.17**	27.15**	92.73**	68.16**	116.09**	88.92**	-1.52	-15.52*	32.48**	32.48*	55.12**	45.91**
6	FY1502AP × MG1506	73.33**	63.71**	2.2	-14.53	-12.62**	-15.42**	19.95**	11.63*	37.2**	23.1*	-7.62	-15.22*	-10.37	-11.88	-14.06*	-15.17
7	FY1502AP × MG1507	9.95**	-16.93**	4.54	-6.31	2.9**	-9.92**	46.82**	46.64**	-14.12**	-32.59**	-7.36	-19.94**	5.45	-4.16	-7.42	-10.17
8	FY1502AP × MG1508	52.24**	47.52**	13.85	-4.56	17.23**	-7.92**	44.24**	14.55*	71.87**	41.26**	-9.71	-19.94*	1.77	-20.2*	15.08	-1.32
9	FY1502AP × MG1509	90.24**	89.56**	12.81	-8.71	32.34**	4.53**	50.59**	34.02**	136.58**	66.86**	-7.86	-8.19	53.22**	22.57*	31.03**	30.34*
10	FY1502AP × MG1510	75.4**	61.04**	27.11	3.73	34.19**	9.07**	46.19**	37.87**	35.23**	28.35*	-16.44*	-17.66*	25.89**	11.68	26.26**	18.52*
11	FY1502 × MY1501	57.56**	43.76**	0.32	-25.14	-11.32**	3.04**	18.27**	2.38*	95.5**	78.62**	-10.76*	-10.19	32.53**	16.77	30.45**	24.86**
12	FY1502 × MY1502	53.67**	51.39**	59.46*	28.58	-3.39**	19.6**	28.41**	3.48*	106**	84.26**	-3.59	-11.73*	46.05**	27.6*	59.87*	37.57
13	FY1502 × MG1503	20.08**	-1.22	30.66	2.07	-8.09**	-20.21**	5.9**	3.26*	50.64**	7.05	-12.75**	-10.14	8.47*	-3.48	3.67	-4.43
14	FY1502 × MG1504	50.46**	47.89**	34.36	11.35	9.12**	14.11**	34.29**	-1.28	195.98**	123.97**	-19*	-10.6	1.47	-11.89	35.67**	12.43
15	FY1502 × MG1505	76.84**	63.25**	48.03	9.88	*-4.64**	22.98**	67.91**	28.32**	181.63**	117.99**	-12.99*	-10.58	17.83**	13.86*	32.47**	27.35*
16	FY1502 × MG1506	65.27**	52.2**	7.41	-17.55	3.15**	-12.08**	4.76	-15.62	59.8**	26.38**	-10.62*	-11.71	3.23	1.43	2.53	-1.03
17	FY1502 × MG1507	17.32**	-1.29	26.22	2.9	6.8**	5.9**	28.96**	10.1*	15.72**	-17.99*	-9.28	-13.59*	28.51*	20.59	13.2	7.44
18	FY1502 × MG1508	76.21**	58.4**	14.28	-12.1	-0.15	-5.01**	32.77**	-5.91	206.24**	187.75**	-11.15*	-14.45	27.18**	2.34	25.12*	9.39
19	FY1502 × MG1509	30.62**	13.84**	5.39	-21.37	15.27**	-2.96**	9.8**	-14.73*	97.02**	53.76**	-10.14	-21.16*	47.8**	21.44*	23.47*	21.33
20	FY1502 × MG1510	52.11**	44.12**	6.56	-19.92	5.05**	4.39**	16.68**	-4.98	65.44**	37.12**	-14.67*	18.26*	23.67*	13.16	7.05	-1.62

Note[1]: FD = flower diameter (cm), FP = number of petals per flower (petals), CL= calyx length (cm), PL= peduncle length (cm), FW = flower weight (g), FF = number of days from sowing to first flowering (days), PH = plant height (cm), PB = plant bush diameter (cm), MP = mid-parent heterosis, HB = heterobeltiosis.
Note[2]: *, ** significant difference at $p \leq 0.05$ and $p \leq 0.01$ levels, respectively.

