

Research

Can flower forms be indicators of pollen diversity and possible gametophytic selection in marigold breeding programs?

Labdhi Dedhia¹ · P. Veeresh¹ · Duleep Samuel² · R. Venugopalan³ · P. Tejaswini¹

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Abstract

Pollen shape has been utilized as a taxonomic attribute since it is thought to be the most constant trait within a plant species. The African marigold (*Tagetes erecta* L.) has substantial variability within the species due to its wider adaptability to various agroclimatic conditions; thus, from a palynological perspective, this species is interesting for studying the variation in its pollen grains. As marigold is a commercial flower crop, the breeding program involves multiple flower forms within the species. Therefore, the study aimed to characterize the typical pollen grain features of marigold and assess the potential variation in pollen morphology across different flower forms for possible gametophytic selection in breeding programs. Pollen grains of nine African marigold genotypes with six different flower forms were imaged using a scanning electron microscope to study their characteristic features. The pollen grains across *Tagetes erecta* genotypes were found to be radially symmetrical, isopolar monads that were oblate spheroidal and suboblate in shape with tricolporate apertures and with echinate erections on the pollen wall. Significant differences were recorded between the genotypes and flower forms for all the pollen morphological traits studied. The variability observed among the pollen grain populations within the genotypes for certain morphometric traits also suggested the potential of using gametophytic selection in marigold breeding programs. Although it is important to establish pollen traits for commercial hybridization programs, little information is available, and to our knowledge, this is the first report on characteristic pollen grain features in African marigolds.

Keywords Asteraceae · Intraspecific variability · Marigold · Male gametophyte · Palynology · Pollen grains

1 Introduction

African marigold (*Tagetes erecta* L.), indigenous to Mexico and Guatemala, is one of the most widely distributed members of the Asteraceae family and is cultivated globally. It is an often-cross-pollinated crop that is recognized as one of the most important traditional flowers in Asian countries and as a bright ornamental flower crop worldwide. The inflorescence of marigold plants is the capitulum, which is a flower head composed of numerous flowers with inner rows of hermaphrodite disc florets and outer rows of male sterile ray florets. Ray florets in general are considered petals that determine the aesthetic value of a flower for its commercial utility. Earlier reports suggested the existence of modified flower forms in marigold viz. single, semidouble, and double types of fertile lines as well as apetaloid and petaloid (modified petals) forms of male sterile lines. These male sterile lines have eased the production of hybrid

✉ Labdhi Dedhia, labdhidedhia@gmail.com; ✉ P. Tejaswini, tejaswinitejuin@yahoo.co.in | ¹Division of Flower and Medicinal Crops, ICAR—Indian Institute of Horticultural Research, Bengaluru, India. ²Division of Crop Protection, ICAR—Indian Institute of Horticultural Research, Bengaluru, India. ³Division of Social Sciences, ICAR—Indian Institute of Horticultural Research, Bengaluru, India.



seeds in marigold, as the sterile forms eradicate the tedious process of emasculation [1–3]. The development of a hybrid and the success of the hybridization program are determined by flowering, cross-fertilization, and detailed knowledge about the parents. Although maternal parents have a pronounced effect on the phenotype of offspring [4, 5], paternal parents can also affect several plant traits, making pollen parents a major contributor to the resulting progeny [6, 7], necessitating the study of pollen grains.

Pollen or microspores are microscopic abundant flower elements that offer an opportunity for male gametophytic selection. These male gametophytes also contain genetic information, which is crucial for sexual reproduction in seed-propagating plants [8]. In the field of palynology, the study of pollen grain characteristics (wall micromorphology, polarity, symmetry, shape and size) is useful because it provides information on the genetic distinctiveness and ancestry of plant genotypes, which is crucial for the exploitation of germplasm [9], and may be useful in the identification of species because the biological makeup of pollen is intricate and species specific [10]. Variability exists in the pollen grains of different species [11–13], and studies have also reported that pollen characteristics, especially the size of pollen grains, vary between genotypes/cultivars within species [14, 15].

