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## GENETIC INHERITANCE PATTERNS OF MALE STERILITY SYSTEMS IN MARIGOLD (*TAGETES ERECTA* L.)

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### ABSTRACT

Marigold (*Tagetes erecta* L.) is a widely cultivated ornamental crop valued for its adaptability, diverse floral traits, and economic importance in floriculture and pigment industries. Hybrid seed production in marigold is challenged by complex floral morphology requiring laborious emasculation. Male sterility systems, including cytoplasmic male sterility (CMS) and genetic male sterility (GMS), provide efficient alternatives for hybridization. This study aimed to elucidate the inheritance pattern of cytoplasmic petaloid male sterility. The progenies from crosses with fertile inbred lines were analyzed for male sterility inheritance using chi-square tests. Results showed genetic analyses confirmed maternal inheritance of male sterility with fertility restoration controlled by a single dominant nuclear gene. These findings provide a foundation for genetic maintenance of male sterile lines, facilitating large-scale hybrid seed production in marigold and contributing to sustainable floriculture development.

**Keywords :** Marigold; Cytoplasmic male sterility; inheritance; Genetic inheritance.

### Introduction

Marigold (*Tagetes erecta* L.) is one of the most popular ornamental crops cultivated across tropical and subtropical regions due to its short crop duration, high adaptability, and diverse floral characteristics. It holds significant economic and cultural importance, being widely used for garland making, decorations during religious and social events, landscaping, and pigment extraction. The rich carotenoid content of marigold flowers supports its industrial utilization in dye, food coloring, and cosmetic formulations, while root-derived nematocidal compounds make it a valuable trap crop in integrated pest management systems (Estrada *et al.*, 2025). Additionally, marigold extracts have applications in traditional medicine, perfumery, and oil industries (Shaik *et al.*, 2023), further emphasizing the crop's multifaceted utility.

Currently, F<sub>1</sub> hybrids dominate marigold cultivation due to their advantages in earliness,

uniform flowering, stress tolerance, and higher yield, although hybrid seed production is challenged by the complexity of emasculation caused by the flower structure. The male sterility system is widely adopted to circumvent emasculation for hybrid seed production, but hand pollination remains necessary. In India, hybrid seed demand exceeds supply, with public sector institutions like ICAR-IIHR releasing promising F<sub>1</sub> hybrids to meet this gap, emphasizing the importance of high-quality seeds in flower seed production for profitability and sustainability. Male sterility system is widely recognized and utilized in the production of hybrid seeds avoiding the process of emasculation (Tejaswini *et al.*, 2016a). Worldwide, the demand for marigold hybrid seeds is approximately 10,000 kg annually (Sukwiwat *et al.*, 2023). At present, in India, the area under flower seed production is approximately 600 – 800 ha (Patil *et al.*, 2023).

The increasing adoption of F<sub>1</sub> hybrids in marigold cultivation has been driven by their superiority in traits such as early and uniform flowering, better stress tolerance, and higher yield potential. However, hybrid seed production in marigold remains labor-intensive and costly because of the complex floral morphology. The disc florets, which contain functional anthers, are tightly enclosed within the capitulum, making manual emasculation challenging and time-consuming (Tejaswini *et al.*, 2016a). The incorporation of male sterility systems has emerged as a promising alternative, offering an efficient approach to bypass emasculation while maintaining genetic purity and reducing production costs (Chen & Liu, 2014; Du *et al.*, 2020).

In marigold, both genetic and cytoplasmic forms of male sterility have been documented. Genetic male sterility (GMS) occurs in two forms—apetaloid and petaloid—each governed by a single gene, recessive and dominant respectively (Gupta *et al.*, 1999; Sumalatha *et al.*, 2024). Conversely, cytoplasmic petaloid male sterility (CMS) is maternally inherited and has gained prominence due to its stable expression and easy maintenance through vegetative propagation (Kumar *et al.*, 2017; Tejaswini *et al.*, 2016b). Morphologically, apetaloid GMS lines exhibit only disc florets and lack ray florets, whereas cytoplasmic petaloid sterility results in flowers composed solely of ray florets.

The present study aims to elucidate the inheritance pattern of cytoplasmic male sterility in marigold and understanding these genetic strengths to strengthen their deployment in commercial hybrid seed production programs for marigold, ensuring cost-effective and large-scale seed supply for sustainable floriculture development.

