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Pollen grain comparative study of *Kalanchoe Blossfeldiana* poelln cultivars

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Abstract
The study of pollen grains, or palynology, includes analyzing the microscopic structure and the application of pollen grain variations in different scientific branches. Seven cultivars including, (*K, Blossfeldiana* Calandiva; Leonardo *K. blossfeldiana* Calandiva; Fonda; *K. blossfeldiana* Calandiva Monro; *K. blossfeldiana* Calandiva Hayworth; *K. blossfeldiana* Calandiva Rosalina; *K. blossfeldiana* Calandiva Bardot; *K. blossfeldiana* Calandiva Middler) of the family Crassulaceae, where studied using microscopic and scanning electron microcsope (SEM) features. Inorder to evaluate interspecific similarities and differences, as a taxonomicindicators. The light microscope results revealed that pollen grains were free, small to medium size varied from subprolate, prolate – spheroidal and perprolate shapes, radially symmetric, isopolar, trisulcate. The Scanning electron microscope results showed simple differences in exine ornamentation, while polar, equatorial, and exine thickness measurements revealed variations among studied cultivars.

Keywords: Crassulaceae, *K. Blossfeldiana*, palynology, pollen grains

Introduction
The male gametophyte of the spermatophyta plants develops in the pollen sac, which contains a tissue act like a connection (a transient, sporophytic, apoptotic tissue) between the anther tissue and the gametophyte [1].
Pollen grains shape variations have taxonomical significance in plant taxonomy that cannot be overlooked. Study of pollen grain have great importance in the spot light over a lot of relationships that are difficult to diagnose in other ways, weather that it is at level of genera or species. Pollen grains considered one of important factors in identifying and linking natural and evolutionary relationships among different plant genera and families [2, 3]. Palynology is involved in the discipline of the study that examines plant pollen grains in both fossil and living organisms [4].
K. Blossfeldiana Poelln. Is one of the family Crassulaceae genera of about (125) species [5], as an ornamental pot plant which has beautiful flowers of different colors and succulent leaves with a long flowering period. The importance of Succulent plants as an economic ornamental and the palynological studies are scarce, this is why groups of uncertain classification appear [6]. One of Crassulacean acid metabolism plants (CAM) plant which have taxonomical importance [7, 8], *K. blossfeldiana* have a large number of cultivars hard to classify because of phenotypic affinity among them, Interspecific hybridization give so many cultivars [7, 9], so it is necessary to find accurate indicators to make a taxonomical comparison like the study of pollen grain of 28 species of the family Crassulaceae by light and electron microscope [10] and discribe the morphology of pollen grain of *K.integra* are sub oblate while the tectum was straight and isopolar. In another study on *K. laetivirens* pollen grain and confirmed that the pollen grain has radial symmetry, tricolporate, and is small [11]. In another article K. densiflora submitted a general description of morphological and measurements of polar, equatorial, and P/E ratio [12]. The pollen grain of the family Crassulaceae has an equatorial view (subspheroidal to prolate), with (convex-triangular) in polar view, 3 – colporate and longate apertures with complete straight smooth tectum [6]. The exine of the family Crassulaceae is thin [13].
Most of the studies on Crassulaceae pollen grain used light microscopy and SEM on Korean Crassulaceae [14]; Sedum [15], as well as in Sempervivum [16]. Three species of *Bryophyllum* (Lam.) and two of *Kalanchoe* were examined and found Psilate sculpturing in all the studied species and observed that pollen grains ranged from (acolpate, monocolpate, bicolpate, and tricolpate). The studied species showed a pollen grain with a circular shape [17].
The research aims to highlight the pollen grain of *K. blossfeldiana* cultivars as a taxonomic indicator, since there is no specialized study on the pollen grain of the mentioned species' cultivars.