The plant life cycle includes a haploid gametophytic phase, during which pollen grains compete for fertilization. This competition, known as gametophytic selection, is hypothesized to be a major driver of plant evolution and genetic variation. The resulting variation in fertilization success due to pollen competition creates the potential for strong directional selection [16, 17]. Earlier studies have reported variations in pollen size, content and vigor within a genotype that are being utilized for gametophytic selection in a breeding program [18–21]. Pollen grains are highly important for determining the taxonomy of flowering plants and have been used in determining the relationships between genera and species in the Asteraceae family [22]. However, there is limited information on morphometric analysis of pollen grains among African marigold genotypes. Considering the multiple flower forms that exist and are essential components in marigold hybrid development, it will be interesting to study the structure of pollen grains in relation to their flower forms and utility in crop improvement. Thus, the present study was carried out to determine the basic pollen morphology of marigold plants and to describe the variability in the pollen population within and between genotypes across flower forms.

2 Materials and methods

Marigold plants with different flower forms were collected from the ongoing marigold breeding program at the ICAR-Indian Institute of Horticultural Research, Bengaluru (13.14°N latitude and 77.50°E longitude), which is located at an elevation of approximately 890 m above mean sea level. The plant material selected for the study comprised nine genotypes representing different flower forms (Table 1). Six genotypes were found to be fertile and maintained by selfing, and the remaining three genotypes were male sterile lines. The sterility observed in the male sterile lines is due to the genetic male sterility, which is the most predominant kind of sterility in marigold and is widely utilized for commercial hybrid seed production. These sterile lines segregated into sterile: fertile (1:1) plants which were maintained by intercrossing between them. Fertile flowers were used for the extraction and estimation of pollen grains. Ten random flower buds from each plant were bagged using butter paper to prevent pollen contamination, and fully bloomed flowers were collected at the forenoon when the pollen sacs dehisced. The flowers were held upside down, tapped gently to collect the pollen grains and placed in the desiccator for an hour [23]. The pollen grain samples representing each genotype were dusted on two-sided copper tape and fastened on an aluminum metal plate, and the images were captured with a scanning electron microscope at 5 kV (Hitachi S3000 N, Japan). The morphometric parameters of 30 pollen grains from each genotype were measured using Fiji and ImageJ software [24]. The shape of the pollen grains was determined by the equation $FI = (P/E) \times 100$, where FI is the form index, P is the length of the polar axis (the line connecting the proximal and distal pole) and E is the length of the equatorial axis (the line perpendicular to the polar axis) [25, 26]. Depending on the FI values derived, the pollens were classified as oblate-spheroidal (0.89–0.99), spheroidal (1.00), prolate-spheroidal (1.01–1.14), subprolate (1.15–1.33) prolate (1.34–2.00). Descriptive analysis of pollen morphometrics was performed, and Duncan's multiple range test was used to investigate the differences between the genotypes. The possibility of pollen selection was analyzed using a frequency distribution superimposed on the expected Gaussian distribution (D'Agostino test). The genetic divergence among the genotypes was quantified by D^2 values using Mahalanobis D^2 statistics [27] based on Tocher's method and the R package "biotools" [28].

Table 1 General description of African marigold genotypes with different flower forms used in the study

Genotype	Description of flower form	
IIHRMO 12	Single fertile-with one whorl of ray florets	
IIHRMO 53	Single fertile -with one whorl of ray florets	
IIHRMO 2330	Semi-double fertile—with more than one whorl of ray florets	
IIHRMY 1–4	Double fertile—with more than three whorl of ray florets	
IIHRMO 2335	Double fertile—with more than three whorl of ray florets	
IIHRMY 2-1	Double fertile—with more than three whorl of ray florets	
IIHRMO 2356 SF	Apetaloid with Double fertile	
	Apetaloid—with only stigma and no petals	
IIHRMO 2337 SF	Double fertile-double with more than three whorl of ray florets	
	Single fertile—single with one whorl of ray florets	
		SterileFertile SterileFertile

Table 1 (continued)

Genotype	Description of flower form		
IIHRM 2–3 SF	Petaloid with single fertile Petaloid—with only stigma and petals Single fertile—single with one whorl of ray florets		
		Sterile	Fertile

3 Results

The results from detailed scanning electron microscopy images processed using FijiJ software revealed that most of the African marigold pollen grains belonged to the oblate spheroidal group, followed by the suboblate and rarely oblate groups, according to the classification of [25, 26] (Fig. 1). Since both mature and immature pollen grains are present during anther dehiscence, the variation in pollen shape may be attributed to developmental stages. However, mature pollen grains typically exhibit a consistent pollen shape. The pollen grains of the marigold plants were isopolar and radially symmetrical with tricolporate apertures. The spines on the walls of the pollen grains were pyramid shaped and often conical, with a wide base at the bottom and a blunt to pointed apical tip.

