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Original Research Paper

Comparative analysis of BLUP and GCA for parental selection in marigold (*Tagetes erecta* L.) for hybrid development

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ABSTRACT

The area under marigold cultivation is increasing over the years and so is the demand for marigold seeds. To meet the increasing demand, hybrid varieties are preferred as they produce higher yields, for which the right parental selection is of major concern. Male sterility being the prerequisite for economical hybrid seed production of marigold, we have attempted to strategize the selection of male sterile seed parent and fertile pollen parent for yield and yield-related traits. The study was undertaken across multiple forms of male sterile lines morphologically varying in apetaloid and petaloid types, therefore use of BLUP and GCA was evaluated as a criterion to select the parents for the hybridization program. Results suggested apetaloid male sterile lines as better seed parents for days to bud initiation, while, petaloid male sterile lines can be selected for the improvement of shelf life and flower diameter. Results from BLUP and GCA were in agreement with each other for the traits studied. However, BLUP-based comparison of different lines is less tedious as it eliminates the laborious procedure of developing multiple hybrids and evaluating them to study the combining ability effects.

Keywords: BLUP, flower yield, GCA, hybrid seed production, marigold

INTRODUCTION

Marigold (*Tagetes erecta* L.), is the hardiest annual crop, cultivated in various agroclimatic conditions (Raghava, 2000). It is in great demand in the Indian market as a traditional loose flower used in various religious and cultural festivals, and extraction of carotenoids as a nutraceutical (Shao & Ren, 2016 and Ren & Reilly, 2018). It is widely cultivated around fruit orchards and commercial vegetable fields as a trap crop against root-knot nematode, aphids and whiteflies (Stavridou & Biezla, 2017; El- Naggar et al., 2017).

Marigold belongs to the family Asteraceae with its characteristic composite flowers consisting of ray and disc florets. Functional anthers in marigold are hidden within disc florets in the center of the flower, making emasculation a difficult process. The use of male sterile lines dramatically decreases the hybrid seed production cost by eliminating the tedious manual emasculation step (Huang et al., 2014; Wu et al., 2016). In marigold, apetaloid and petaloid types are the major morphological male sterile forms reported. In apetaloid male sterility, petals and androecium are modified into filament like structure (Gupta et al., 1999; He et al., 2009), while, flowers with only ray florets and absence of androecium are associated with petaloid male sterility (Tejaswini et al., 2016). Pre-breeding plays a pivotal role in developing parents for hybrids, providing a source of male sterility and fertility controlling genes, allowing efficient hybrid seed production. By incorporating male sterility, breeders can streamline the production of hybrid seeds, ensuring a consistent supply of high-quality planting materials for commercial flower cultivation.

Selection of breeding lines and the development of hybrids that show better and stable performance is a long-term strategy to cater immediate, medium and long-term needs of the farmers. Best Linear Unbiased Prediction (BLUPs) allows the comparison of lines over time (generation, year) and space (location, block) by minimizing their effects (Tajalifar & Rasooli, 2022). Also, BLUP can help in the selection of stable and better-performing genotypes/lines in a breeding program (Asfaw et al., 2020; Acharya et al., 2020). Furthermore, general combining ability (GCA) also suggests the suitability of genotypes/lines for the hybridization program. Estimated GCA by analyzing the average performance of genotypes in a series of crosses, provides the strength of the genotypes/lines to be used as a parent in the hybrid development program (Sprague & Tatum, 1942). The present study was attempted to identify reliable strategy for the selection of parents for yield and yield-related traits to be used in the hybridization program.

MATERIALS AND METHODS

Fifteen marigold breeding lines representing different categories of sterility and fertility (Table 1 & Fig. 1) were selected from pre-breeding population maintained at ICAR- Indian Institute of Horticultural Research, Bengaluru, India which is situated at 13° 08' 26.6" N latitude and 77° 30' 2.2" E longitude and 890 m above mean sea level.

Stabilisation and maintenance of breeding lines used in the study is presented in Table 1. Male sterility of vegetatively propagated petaloid lines are cytoplasmically inherited, while, apetaloid male sterility is controlled by nuclear genes and are seed propagated (Tejaswini et al., 2016). In the present study, petaloid male sterile lines were also used that are seed propagated and controlled by nuclear genes (unpublished data). Breeding lines were evaluated for morphological traits viz., days to bud initiation, flower diameter, shelf life, number and yield of flowers per plant, for three consequent years 2020 (summer), 2021 (winter) and 2022 (rainy).