### Materials & Methods

The present study was carried out at the ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru (13.13°N latitude, 77.49°E longitude) at an elevation of approximately 890 meters above mean sea level. For genetic inheritance studies, six fertile inbred lines were crossed with each of the two male sterile seed parents: IIHRMYS-1 was crossed with six yellow fertile inbred lines (MYP1 to MYP6), and IIHRMOS-1 was crossed with six orange fertile inbred lines (MOP1 to MOP6). The resulting seeds from six yellow and six orange combinations were sown and the resulting six progeny populations in each colour group were checked for presence of fertile and sterile flowers in each of the progeny plants. Since, the resulting populations were 100 per cent sterile in all the

combinations, they were maintained by vegetative propagation and for confirmation as possible maintainer line, they were further crossed with pollen parents of hybrid combination under study; *viz.*, yellow combinations with IIHRMY 2-1, IIHRMY 1-4 and orange combinations with IIHRMO 12, IIHRMO 53.

To understand the genetics of male sterility and identify maintainer/restorer relationships, four orange sterile F<sub>1</sub> lines were selected for backcrossing with the fertile parent IIHRMO 12 to develop BC<sub>1</sub> populations. These crosses included the combinations (MOS1 × IIHRMO 12) × IIHRMO 12, (MOS4 × IIHRMO 12) × IIHRMO 12, (MOS5 × IIHRMO 12) × IIHRMO 12, and (MOS6 × IIHRMO 12) × IIHRMO 12. Seeds from these BC<sub>1</sub> populations were sown, and segregation of fertile and sterile plants was recorded for genetic analysis.

### Statistical Analysis

The experiments were laid out in completely randomized design with three replications. For genetic segregation ratio of the male sterile progenies was subjected to chi-square test. The test on goodness of fit between expected and observation segregation ratio was calculated using R software and statistically significance values taken at  $\leq 0.05$ . The chi-square value was calculated and compared with the table value with n-1 degrees of freedom.

## Results

### Identification of maintainer line & inheritance pattern of petaloid male sterile system

All the F<sub>1</sub> progenies resulting from IIHRMYS-1 (yellow) male sterile seed parent crossed with six yellow fertile inbred lines *viz.*, MYP1, MYP2, MYP3, MYP4, MYP5, MYP6 and IIHRMOS-1 (orange) male sterile seed parent crossed with six orange fertile inbred lines *viz.*, MOP1, MOP2, MOP3, MOP4, MOP5 and MOP6 were 100% male sterile without any segregation (Table 1). Further, six yellow colour male sterile F<sub>1</sub> progenies crossed with pollen parents of hybrids under study which contain two yellow pollen lines (IIHRMY 2-1 & IIHRMY 1-4) resulting in a segregating population of 1 fertile: 1 sterile ratio. Similarly, six orange sterile F<sub>1</sub> progeny crossed with pollen parents of hybrids under study which contain two orange pollen lines (IIHRMO 12 & IIHRMO 53) also resulting in a segregating population of 1 fertile: 1 sterile ratio (Table 2). Further, BC<sub>1</sub> progenies also resulted in a segregating population of 1 fertile: 1 sterile ratio (Table 3). Chi-square test, when applied to these progenies exhibited good fit for the expected ratio of 1:1. Cytoplasmic inheritance patterns of petaloid male sterile are presented in Fig. 1 & Fig. 2.

The seed parents IIHRMYS-1 and IIHRMOS-1 were 100% male sterile and all the inbred lines (six orange & six yellow) were 100% male fertile. Assuming all the inbred lines have normal cytoplasm (N) with a fertility restorer gene in a homozygous dominant condition (RfRf) and the correspondingly male sterile lines (IIHRMOS-1 & IIHRMYS-1) in homozygous recessive (rfrf) with sterile cytoplasm. All the resulting progeny are 100% male sterile without any segregation. Further, six yellow coloured male sterile F<sub>1</sub> progenies crossed with pollen parents of hybrids under study which contain two yellow pollen lines (IIHRMY 2-1 & IIHRMY 1-4) resulting in a segregating population of 1 fertile: 1 sterile ratio. Similarly, six oranges sterile F<sub>1</sub> progeny crossed with pollen parents of hybrids under study which contain two orange pollen lines (IIHRMO 12 & IIHRMO 53) also resulting in a segregating population of 1 fertile: 1 sterile ratio. Further, BC<sub>1</sub> progenies also resulted in a segregating population of 1 fertile: 1 sterile ratio. The results indicate that the petaloid male sterility in marigold is maternally inherited via cytoplasmic factors, while fertility restoration follows a nuclear gene interaction with a dominant fertility restorer gene (Rf) and recessive sterile allele (rf), validating the genetic mechanism underlying male sterility maintenance and restoration in this crop.