Descriptive statistics for pollen morphometrics were calculated to determine the general characteristic features of the marigold pollen grains (Table 2). The results revealed lower variance and range for all the traits under study, except

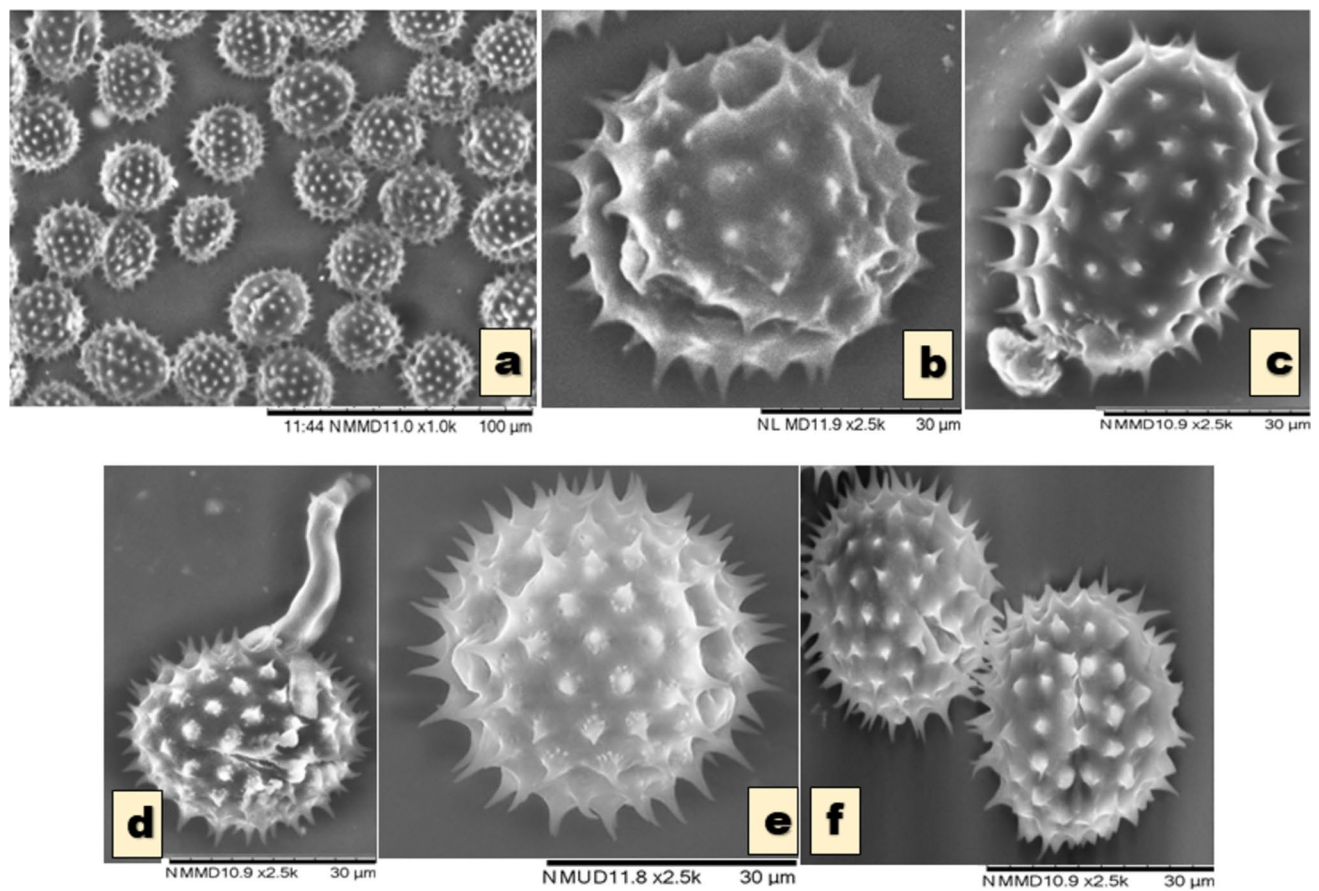


Fig. 1 Characteristics of pollen grain morphology in African marigolds. Mass of pollen grains at lower magnification (a); polar view of pollen (b); equatorial view of pollen (c); germination of pollen tubes from ectoaperture (d); tricolporate aperture (e, f)