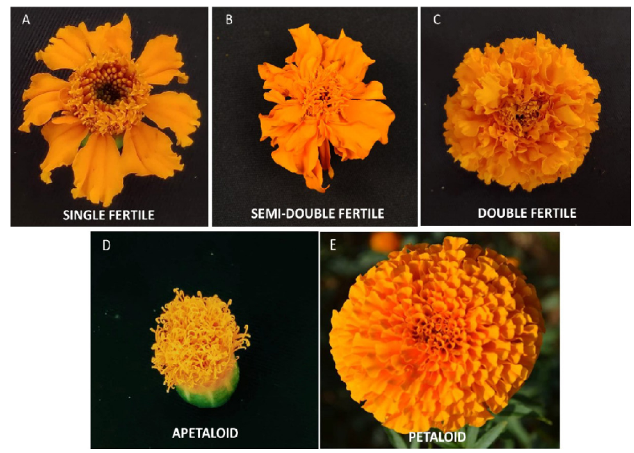

Fig. 1 : Male fertile flower types in marigold, A. single fertile, B. semi-double fertile, C. double fertile; Male sterile flower types, D. apetaloid, E. petaloid

Table 1 : Description of breeding lines used as pollen and seed parents for derivation of hybrids of marigold

Breeding Line	Flower form	Male Sterile: fertile plants	Propagation	Maintenance of breeding lines	Flower color
IIHRM 4-4	Single fertile	0:1	Seed	Selfing	Orange
IIHRM 5-2	Single fertile	0:1	Seed	Selfing	Yellow
IIHRM 4-13	Semi-double fertile	0:1	Seed	Selfing	Orange
IIHRM 1-11	Semi-double fertile	0:1	Seed	Selfing	Yellow
IIHRM 1-11-2	Semi-double fertile	0:1	Seed	Selfing	Yellow
IIHRM 5-4	Double fertile	0:1	Seed	Selfing	Orange
IIHRM 1-12	Double fertile	0:1	Seed	Selfing	Yellow
IIHRM 7-4	Apetaloid	1:1	Seed	Intercrossing between sterile and fertile plants within the line	Orange
IIHRM 7-5	Apetaloid	1:1	Seed	Intercrossing between sterile and fertile plants within the line	Orange
IIHRM 3-2	Apetaloid	1:1	Seed	Intercrossing between sterile and fertile plants within the line	Yellow
IIHRM 3-5	Apetaloid	1:1	Seed	Intercrossing between sterile and fertile plants within the line	Yellow
IIHRM 6-5	Petaloid	1:1	Seed	Intercrossing between sterile and fertile plants within the line	Orange
IIHRM 2-9	Petaloid	1:1	Seed	Intercrossing between sterile and fertile plants within the line	Yellow
IIHRMOs-1	Petaloid	1:0	Vegetative	Vegetative	Orange
IIHRMYs-1	Petaloid	1:0	Vegetative	Vegetative	Yellow

Hybrids were developed within the yellow and orange color group, resulting in a total of 34 hybrid combinations. Lines studied comprised petaloid and apetaloid male sterile lines as seed parents and single, double and semi-double fertile as pollen parents (Table 5). As seed propagated male sterile lines segregates into sterile and fertile lines; such lines were used both as seed and pollen parents. Hybrids developed were evaluated in randomized complete block design with three replications along with the parents to analyze the combining ability and contribution of parents to hybrids.

The phenotypic data recorded were analyzed using R software version 4.2.0. Levene's test (Levene, 1960) was carried out to confirm the homogeneity of error variances. Within and across the seasons, analysis of variance (ANOVA) was carried out in the 'metan' R package. For all the traits, best linear unbiased predictors (BLUPs) were calculated using the META-R software version 6.0 (Alvardo et al., 2020).

RESULTS AND DISCUSSION

The significance of mean sum of squares for seasons and genotypes x season in pooled ANOVA revealed the influence of the environment on the expression of these traits (Table 2). BLUPs were calculated within and across seasons for all the parental lines for five traits, as it minimizes the seasonal and space (location, block) effects allowing to compare the parents across five seasons.