Materials and Methods
Pollen grain were collected from flowers of K. blossifeldiana cultivars which have different flowers colors from university of Mosul gardens after being diagnosed depending on [5], fresh pollen grain prepared for light microscopy depending on [18], by staining it with (safranin – tincture glycrerin) after keeping the florets in formalin-aceto-Alcohol (FAA) and excision of the mature anther, put it on a clean slide, adding a drop of safranin –glycerine, which prepared by putting the sample in plastic tube with (0.1ml) water and adding a drop of glycerol with a drop of safranin carfully, re-cap the tubes, vortex (10) seconds then let them over night, wash with ethanol, with centrifuge, placed them on slides, covering with cover slide or without staining and check using light microscope (Type Ogawa)

under (40 x) and (oil immersion 100x) and ocular piece for measuring (using quantitative and qualitative characteristics) polar axis, equatorial axis and exine thickness, by taking the average of (30) samples, (SEM) sample prepared by mounting pollen grains on double side carbon tab and using aluminum for fixation,and the gold as a cover depending on [16, 19]. The data were analized using a simple experimental system and completely randomized desighn, employing (Multivariate analysis of variance MANOVA), caculation of significant deferences at (p-value ≥ 0.05).

Results

1. Pollen Grain Size and Shape

The results are presented in (Table 1) and (Figs. 1-2) showed that the pollen grains of the seven studied cultivars were monads and ranged from small to medium in size. Based on the P/E ratio, pollen shape varied from subprolate to prolate-spheroid among the cultivars.

Table 1: Pollen grain general characteristics of studied *Kalanchoe blossfeldiana* cultivares.

Species	No. of stamens \ flower	No. of petals av.	Shap of pollen grain	Thickness of exine av. (µm)	Polar axis av. (µm)	Equatorial axis av.(µm)	P/E
K. blossfeldiana Calandiva Monro	4	25	Subprolate	0.5	30.345	24.415	1.242
K. blossfeldiana Calandiva Hayworth	8	16	Prolate-spheroidal	0.4	28.656	25.160	1.138
K. blossfeldiana Calandiva Fonda	8	19	Perprolate	0.9	16.270	7.650	2.126
K. blossfeldiana Calandiva Bardot	4	14	Subprolate	0.5	49.090	35.15	1.396
K. blossfeldiana Calandiva Leonardo	8	25	Prolate-spheroidal	0.8	25.520	24. 262	1.051
K. blossfeldiana Calandiva Rosalina	8	23	Preprolate	1.25	14.195	6.854	2.071
K. blossfeldiana Calandiva Middler	12	30	Subprolate	1.30	15.400	12.384	1.243

Each value represent average of 30 replicate

The results in (Table 1) also showed variation in both polar axis length and equatorial diameter. According to these measurements, the cultivars could be classified into 2 groups. The first group, with smaller average dimensions, included (*K. Blossfeldiana* Calandiva Fonda, *K. blossfeldiana* Calandiva Rosalina, and *K. blossfeldiana* Calandiva Middler. The second group, with medium average dimensions, included *K. blossfeldiana* Calandiva Leonardo, *K. blossfeldiana* Calandiva Hayworth, *K. blossfeldiana* Calandiva Monro, and *K. blossfeldiana* Calandiva Bardot).

Table 2: Multivariate tests of variance (MANOVA) of studied traits

Multivariate Tests[c]							
Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	1.000	6.887E4[a]	6.000	9.000	.000	1.000
	Wilks' Lambda	.000	6.887E4[a]	6.000	9.000	.000	1.000
	Hotelling's Trace	4.591E4	6.887E4[a]	6.000	9.000	.000	1.000
	Roy's Largest Root	4.591E4	6.887E4[a]	6.000	9.000	.000	1.000
groups	Pillai's Trace	4.671	8.200	36.000	84.000	.000	.778
	Wilks' Lambda	.000	412.918	36.000	42.283	.000	.986
	Hotelling's Trace	3.954E3	805.409	36.000	44.000	.000	.998
	Roy's Largest Root	3.161E3	7.377E3[b]	6.000	14.000	.000	1.000
a. Exact statistic							
b. The statistic is an upper bound on F that yields a lower bound on the significance level.							
c. Design: Intercept + groups							

Table 3: Levene's Test of Equality of Error of variance (MANOVA) of studied traits

Levene's Test of Equality of Error Variances[a]				
	F	df1	df2	Sig.
No. of stamens	.392	6	14	.872
No. of petals av.	1.519	6	14	.243
Thickness of exine av.	5.930	6	14	.003
Polar axis av. (µm)	1.988	6	14	.136
Equatorial axis av. (µm)	3.026	6	14	.041
P/E ratio	5.569	6	14	.004
Tests the null hypothesis that the error variance of the dependent variable is equal across groups; a. Design: Intercept + groups.				