negative heterosis and heterobeltiosis in progenies of four and six crosses, respectively, and FY1502 showed significant heterosis in seven and four crosses. Most progenies' heterosis in the vegetative characteristics, plant height, and plant bush diameter was significantly different. For the plant height, progenies of FY1502AP × MG1509 showed the highest positive and significant heterosis and heterobeltiosis with a plant height of 53.22% and 22.57%, respectively. Progenies of FY1502AP showed positive plant height heterosis in five crosses and heterobeltiosis in two crosses. FY1502, used as the female parent, gave significant heterosis in eight and three crosses. Progenies of FY1502 × MY1502 showed 59.87% heterosis in plant bush diameter, while FY1502AP × MG1505 had 45.91% heterobeltiosis. FY1502AP, as a female parent, showed significant heterosis in progenies from five and three crosses for plant bush diameter. When using FY1502 as the female parent, six crosses showed significant heterosis and one heterobeltiosis.

DISCUSSION

The plant and flower characteristics of two females, 10 males, 20 progenies, and commercial varieties underwent investigation. Horticultural characteristics revealed that 12 parents, including females and males, differed in some traits, such as, flower size and flower form; however, when crosses ensued, progenies from these 12 parents had similar characteristics, such as, flower color and double flowers. Marigold flowers are generally large with bright shades, ranging from yellow to orange, and suitable for various purposes such as, garlands, cut flowers, decorations, ceremonial functions, and landscape gardening (Ramappa *et al*., 2022). The flower color determination used the RHS color chart. In the presented study, two major colors were evident: golden-yellow in crosses using MG1503 to MG1510 as male parents and yellow in crosses using MY1501 and MY1502 as male parents. It indicated that the color of progenies has expression according to the male parent. Pludtongsri and Potapohn (2016) reported that the genes governing marigold flower color may have influences from three or more genes. Yellow gold and orange petal colors may have the control of genes Y, G, and O, respectively. Furthermore, it could also involve epistasis expression.

Male sterility is a valuable approach in the seed production of marigolds (Hou *et al*., 2016). This study employed two forms of male-sterilize system in the breeding program. Genetic controlling male sterile was different. Sukwiwat *et al*. (2021) studied the genetic background of two different male sterility lines in American marigolds, apetaloid and petaloid male sterility sterile. The results indicated that F_1 and gynomonoecious self-fertilization showed that CMS determines male sterility in the case of petaloid flowers. On the other hand, controlling and governing apetaloid flowers by GMS as F_2 and gynomonoecious self-fertilization has produced the predicted relationship ratio.

Progenies from petaloid females, FY1502, had all fully double flower forms. A fully double flower is valuable in many species, such as, carnations, roses, and lilies (Wang *et al*., 2020). Double flowers without disc florets are desirable in commercial marigold production (Bisht *et al*., 2021). Tejaswini *et al*. (2016) found that crossing petaloid sterile flowers with the fertile line resulted in 100% petaloid sterile progenies, demonstrating that male sterility can be inherited cytoplasmically. FY1502AP's generation came from repeated backcrossing with FY1502 female lines for five generations continuously. The backcrossed population plants having similar flower morphology to the recurrent parent in each generation gained selecting and backcrossing with the recurrent parent line.

The results showed that the flower morphology of two female varieties, except for flower form, had the same plant morphology; there was no statistically significant difference in the horticulture characteristics, especially flower diameter, number of petals, calyx length, flower stalk length, flower weight, number of days from seed sowing to first flowering, plant height, and bush diameter. It demonstrated that producing the double-flower male sterility line can be backcrossing a

gynomonoecious plant. Progenies of the petaloid female, FY1502, and 10 types of males were entirely sterile, demonstrating the passing of male sterility from the female parent; however, the male parent's fertility did not have any effect (Thomas, 2007). The progenies of the apetaloid female, FY1502AP, and the 10 male parents were all double-flowered males, indicating that the FY1502AP female shares the same genetic background as the petaloid female, FY1502. The results showed that apetaloid and petaloid females could yield all double flower progenies.