Performance of different male sterile and fertile lines as parents for hybridization based on BLUP values

The use of BLUP generates more accurate estimation of genetic parameters and unbalanced experimental designs can also be used to predict the genotypic values, which also helps in evaluating the performance of the same genotypes/lines in different conditions by estimating the genetic correlations (Abu-Ellail et al., 2018).

Based on BLUP values, apetaloid male sterile lines were early to flower and took shorter time for bud initiation. Flower diameter and shelf life were recorded maximum in petaloid male sterile group except IIHRMOs-1 (Table 3). Petaloid lines have no functional pollen in turn exhibited longer life than other lines, as increase in ethylene production following pollination and fertilization regulates flower senescence (Serek et al., 1995). Semi-double fertile and apetaloid sterile lines produced a relatively more number of flowers and yield per plant than other groups as their flower diameter and per flower weight were higher compared to apetaloid lines (Table 3). Pedigree-based BLUP procedure was reported to enhance selection efficiency for production-related traits in *P. zonale* and shelf-life-related traits in *D. caryophyllus* L. (Molenaar et al., 2018), while, Acharya et al. (2020) and Ashwini et al. (2021) used the BLUP method to select genotypes with preferable agronomic traits in alfalfa and horse gram, respectively.

Performances of breeding lines and crosses based on combining ability effects

Among the breeding lines, apetaloid male sterile lines were good general combiners for days to bud initiation but were poor combiners for other traits. Seed propagated petaloid male sterile line (IIHRM 3-2) as female parent was found to be good general combiner for days to bud initiation and flower diameter.

Table 2 : Pooled analysis of variance of marigold breeding lines for yield and quality-related quantitative traits

Source of variation	df	Mean sum of squares				
		Days to bud initiation	Flower diameter (cm)	Shelf life (days)	Flowers per plant (Nos.)	Flower yield per plant (g)
Seasons	4	2073.93**	1.19*	6.51*	1185396**	71672**
Treatment	14	778.69**	6.07*	15.50**	399603**	2965494**
Replication within seasons	10	4.51	0.01	0.02	3630	12456
Season x Treatment	56	37.67**	0.40*	0.43*	257027**	211468**
Error	140	1.08	0.02	0.03	411	1495

Table 3 : Parental lines BLUP values and their ranges for five quantitative traits

Breeding line	Days to bud initiation	Range	Flower diameter (cm)	Range	Shelf life (days)	Range	Flowers per plant (Nos.)	Range	Flower yield per plant (g)	Range
Single fertile										
IIHRM 4-4	53.26	36.00 - 64.00	4.04	3.00 - 4.50	2.84	2.00 - 4.00	101.38	59.00 - 190.00	270.45	118.00 - 380.00
IIHRM 5-2	65.00	56.00 - 74.00	4.06	4.00 - 4.40	2.61	2.00 - 3.00	63.82	51.00 – 95.00	209.25	153.00 - 285.00
Semi-double fertile										
IIHRM 4-13	47.88	38.00 - 56.00	3.94	3.00 - 4.00	2.84	2.00 - 4.00	286.60	236.00 - 338.00	1116.37	708.00 - 2690.00
IIHRM 1-11	50.00	42.00 - 57.00	4.72	4.30 - 5.00	2.68	2.00 - 3.00	222.97	200.00 - 260.00	1003.70	700.00 - 2088.00
IIHRM 1-11-2	69.33	61.00 - 77.00	4.05	4.00 - 4.30	3.53	2.00 - 5.00	51.96	40.00 - 75.00	192.61	120.00 - 192.50
Double fertile										
IIHRM 5-4	52.46	34.00 - 64.00	4.07	4.00 - 4.50	3.48	3.00 - 4.00	144.06	236.00 - 338.00	628.68	448.00 - 804.00
IIHRM 1-12	54.00	45.00 - 63.00	5.07	5.00 - 5.30	4.52	4.00 - 5.00	120.02	101.00 - 143.00	526.46	408.00 - 758.00
Apetaloid										
IIHRM 7-4	43.07	35.00 - 49.00	2.87	2.00 - 3.00	2.53	2.00 - 4.00	196.25	162.00 - 245.00	740.20	567.00 - 1040.00
IIHRM 7-5	46.35	38.00 - 53.00	3.20	2.00 - 3.50	2.76	2.00 - 4.00	200.27	174.00 - 240.00	716.31	556.80 - 1217.00
IIHRM 3-2	47.67	38.00 – 56.00	3.78	3.50 – 4.00	2.81	2.00 - 4.00	188.81	166.00 - 211.00	597.49	498.00 – 750.00
IIHRM 3-5	51.67	43.00 - 60.00	3.98	3.70 - 4.00	2.68	2.00 - 4.00	134.34	103.00 - 160.00	411.67	309.00 - 480.00
Petaloid										
IIHRM 6-5	53.82	47.00 - 62.00	4.99	4.30 - 5.50	4.19	3.00 - 5.00	146.71	116.00 - 222.00	765.32	575.00 - 1110.00
IIHRM 2-9	54.33	45.00 - 64.00	4.39	4.00 - 5.00	5.32	4.00 - 6.00	197.26	170.00 - 224.00	1917.58	1530.00 - 2835.00
IIHRMOs-1	57.22	30.00 - 72.00	3.50	1.50 - 4.00	2.61	2.00 - 3.00	73.60	12.00 - 120.00	263.50	12.00 - 333.00
IIHRMYs-1	59.00	50.00 - 67.00	4.02	4.00 - 4.20	4.39	3.00 - 5.00	113.83	102.00 – 130.00	638.08	408.00 - 1440.00