As shown from the tables 2.3 each of (No. of stamens av; No. of petals av.; Polar axis av. (µm)) have no significant defernces at level 1%, while both of (Thickness of exine av. and P/E ratio) have significant defernces at level 1% and the Equatorial axis av. (µm) charecter have significant defernces at level 5 %.

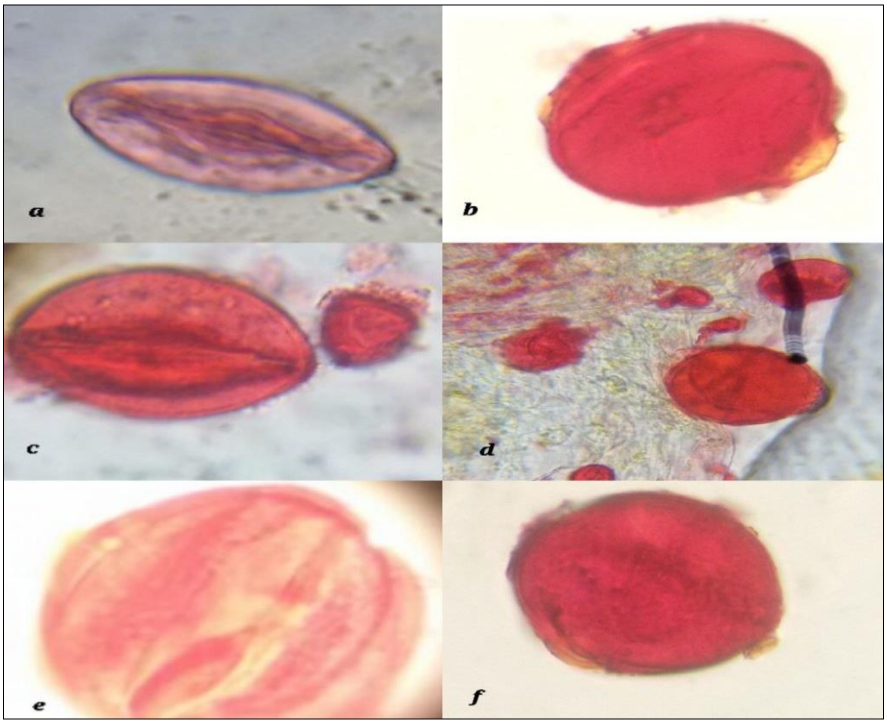

Fig 1: Shows the pollen grain morphological characters, *K. blossfeldiana* Calandiva Bardot

a. Equatorial axis
b. Polar axis, *K. blossfeldiana* Calandiva Monro
c. Equatorial axis
d. Polar axis, *K. blossfeldiana* Calandiva Leonardo
e. Equatorial axis
f. Polar axis. on light microscope oil lens (100x).