Heterosis is the inbred parents' combination of their different and superior gene content, contributing to supreme performance (Mrutyunjaya *et al*., 2021)**.** A lot of research has proceeded using heterosis in the breeding of marigolds. Priyanaka *et al.* (2016) carried out the study of heterobeltiosis of crossings *Tagetes erecta* × *T. patula* using a line × tester crossing program, which produced and evaluated 72 F_1 hybrids with 22 parental lines during the summer and rainy seasons in randomized block design. The results during both seasons showed the same significant positive heterobeltiosis in flower diameter and plant bush diameter. Zhang *et al.* (2019) demonstrated that the progeny of crossings *Tagetes erecta* × *T. patula* showed positive heterosis compared with the male parent in plant height, plant bush diameter, flower diameter, ray floret number, and days leading to flowering, showing both positive and negative heterosis over their male parents. Lahkar *et al.* (2020) studied heterosis in several growth and flowering traits of 13 marigolds' parents (three male sterile lines and 10 pollen parents) and their 30 hybrids. The results showed significant positive mid-parent heterosis and heterobeltiosis for flower diameter, flower weight per flower, and plant height but had significantly negative heterosis for days to 50% flowering.

The presented study showed that both maternal parents, FY1502 and FY1502AP, had similar positive heterosis and heterobeltiosis effects on various characteristics, including flower diameter, calyx length, plant height, and plant bush diameter. Based on heterobeltiosis, the FY1502AP produced more peduncle-length progenies than the FY1502. Mrutyunjaya *et al*. (2021) found significant positive mid-parent heterosis and heterobeltiosis in the peduncle length of the Chinese aster, a considerably important characteristic in cut flower production. One advantage of FY1502AP over FY1502 is genetic control of male sterility. FY1502AP is nuclear male sterility. Within the situation of global warming, the stability of female parents is very crucial (Sindhuja *et al*., 2018). Heat stress on FY1502, cytoplasmic sterility, might cause pollen contamination for hybrid seed production (Hampton *et al*., 2016). Thus, the apetaloid is more suitable for employment in hybrid seed production (Aziz *et al*., 2016; Mohsin *et al*., 2023).

CONCLUSIONS

Progenies derived from apetaloid and petaloid flowers with the same male gave similar flower and plant characteristics. Both females gave the same superior F_1 progenies in flower diameter compared with commercial varieties. The female types showed a significant increase in value for flower traits, plant size, and early flowering. The female FY1502AP gave a slightly better peduncle length. Even though petaloids and apetaloids could be valuable in marigold seed production, apetaloids are preferable due to their stability in the genetic background. Research findings demonstrated that GMS marigolds could benefit from utilizing the CMS line backcross method, obtaining stable marigold male sterile.

ACKNOWLEDGMENTS

The authors thank the Center of Excellence on Agricultural Biotechnology at Chiang Mai University, which supported this research. They also thank the Research Management Department, Chiang Mai University, for the project funding for Ph.D. students for the 50th anniversary of Chiang Mai University-supported research and the AmeriSeed Company, Chiang Mai, Thailand, for providing the materials and field trials. The authors acknowledge the help of Dr. Vernon Gracen, Cornell University, for his editorial assistance.

REFERENCES

Anonymous (2021). 2017-2021 Sales analysis by country and customers. Ameriseed (International) Company Limited.

Ashok AD, Velmurugan S (2020). Review on seed production techniques in flowering ornamentals. *J. Pharmacogn. Phytochem*. 9(5): 190-198.

Aziz SA, Azmi TKK, Sukma D, Qonitah FZ (2016). Morphological characters of triploids and tetraploids produced by colchicine on buds and flowers of *Phalaenopsis amabilis*. *SABRAO J. Breed. Genet*. 48(3): 352-358.

Bharathi TU, Jawaharlal M, Kannan M, Manivannan N, Raveendran M (2014). Screening of African marigold (*Tagetes erecta* L.) for novelty. *Asian J. Hort*. 9(2): 492-495. doi: 10.15740/HAS/TAJH/9.2/492495.

Bisht A, Rao VK, Dimri DC (2021). Studies on flowering behavior of double-type varieties of African marigold (*Tagetes erecta* L.) in different seasons under Uttarakhand conditions. *Pantnagar. j. res*. 19(2): 159-165.

Chaudhary M, Beniwal BS, Kumari A (2015). Character association and path coefficient analysis studies in Marigold. *Ecol. Environ. Conserv*. 21(1): 165-171.