Table 2 General descriptive statistics of African marigold pollen grains

	Perimeter (μm)	P: polar axis (μm)	E: equatorial axis (μm)	Length of ectoaperture (μm)	P/E ratio
Mean	101.42	29.39	32.86	4.51	0.90
Range	82.33–133.14	22.64–39.57	25.37–49.74	1.86–10.61	0.64–1.05
Sample variance	93.39	9.36	11.74	2.79	0.01
Skewness	0.62	0.22	0.59	0.95	– 0.91

for the perimeter of the pollen grains. The lengths of the polar and equatorial axis ranged from 22.64 to 39.57 μm and 25.37 to 49.74 μm, respectively, while the perimeter of the pollen grains ranged from 82.33 to 133.14 μm. Furthermore, the results revealed that the data pertaining to the polar axis followed a normal distribution, as the values obtained for skewness were near zero. In contrast, all the other traits studied had skewed distribution.

Analysis of variance was used to assess the statistical significance of differences in pollen morphometrics among various flower forms of marigold. The results revealed significant differences among the different flower forms for the traits studied (Table 3). Fertile counter parts to petaloid flowers exhibited significantly greater values for almost all the traits than did the other flower forms. The semi-double fertile form displayed the lowest values for perimeter and both polar and equatorial axis lengths. Fertile counter parts to petaloid flowers were found to be at par with the double fertile form for the equatorial axis. Similarly, the double fertile counter part of apetaloid flowers and double fertile flowers were at par for the polar axis.

Furthermore, Duncan's multiple range test was used to analyze the relationships and differences between the genotypes. Significant variations were observed for all the traits among the genotypes (Table 4). The genotype IIHRM 2–3 SF was significantly different for most of the traits under study except for the equatorial axis. The genotype IIHRMO 2330 was significantly different in terms of the trait perimeter of the pollen grains. Across the genotypes, the trait P/E ratio did not vary significantly. The pollen grains, classified according to Edrman's classification, revealed three shapes: oblate spheroidal, suboblate and rarely oblate. Moreover, across the genotypes, oblate spheroidal-shaped pollen grains were dominant, followed by suboblate pollen grains.

Tocher's method based on Mahalanobis D^2 statistics was used to estimate the genetic distances among the marigold genotypes belonging to the same as well as different flower forms, and to investigate whether genotypes belonging to the same flower form were grouped together. The results revealed that the genotypes, based on all the traits studied, were grouped into five distinct clusters (Table 5). Five genotypes belonging to the double fertile and semi-double fertile groups were grouped in the first cluster, whereas the remaining four genotypes were individually separated into four different clusters without exhibiting a definite trend.

The variability observed for pollen morphological traits across the genotypes is represented in a box plot (Fig. 2). A symmetric distribution was recorded only for the polar axis, while an asymmetric distribution was recorded for all the

Table 3 Pollen morphometric features of different flower forms in African marigolds

Flower forms	Perimeter (μm)	P: polar axis (μm)	E: equatorial axis (μm)	Length of ectoaperture (μm)	P/E ratio
Single fertile	101.26 ^b	28.26 ^c	32.70 ^b	4.86 ^b	0.87 ^c
Semi-double fertile	92.04 ^d	27.35 ^c	30.21 ^d	5.14 ^b	0.91 ^b
Double fertile	103.62 ^b	30.18 ^b	34.09 ^a	3.88 ^c	0.89 ^{bc}
Apetaloid with single fertile	96.54 ^c	27.54 ^c	30.64 ^{c,d}	3.72 ^c	0.90 ^{b,c}
Apetaloid with double fertile	96.25 ^c	30.39 ^b	31.92 ^{b,c}	3.75 ^c	0.95 ^a
Petaloid with single fertile	114.55 ^a	32.16 ^a	35.26 ^a	6.36 ^a	0.91 ^b
<i>F value</i>	33.58*	17.27*	14.96*	14.89*	4.88*
SE (m)±	1.39	0.49	0.56	0.28	0.01
SE (d)	1.97	0.69	0.79	0.40	0.02
CD (1%)	5.11	1.78	2.03	0.78	0.05