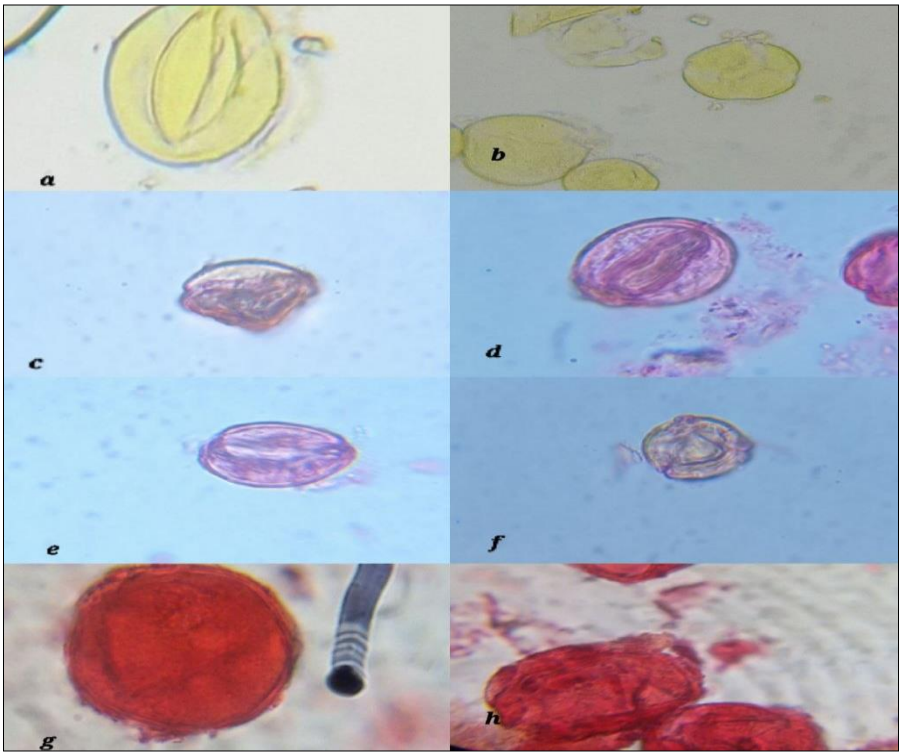

Fig 2: Shows the pollen grain morphological characters, *K. blossfeldiana* Calandiva Hayworth (unstained)

a. Equatorial axis b. Polar axis, *K. blossfeldiana* Calandiva Fonda
b. Equatorial axis d. Polar axis, *K. blossfeldiana* Calandiva Rosalina e. Equatorial axis
c. f. Polar axis, on light microscope oil lens (100x).
d. *K. blossfeldiana* Calandiva Middler. g. Equatorial axis h. Polar axis, on light microscope oil lens (100x).

2. The relationship between Exine thickness and number of petals per flower

Exine thickness varied among the studied cultivars and ranged from 0.4 µm to 1.30 µm. The lowest value was recorded in *K. blossfeldiana* Calandiva Hayworth (0.4 µm), whereas the highest value was recorded in *K. blossfeldiana* Calandiva Middler (1.30 µm). Other cultivars showed intermediate values, including *K. blossfeldiana* Calandiva Leonardo (0.8 µm), *K. blossfeldiana* Calandiva Fonda (0.9 µm), and *K. blossfeldiana* Calandiva Rosalina (1.25 µm) (Table 1), Observation from (fig.3) that the pattern of variation of exine thickness is somewhat similar to the variation pattern of number of petals per flower, except for the cultivar *K. blossfeldiana* Calandiva Rosalina which showed a clear difference, exhibiting an increase in the average of exine thickness and decrease in the unmber of petals per flower.