East-West Seed (2017). Accelerating imports of marigold seeds in Thailand. Retrieved from http://www.eastwestseed.com/news/accelerating-import-of marigold-seed-in Thailand. July 3, 2017.

Giri TK, Kumar M, Sharma VR, Malik S, Chand P, Naresh RK, Prakash S (2019). Phenotypic variation, and genetic divergence in marigold (*Tagetes erecta* L.) based on agro morphic traits*. J. Pharmacogn. Phytochem.* 8(1): 1298-1302.

Hampton JG, Conner AJ, Boelt B, Chastain TG, Rolston P (2016). Climate change: Seed production and options for adaptation. *Agriculture*. 6(3): 1-17. https://doi.org/10.3390/agriculture6030033.

He Y, Sun Y, Zheng R, Ai Y, Cao Z, Bao M (2016). Induction of tetraploid male sterile *Tagetes erecta* by colchicine treatment and its application for interspecific hybridization. *Hortic. Plant. J*. 2(5): 284-292. https://doi.org/10.1016/j.hpj.2017.01.002.

Hou Z, Tang D, Tang N (2016). Isolation and analysis of differentially expressed genes between male fertile and male sterile flower buds of marigold (*Tagetes erecta* L.). *Pak. J. Bot*. 48: 2423-2431.

Karladee D (2002). Plant Improvement Technology. (2nd ed.) Ming Muang Publisher, Chiang Mai. Thailand.

Kempthorne O (1957). An Introduction to Genetic Statistics. John Wiley and Son, Inc. New York. USA.

Lahkar C, Borkakati RP, Sharma G (2020). The exploitation of heterosis for growth and flowering traits in Kharif marigold (*Tagetes erecta* L.). *Int. J. Curr. Microbiol. Appl. Sci*. 9(2): 808-820. https://doi.org/10.20546/ijcmas.2020.902.098.

Lohar A, Majumder J, Sarkar A, Rai B (2018). Evaluation of African marigold (*Tagetes erecta* L.) varieties for morphological and biochemical characters under West Bengal condition. *Int. J. Curr. Microbiol. Appl. Sci*. 7(10): 241-248.

Lou XY, Hu QS, Bao MZ, Ye YM (2010). Analysis of combining ability of two types of male sterile and four restorer lines of *Zinnia elegans*. *Euphytica*. 174:91-103. https://doi.org/10.1007/s10681-010-0143-x.

Meena K, Visarada KBRS (2021). Induction of male sterility: A boon for plant breeding. *Int. J. Curr. Microbiol. App. Sci*. 10(2): 1084-1101. https://doi.org/10.20546/ijcmas.2021.1002.128.

Mohsin RM, Abd Asal KN, Kamaluddin AA, Zaky AA (2023). Genotypes and storage duration effects on the quality of cut flower - gerbera (*Gerbera jamesonii* Hook). *SABRAO J. Breed. Genet*. 55(1): 260-267. http://doi.org/10.54910/sabrao2023.55.1.24.

Mrutyunjaya SH, Shiragur M, Patil BC, Rathod V, Lakshmidevamma TN, Preetham SP (2021). The exploitation of heterosis for yield and quality traits in China aster (*Callistephus chinensis* (L.) Nees). *J. Pharm. Innov*. 10(2): 350-354.

Nilima G, Gajabhiye RP, Ravi G, Sowjanya P, Moom SS (2017). Evaluation of marigold genotype for growth and flowering parameters under Vidharbha condition. *J. Soils. Crop.* 27(1): 132-135.

Pludtongsri P, Potapohn N (2016). Petal color inheritance of marigold. In The proceedings of the 54th Kasetsart University Annual Conference: Plant, Agricultural Innovation for Global Value Chain, Kasetsart University, Bangkok: 95-99.

Priyanaka K, Gupta YC, Dhiman SR, Dogra RK, Madhu S, Monika B (2016). Heterobeltiosis in French marigold (*Tagetes patula* L.). *J. Agric. Sci*. 8(8): 182-193. https://doi.org/10.5539/jas.v8n8p182.

Ramappa S, Jain SK, Vijay D, Lal SK, Singh KP, Anjali A (2022). Floret position in the

capitulum determines the seed quality in marigold. *J. Pharm. Innov*. 11(11): 1318-1323.