Similarly, vegetatively propagated petaloid male sterile line IIHRMYs-1 was a good general combiner for shelf life. For the number of flowers per plant and yield per plant, IIHRM 6-5, a seed propagated petaloid male sterile line showed good general combining ability. Based on these results, seed propagated petaloid male sterile lines were good general combiners for days to bud initiation, flower diameter, number of flowers and yield per plant. Among pollen parents, semi-double fertile and double fertile lines were good general combiners for days to bud initiation. In addition, semi-double fertile lines were good combiners for flower diameter, shelf life, number and yield of flowers per plant. IIHRM 4-13, a semi-double fertile line showed an excellent general combining ability as a tester for all the traits under study (Table 4).

The performance of parental lines in combination with all other lines is reflected by GCA effects, parents with the highest GCA effects have a greater impact on the trait improvement. Singh & Misra (2008) identified good combiners for earliness in flowering, yield and yield attributes in African marigold.

Table 4 : General combining ability effects of marigold hybrids

Breeding line	Days to bud initiation	Flower diameter (cm)	Shelf life (days)	Flowers per plant (Nos.)	Flower yield per plant (g)
Male sterile seed parents					
Apetaloid					
IIHRM 3-2	-8.13	-1.15	-1.17	100.15	78.84
IIHRM 3-5	0.78	-0.80	-1.33	23.77	-381.25
IIHRM 7-4	-2.51	-0.11	-0.27	-17.31	-236.60
IIHRM 7-5	-5.73	0.28	-0.27	7.02	-127.71
Petaloid					
IIHRM 2-9	-6.79	1.34	0.67	28.23	247.67
IIHRM 6-5	-6.73	0.25	-0.27	103.13	387.73
IIHRMOs-1	7.49	-0.21	0.40	-46.42	-11.71
IIHRMYs-1	8.08	0.35	1.05	-59.78	31.28
Pollen parent					
Single fertile					
IIHRM 5-2	3.70	-0.09	-0.25	-18.60	-163.83
IIHRM 4-4	-1.46	0.22	-0.60	-10.87	-256.43
Semi double fertile					
IIHRM 1-11	-5.97	0.12	-0.25	69.07	186.42
IIHRM 1-11-2	0.20	0.26	-0.25	-39.18	-401.83
IIHRM 4-13	-3.96	0.22	0.40	23.88	201.73
Double fertile					
IIHRM 5-4	-1.21	-0.06	-0.10	24.80	57.73
IIHRM 1-12	-3.88	-0.19	0.00	23.23	169.00

Comparison of BLUP and GCA as a criterion for selection of parents

Performance of hybrids indicated that BLUP and GCA results were in agreement with each other for the traits studied. Both BLUP and GCA showed that apetaloid lines for days to bud initiation; petaloid lines for shelf life and flower diameter when used as parents to develop hybrids could give desirable results. For traits like number of flowers and yield per plant, petaloid line IIHRM 6-5 and semi-double fertile IIHRM 1-11 manifested relatively high BLUP and GCA.