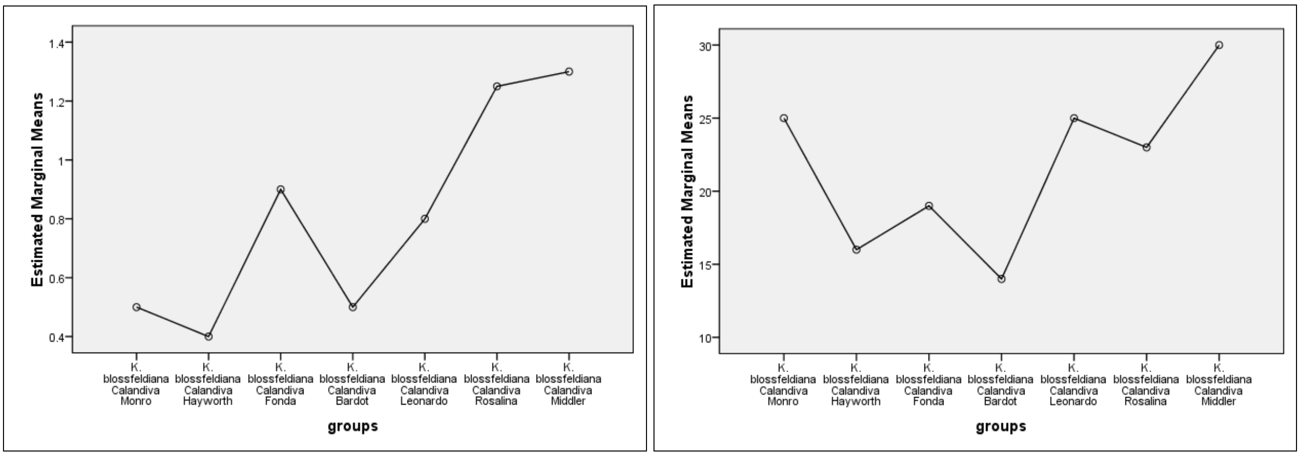

Fig 3: The relationship between Exine thickness and number of petals per flower

3. Relationship Between Pollen Dimensions and Stamen Number

The results also indicated AN variable relationship between patteren of variation of stamen number and pattern of variation of pollen grain dimensions. Cultivars with a smaller number of stamens (*K. blossfeldiana* Calandiva Monro; *K. blossfeldiana* Calandiva Bardot) showed greater values of polar axis length average and equatorial diameter average, whereas cultivars with a higher number of stamens average (*K. blossfeldiana* Calandiva Hayworth; *K. blossfeldiana* Calandiva Fonda; *K. blossfeldiana* Calandiva Rosalina; *K. blossfeldiana* Calandiva Leonardo; *K. blossfeldiana* Calandiva Middler) showed smaller pollen dimensions. The cultivars with 4 stamens had the largest pollen measurements, P/E ratio showed variable patteren and non – dependent based on previosly mentioned dimentions (Table 1) (fig. 5).