### Discussion

#### Identification of maintainer line & inheritance pattern of petaloid male sterile system

The identification of an appropriate maintainer line is critical in hybrid breeding programs to effectively exploit heterosis and ensure the vigor of male sterile lines over successive propagations. Continuous vegetative propagation can potentially lead to loss of vigor in male sterile lines, underscoring the importance of selecting the correct maintainer. In this study, it is assumed based on prior work by Sumalatha *et al.* (2024) that all inbred lines used as pollen parents possess normal cytoplasm (N) and carry a homozygous dominant fertility restorer gene (RfRf), while the male sterile lines IIHRMYS-1 and IIHRMOS-1 harbor sterile cytoplasm and homozygous recessive fertility alleles (rfrf). Crosses between these male sterile lines and inbred maintainers resulted in 100% male sterile progenies with no segregation, suggesting that these inbred lines serve effectively as maintainers of male sterility. Further, crosses of male sterile progenies with fertile pollen parents from hybrid lines produced segregating populations with a 1:1 ratio of fertile to sterile plants, confirmed statistically by chi-square

analysis. This supports the hypothesis that fertility restoration is governed by a single dominant nuclear gene. Understanding this inheritance pattern is vital for optimizing hybrid seed production, especially as cytoplasmic male sterility (CMS) is maternally inherited through mitochondrial genes, causing pollen abortion via premature tapetum degeneration during microspore development (Budar and Pelletier, 2001; Roberts *et al.* 1995).

Restorer (Rf) genes located in the nucleus counteract the CMS effect by suppressing the mitochondrial male sterility phenotype and thus restoring pollen viability (Popova *et al.* 2007). In this study, the behavior of restorer genes appears cytoplasm-dependent, with heterozygous conditions leading to complete sterility, indicating differential gene expression influenced by cytoplasmic background. While, CMS has been reported previously in petaloid and apetaloid male sterile marigold lines (Tejaswini *et al.* 2016b; Sukwiwat *et al.* 2023), the nature of fertility restorer genes remained unclear. Similar CMS-restorer gene interactions have been documented in other crops such as sunflower and various vegetables, typically involving either single or complementary dominant nuclear restorer genes (Chandra *et al.* 2010; Dash *et al.* 2001; Pradeepkumar *et al.* 2012; Varalakshmi and Rajashekharan, 2022). However, the precise molecular mechanism by which restorer genes counteract mitochondrial CMS remains to be fully elucidated (Hanson and Bentolila, 2004). This study confirms earlier findings by Sumalatha *et al.* (2024) regarding the differential expression of restorer genes modulated by cytoplasmic background, thus providing essential insights into the genetic control of male sterility in marigold. These findings offer a practical foundation for selecting suitable maintainer and restorer lines to enhance hybrid seed production efficiency in marigold breeding programs.

### Conclusion

The study conclusively demonstrated that genetic analysis of the petaloid male sterility system confirmed its cytoplasmic maternal inheritance, while fertility restoration is governed by a single dominant nuclear gene, supporting an efficient maintainer-restorer model. Identifying appropriate maintainer lines capable of producing 100% male sterile progeny without segregation is pivotal for hybrid seed production. This research provides critical insights into the genetic male sterility maintenance for hybrid seed production systems in marigold breeding programs.