CONCLUSION

Selection of the right parents to develop desirable hybrids is an important decision-making step in a hybrid breeding program. The ability of parents to produce superior hybrids is confirmed by assessing their progeny performance to study the combining abilities of parents which is a tedious and time-consuming procedure. BLUP-based comparison of different lines is a much easier way as it eliminates the laborious procedure of developing hybrids and evaluating them to study the combining ability effects. Based on the results, BLUPs could be used as a criterion to select parents for hybridization.

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Table 5 : Mean performance of parents and their hybrids for yield and yield contributing characters

Breeding line	Days to bud initiation	Flower diameter (cm)	Shelf life (days)	Flowers per plant (Nos.)	Flower yield per plant
Orange hybrids					
IIHRM 6-5 x IIHRM 4-4	49.33	6.07	2.67	208.33	637.67
IIHRM 6-5 x IIHRM 4-13	45.67	6.07	3.67	293.67	1546.33
IIHRM 6-5 x IIHRM 5-4	45.67	6.33	3.33	333.00	1199.00
IIHRM 7-4 x IIHRM 4-4	50.00	6.30	2.67	182.33	496.00
IIHRM 7-4 x IIHRM 4-13	45.00	6.17	3.67	192.33	818.00
IIHRM 7-4 x IIHRM 5-4	58.33	4.93	3.00	99.00	196.00
IIHRM 7-5 x IIHRM 4-4	48.33	5.17	2.00	149.33	331.00
IIHRM 7-5 x IIHRM 4-13	49.00	7.27	4.00	193.33	934.33
IIHRM 7-5 x IIHRM 5-4	46.33	6.13	4.33	204.00	571.33
IIHRMOs-1 x IIHRM 4-4	61.00	7.00	3.67	117.33	469.33
IIHRMOs-1 x IIHRM 4-13	59.00	5.00	4.33	117.00	468.00
IIHRMOs-1 x IIHRM 5-4	59.33	6.00	3.67	164.00	1224.33
IIHRMOs-1 x IIHRM 6-5	65.33	6.00	3.33	129.67	933.67
IIHRMOs-1 x IIHRM 7-4	61.00	5.17	4.00	132.67	676.67
IIHRMOs-1 x IIHRM 7-5	61.00	5.00	4.33	112.00	597.33
Yellow hybrids					
IIHRM 2-9 x IIHRM 5-2	57.00	5.83	3.67	157.67	1324.33
IIHRM 2-9 x IIHRM 1-11	45.00	6.70	4.00	280.00	1890.00
IIHRM 2-9 x IIHRM 1-11-2	46.67	8.77	4.33	189.33	1111.00
IIHRM 2-9 x IIHRM 1-12	46.67	7.00	4.00	168.67	899.67
IIHRM 3-2 x IIHRM 5-2	56.00	4.00	2.00	178.33	610.00
IIHRM 3-2 x IIHRM 1-11	42.67	4.97	2.33	387.33	1975.67
IIHRM 3-2 x IIHRM 1-11-2	48.67	4.20	2.00	190.00	428.33
IIHRM 3-2 x IIHRM 1-12	42.67	5.20	2.33	327.67	1535.67
IIHRM 3-5 x IIHRM 5-2	59.00	5.73	2.00	175.67	871.33
IIHRM 3-5 x IIHRM 1-11	45.33	5.00	2.00	205.33	492.67
IIHRM 3-5 x IIHRM 1-11-2	65.33	5.00	2.00	70.00	525.33
IIHRM 3-5 x IIHRM 1-12	56.00	4.00	2.00	136.67	820.00
IIHRMYs-1 x IIHRM 5-2	65.33	7.00	4.67	96.67	773.33
IIHRMYs-1 x IIHRM 1-11	65.67	6.77	4.00	86.33	621.67
IIHRMYs-1 x IIHRM 1-11-2	62.67	6.00	4.00	76.67	562.33
IIHRMYs-1 x IIHRM 1-12	61.67	6.00	5.00	142.67	1655.00
IIHRMYs-1 x IIHRM 2-9	60.00	6.00	5.00	170.67	1979.67
IIHRMYs-1 x IIHRM 3-2	63.00	5.83	4.00	140.67	1577.33
IIHRMYs-1 x IIHRM 3-5	67.67	5.00	4.00	62.67	459.67
CV	3.07	3.13	10.31	6.85	7.73
CD at 5%	2.73	0.30	0.59	19.35	115.70

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