(* $p \leq 0.01$); *Superscript letters denote grouping through Duncan Multiple Range test

Table 4 Morphometric analysis of pollen grains from African marigold genotypes

Genotypes	Perimeter (μm)	P: polar axis (μm)	E: equatorial axis (μm)	Length of ectoaperture (μm)	P/E ratio	Shape of pollen
IIHRMO 12	98.76 ^{d,e}	26.70 ^e	31.85 ^{c,d}	6.36 ^a	0.84 ^e	Oblate spheroidal, suboblate and oblate
IIHRMO 53	103.77 ^c	29.81 ^{c,d}	33.54 ^b	5.15 ^b	0.89 ^{b,c,d}	Oblate spheroidal and suboblate
IIHRMO 2330	92.04 ^f	27.35 ^e	30.21 ^e	4.91 ^{b,c}	0.91 ^{b,c,d}	Suboblate and oblate
IIHRMY 1–4	100.59 ^{c,d}	29.02 ^d	33.37 ^{b,c}	3.66 ^d	0.88 ^{c,d,e}	Oblate spheroidal and suboblate
IIHRMO 2335	101.96 ^{c,d}	30.10 ^{b,c,d}	32.58 ^{b,c}	4.81 ^{b,c}	0.92 ^{a,b}	Oblate spheroidal
IIHRMY 2-1	108.32 ^b	31.40 ^{a,b}	36.33 ^a	3.62 ^d	0.87 ^{d,e}	Oblate spheroidal, suboblate and oblate
IIHRMO 2356 SF	96.25 ^e	30.39 ^{b,c,d}	31.92 ^{c,d}	3.81 ^d	0.95 ^a	Oblate spheroidal
IIHRMO 2337 SF	96.54 ^e	27.54 ^e	30.63 ^{d,e}	3.84 ^d	0.90 ^{b,c,d}	Oblate spheroidal and suboblate
IIHRM 2–3 SF	114.55 ^a	32.15 ^a	35.26 ^a	4.18 ^{c,d}	0.91 ^{a,b,c}	Oblate spheroidal, suboblate and oblate
SE (d)±	1.89	0.65	0.75	0.41	0.02	
CD 5%	3.72	1.28	1.47	0.80	0.04	

*Superscript letters denote grouping through Duncan Multiple Range test

other traits across the genotypes. Considerable variability was observed among the genotypes for all the traits under study, suggesting success of differential fertilization. Among the genotypes, IIHRM 2–3 SF exhibited the highest variability for all the traits, followed by the genotype IIHRMO 2330, and the lowest variability was observed in genotype IIHRMO 2356 SF. Furthermore, for the trait polar and equatorial axis outliers were recorded in the genotype IIHRMO 2330, although the range of variability was narrower. Similarly, for the trait perimeter, genotype IIHRMO 2335 recorded highest variability as both upper and lower outliers were observed, this variability in the perimeter of the pollen grains may be linked to genetic variation, environmental conditions, or floral form influences.

The findings from the box plot were further supported by the observed frequency distribution for the trait polar and equatorial axis. The distribution graph for certain genotypes was found to be deviating from the expected normal distribution. Within the pollen population of certain genotypes, a bimodal distribution pattern was observed with distinct peaks for both polar and equatorial axis, rather than an unimodal distribution skewed above the mean. This bimodal distribution categorized by two separate mean values for each trait, suggests the possibility of a selective mechanism acting on pollen morphology, indicating the possibility of selecting pollen grains for fertilization success. Two separate peaks were observed for the equatorial axis in pollen population of genotype IIHRMY1-4 and IIHRMO 2337 SF. Similarly, two peaks were observed for the polar axis in genotype IIHRMO 2330 and IIHRM 2–3 SF. The presence of this intra-genotypic variability for differences in pollen grain shapes within the genotype (Fig. 3) suggests the potential for gametophytic selection within the genotypes.