**Table 1 :** Segregation pattern observed in the crossing program between vegetatively propagated petaloid male sterile lines (IIHRMOS-1 & IIHRMYS-1) with inbred lines

Male sterile line × Inbred line	Resulting Progeny	Total plants	(Resulting progeny) Number of plants (Sterile/ fertile)		Expected	Observed	
			Sterile	Fertile			
IIHRMYS-1	MYP1	MYS1	20	20	0	1:0	1:0
	MYP2	MYS2	20	20	0	1:0	1:0
	MYP3	MYS3	20	20	0	1:0	1:0
	MYP4	MYS4	20	20	0	1:0	1:0
	MYP5	MYS5	20	20	0	1:0	1:0
	MYP6	MYS6	20	20	0	1:0	1:0
IIHRMOS-1	MOP1	MOS1	20	20	0	1:0	1:0
	MOP2	MOS2	20	20	0	1:0	1:0
	MOP3	MOS3	20	20	0	1:0	1:0
	MOP4	MOS4	20	20	0	1:0	1:0
	MOP5	MOS5	20	20	0	1:0	1:0
	MOP6	MOS6	20	20	0	1:0	1:0

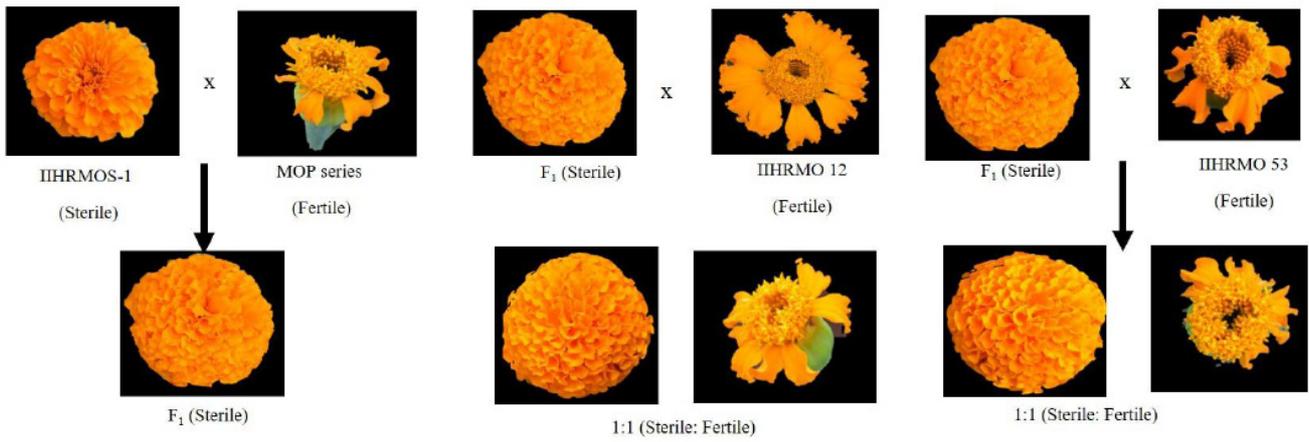
**Table 2 :** Segregation pattern of resulting progenies of male sterile lines in combination with pollen parents of hybrids under study

Male sterile (MS line x inbred line)	Pollen parents of hybrids under study	Total plants	Number of plants (Sterile/ fertile)		Expected ratio	Chi-square test ( $\chi^2$ )		Chi-square test ( $\chi^2$ ) (Table)
			Sterile	Fertile		$\chi^2$ (calculated)	<i>p</i>	
Mys1	IIHRMY 2-1	30	19	11	1:1	2.13	0.14	3.84*
Mys2		21	11	10	1:1	0.05	0.83	
Mys5		30	14	16	1:1	0.13	0.72	
Mys6		30	17	13	1:1	0.53	0.47	
Mys2	IIHRMY 1-4	30	18	12	1:1	1.20	0.27	
Mys4		22	12	10	1:1	0.18	0.67	
Mys5		17	08	09	1:1	0.06	0.81	
Mys6		33	21	12	1:1	2.45	0.12	
Mos1	IIHRMO 12	28	15	13	1:1	0.14	0.71	
Mos2		26	12	14	1:1	0.15	0.69	
Mos4		24	13	11	1:1	0.17	0.68	
Mos6		36	19	17	1:1	0.11	0.74	
Mos1	IIHRMO 53	23	10	13	1:1	0.39	0.53	
Mos 3		17	08	09	1:1	0.06	0.81	
Mos4		34	19	15	1:1	0.47	0.49	
Mos5		18	08	10	1:1	0.22	0.64	