Royal Horticultural Society (2015). Royal Horticultural Society color chart. (6th Ed.) RHS, London, UK.

Sindhuja S, Geetha A, Saidaiah P (2018). Chapter-6 Recent advances in male sterility and its exploitation in marigold. In: Research Trends in Horticulture Sciences, Vol.3. M.L. Meena (ed.), Aki Nik Pub, New Delhi. 47: 87.

Singh D, Misra KK (2017). Heterosis breeding among different crosses in marigold. (*Tagetes* spp.). *Contemp. Res. India*. 7(1): 48-56.

Singh D, Vishwakarma A (2021). Heterosis breeding in marigold: An efficient tool towards crop improvement. Retrieved from http://www.vidyakutirfoundation.org June 15 (2021).

Soriguer IC, Buide ML, Narbona E (2013). The roles of female and hermaphroditic flowers in the gynodioecious-gynomonoecious *Silene littorea*: Insights into the phenology of sex expression. *Plant Biol*. Plant biology (Stuttgart Germany). 15(6): 941-947. https://doi.org/10.1111/j.1438 8677.2012.00697.x.

Sukwiwat K, Potapohn N, Kumchai J, Bundithya W (2021). Analysis of genetic controlling male sterility in American marigold. In The Proceedings of the 18th KU-KPS National Conference: Kasetsart University, Nakhon Pathom, Thailand. pp. 38-44.

Tang N, Liu W, Zhang W, Tang D (2020). Integrative analysis of transcriptomic and proteomic changes related to male sterility in *Tagetes erecta*. *Physiol. Mol. Biol. Plants*. 26(10): 2061-2074 https://doi.org/10.1007/s12298-020-00886-z.

Tejaswini T, Gadre K (2016). An IIHRMGYP-1 (IC0613361; INGR15036), A marigold (*Tagetes erecta* L.) germplasm with petaloid sterile flowers; ability to be multiplied by cuttings. *Indian. J. Plant. Genet*. *Resour.* 29(2): 201-222.

Tejaswini T, Sane A, Gadre A, Ghatke M (2016). Characterization and utilization of three distinct male sterile systems in marigold (*Tagetes erecta*). *Indian J. Agric. Sci*. 86(10): 1271-5.

Thomas B (2007). Flowering and its manipulation: Annual Plant Reviews, Volume 20. *Ann. Bot*. 99(1): 206-207. https://doi.org/ 10.1093/aob/mcl247.

Wang Q, Zhang X, Lin S, Yang S, Yan X, Bendahmane M, Bao M, Fu X (2020). Mapping a double flower phenotype-associated gene DcAP2L in *Dianthus chinensis. J. Exp. Bot*. 71(6): 1915-1927. https://doi.org/10.1093/jxb/erz558.

Whankaew S, Hasthanasombut S, Thaikert R, Tappiban P, Smith DR, Triwitayakorn K (2014). Molecular characterization and genetic relationship of marigolds *(Tagetes spp*.) based on simple sequence repeat markers. *Plant Genet. Res*. 12(3): 317-322. https://doi.org/10.101 7/S14792621 14000100.

Xiong H, Chen P, Zhu Z, Chen Y, Zou F, Yuan D (2019). Morphological and cytological characterization of petaloid-type cytoplasmic male sterility in *Camellia oleifera*. *HortScience*. 54(7): 1149-1155. https:// doi.org/10.21273/HORTSCI14005-19.

Zhang C, Wei L, Wang W, Qi W, Cao Z, Li H, Bao M, He Y (2020). Identification, characterization, and functional analysis of AGAMOUS subfamily genes associated with floral organs and seed development in marigolds (*Tagetes erecta*). *BMC Plant Biol*. 20(1): 439. https://doi.org/10.1186/s12870-020-02644-5.

Zhang H, Xin H, Cong R, Li Z, Song L, Wei Z (2019). Cross-compatibility analysis to identify suitable parents of *Tagetes erecta* and *T. patula* for heterotic hybrid breeding. *Not. Bot. Horti. Agrobo*. 47(3): 676-682. https://doi.org/10.15835/nbha47311388.