4 Discussion

Pollen studies help researchers understand the systematic and evolutionary relationships of various groups of flowering plants [29], and in the Asteraceae family, studies have confirmed that pollen morphological characteristics are useful tools for taxonomic classification [30]. The goal of the present study was to study the characteristic features of African marigold plants and to understand the variability among the flower forms across the genotypes. Pollen grains of marigold were found to be more or less spheroidal, tricolporate, with conical pointed or blunt spines irregularly distributed on the pollen wall, which was similar to the characteristics reported in the Asteraceae family [12, 31–33] and its tribe Coreopsidae [34]. The most dominant shape observed for marigold pollen grains was oblate spheroidal, followed by suboblate. Similar pollen shapes, viz. oblate-spheroidal plants were also recorded in *Dahlia* and *Hidalgoa* species, which belong to Asteraceae [32], while *Centaurea* species, which also belong to the Asteraceae family, had prolate spheroidal shapes in their pollens [12], revealing the species-specific nature of the pollens.

Significant differences were observed among the different flower forms and genotypes for the traits studied; however, the relationship between the genotypes and the flower forms could not be easily established. The estimation of genetic diversity is crucial for identifying distinct genotypes. D² statistics is the easiest biometrical tool for identifying genetically

Table 5 Mahalanobis D² distance between African marigold genotypes based on their pollen morphometric traits

	Cluster I				Cluster II		Cluster III	Cluster IV	Cluster V
	IHRMO 2330	IHRMO 2335	IHRMY 1–4	IHRMO 2356 SF	IHRMY 2-1	IHRMO 53			
IHRMO 2335	9.16								
IHRMY 1–4	2.44	7.24							
IHRMO 2356 SF	10.79	3.65	9.29						
IHRMY 2-1	8.21	8.56	4.45	9.67					
IHRMO 53	6.30	11.53	8.98	8.36	14.71				
IHRMO 2337 SF	7.67	5.43	5.44	12.03	15.34	13.52			
IHRMO 12	13.44	8.99	10.42	15.16	11.07	10.63	12.02		
IHRM 2–3 SF	7.40	7.64	12.61	13.71	12.78	13.60	13.93	13.81	–