**Note:** Male sterile (MS line x inbred line) are the resulting progenies from Table 2

**Table 3 :** Segregation pattern in back cross with pollen parents of hybrids under study

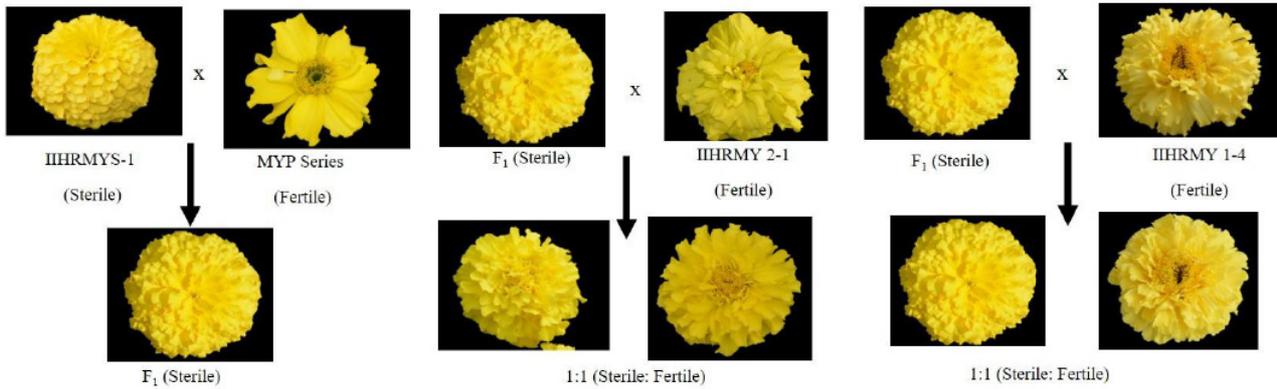
Male Sterile (Resulting progeny of segregated population)	Pollen parents of hybrids under study	Total plants	Number of plants (Sterile/ fertile)		Expected ratio	Chi-square test ( $\chi^2$ )		Chi-square test ( $\chi^2$ ) (Table)
			Sterile	Fertile		$\chi^2$ (calculated)	<i>p</i>	
(MOS1 × IIHRMO 12)	IIHRMO 12	67	39	28	1:1	1.81	0.18	3.84
(MOS6 × IIHRMO 12)		39	21	18	1:1	0.23	0.63	
(MOS4 × IIHRMO 12)		42	22	20	1:1	0.10	0.76	
(MOS5 × IIHRMO 12)		29	14	15	1:1	0.03	0.85	



	Fertile (MOP1 to MOP6) N(RfRf)	
Sterile (IIHRMOS-1) S(rfrf)	N(Rf)	N(Rf)
S(rf)	S(Rfrf) - Sterile	S(Rfrf) - Sterile
S(rf)	S(Rfrf) - Sterile	S(Rfrf) - Sterile

	Fertile (IIHRMO 12 & IIHRMO 53) N(RfRf)	
F <sub>1</sub> Sterile S (Rfrf)	N(Rf)	N(Rf)
S(Rf)	S(RfRf) - Fertile	S(RfRf) - Fertile
S(rf)	S(Rfrf) - Sterile	S(Rfrf) - Sterile

Fig. 1 : Inheritance pattern of male sterility in IIHRMOS-1 in combination with pollen parent MOP1 to MOP6 (MOP series)



	Fertile (MYP1 to MYP6) N(RfRf)	
Sterile (IIHRMYS-1) S(rfrf)	N(Rf)	N(Rf)
S(rf)	S(Rfrf) -Sterile	S(Rfrf) -Sterile
S(rf)	S(Rfrf) -Sterile	S(Rfrf) -Sterile

	Fertile (IIHRMY 2-1 & IIHRMY 1-4) N(RfRf)	
F <sub>1</sub> Sterile S (Rfrf)	N(Rf)	N(Rf)
S (Rf)	S(RfRf) - Fertile	S(RfRf) - Fertile
S(rf)	S(Rfrf) - Sterile	S (Rfrf) - Sterile

Fig. 2 : Inheritance pattern of male sterility in IIHRMYS-1 in combination with pollen parent MYP1 to MYP6 (MYP series)

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### Author contributions statement

**V:** Data curation; Formal analysis; Investigation; Writing-original draft; **T P:** Conceptualization; Resources; Supervision; Review & editing; **R K:** Resources; **U B T:** Resources; **H B K:** Resources; **V R:** Methodology; Resources; Software; **R H L:** Resources; **V R R P:** Resources.

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### Data availability statement

Data will be made available by corresponding author on reasonable request.

### Conflict of interest

The authors declare that they have no conflict of interest.

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