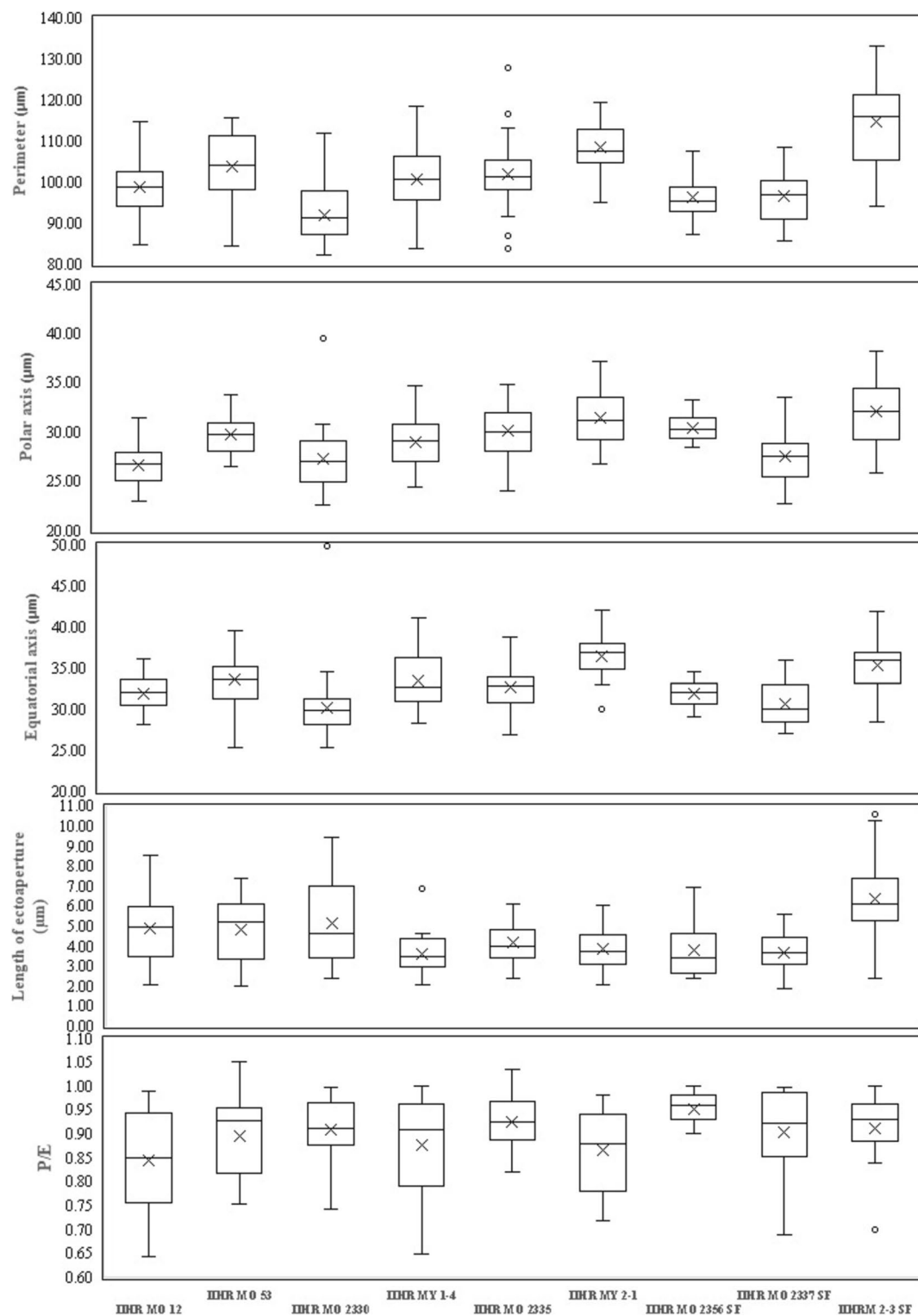


Fig. 2 Box plot for categorization of morphometric traits of the marigold pollen grains

similar and distinct genotypes and could be useful in future breeding programs. Based on the results obtained from Tocher's method, five distinct clusters were formed based on all the pollen morphological traits, with the maximum number of genotypes occurring in the first cluster, and all the genotypes were found to be double fertile. Thus, all the double types of fertile flowers, including those with a fertile counter to apetaloid types, exhibited similar pollen morphologies. In

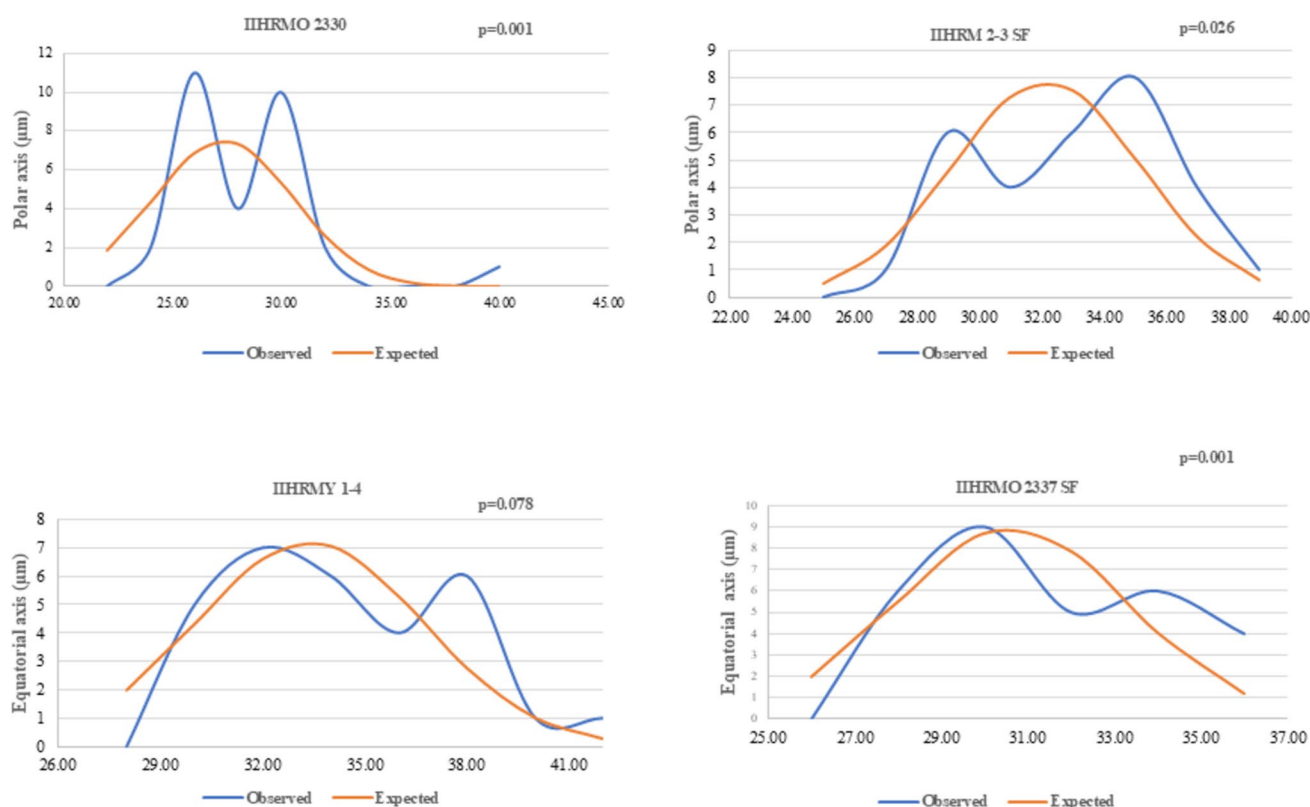


Fig. 3 Observed frequency distribution of pollen grain size in comparison with the expected normal distribution

contrast, the genotypes belonging to the single fertile group or sterile forms where the fertile flowers were of single types did not form a single cluster. Thus, the analysis did not strictly categorize the genotypes based on their flower forms. In contrast, using a similar method, Magdalena [13], were able to group *Linum usitatissimum* genotypes based on their utility.

The results obtained from the box plot showed variation across the genotypes, while frequency distribution studies revealed variation within the genotype. Pollen grain size, determined by the length of polar and equatorial axis, is the critical factor that determines the success of pollination and is influenced by postpollination male–male competition [35]. Deviation from a normal distribution within the genotype was observed for these traits, indicating the possibility of gametophytic selection. This breeding approach allows trait selection in its haploid state, preventing dominant alleles from concealing recessive alleles [36]. In majority of the genotypes under investigation, a greater number of genotypes with longer polar and equatorial axis were observed. These differences are crucial to breeding efforts because they increase the prospect of pollen selection within the population. Earlier studies have demonstrated the success of gametophytic selection in breeding different crops for various characteristics [20, 22, 37–39]. The success of gametophytic selection is attributed to the intense selection pressure imposed due to relatively larger pollen population compared to the sporophyte population size. The gametophytic stage also has potential in combining the desired genes, thereby increasing the chances of selecting complex allele combination.

5 Conclusion

The palynological description reported here is significant for advancing our knowledge of reproductive biology in African marigolds. The characteristic features of African marigold pollen grains are an isopolar monad mostly oblate-spheroidal in shape, with three colpi and sharp to blunt spines on the pollen walls. Significant variability was observed across the flower forms and within the genotype. Deviations from the normal distribution for traits such as polar and equatorial axis, which determine the shape and size of the pollen grains, suggest the possibility of gametophytic selection within the genotype for crop improvement programs in marigolds. The variability observed for pollen traits across the genotypes and flower forms suggests the possibility of exploring similar studies in many other commercially important species.

An elaborate study of pollen wall ornamentation and exine thickness and its relationship with pollen grain vigor and germination may further help in the assessment of the inter- and intraspecific variability of the use of pollen wall ornamentation in hybrid seed development.

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Author Contributions PT conceived and designed the experiment. LD performed the experiments, LD and VP assisted SD for imaging the pollen grains in SEM. LD analyzed the data with assistance from PT and RV. LD wrote the draft manuscript under guidance of PT. All the authors discussed the results and commented on the manuscript.

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Data availability All the data generated and analyzed during this study are included in this manuscript.

Declaration

Ethics approval and consent to participate The genotypes evaluated in this study were developed and owned by Dr. Tejaswini Prakash, Principal Scientist (and one of the authors) who leads the marigold breeding work at ICAR-Indian Institute of Horticultural Research, Bengaluru, India, where the study was conducted. Accordingly, the collection and use of plant materials used in this study comply with the local and national guidelines, and no further permissions were required. This article does not contain any studies with human participants or animals performed by any of the authors.

Competing interests The authors declare no competing interests.

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