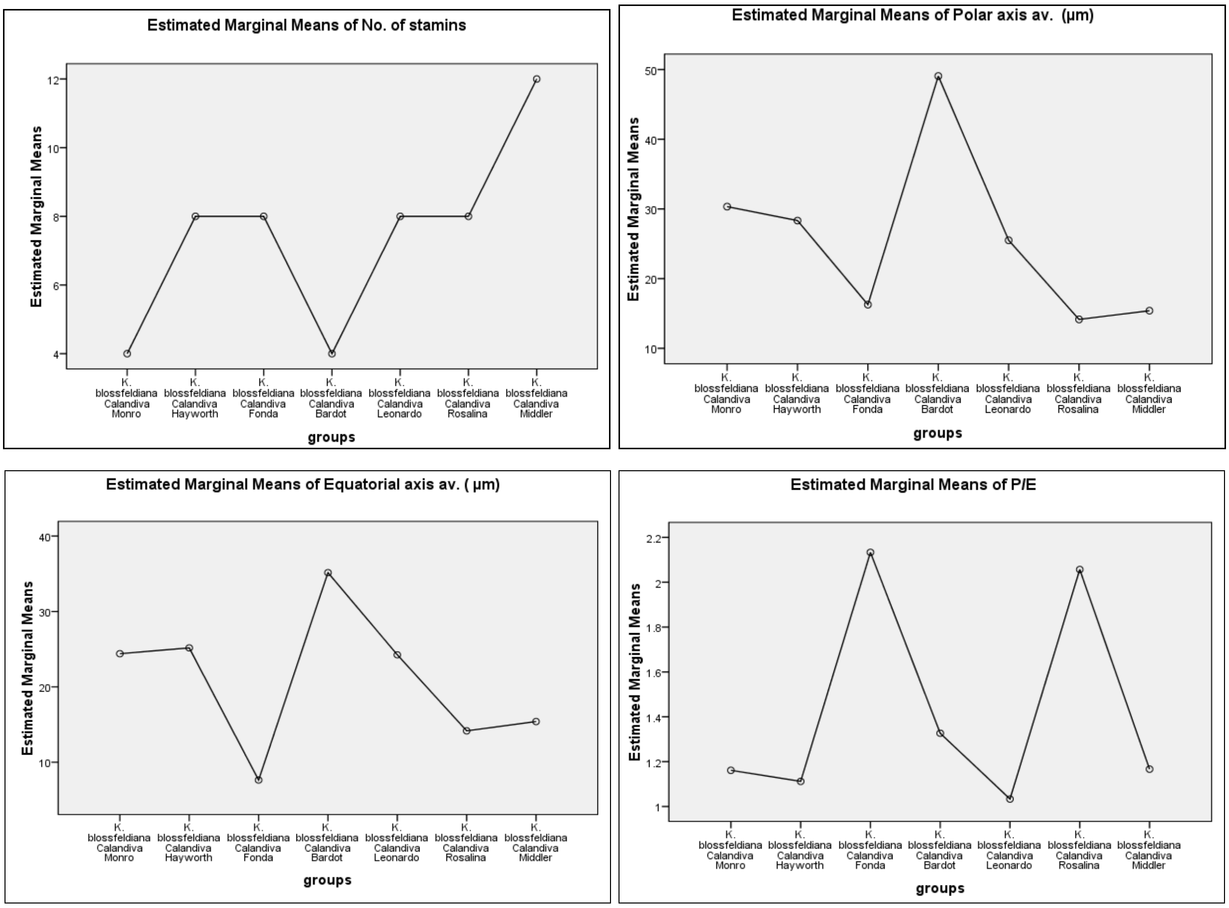

Fig 4: The relationship between pollen dimensions and stamen number

4. Pollen Ornamentation

Scanning electron microscopy (SEM) was used to examine pollen wall ornamentation, apertures, and furrows.

The Results Revealed 2 Types of Ornamentation Among the Studied Cultivars:

- Striate ornamentation, which was observed in all studied cultivars (Figs. 5, 6)
- Verrucate ornamentation, which was abundant in *K. blossfeldiana* Calandiva Bardot (Fig. 3b), less evident in *K. blossfeldiana* Calandiva Monro (Fig. 5a), and also present in *K. blossfeldiana* Calandiva Leonardo (Fig. 6c)

The presence of verrucate ornamentation distinguished these 3 cultivars from the others, whereas striate ornamentation represented a common feature shared by all cultivars.

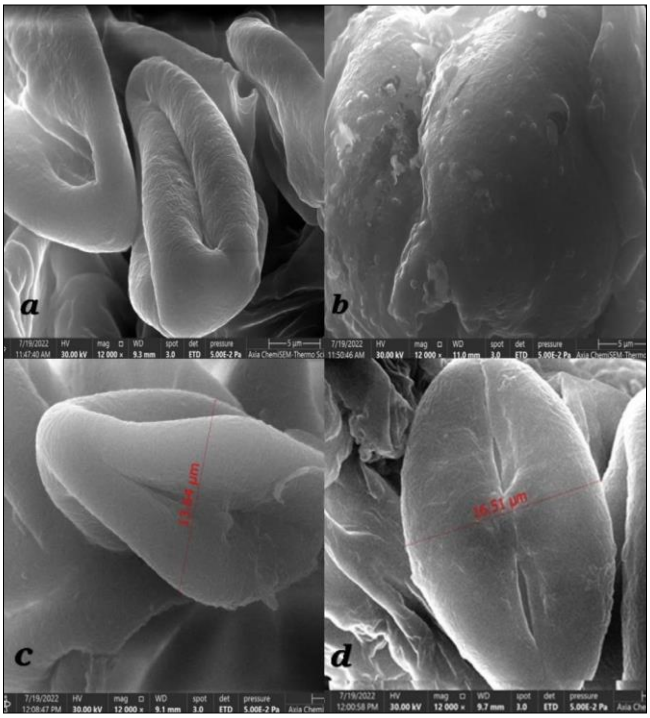

Fig 5: Scanning electron (S.E.M) of pollen grain micrographs of studied cultivars

mag: Magnification power (% image aerea; scanned area); HV: High voltage; WD: The working distance between the sample and the objective lens; det ETD (Everhart –Thornly Detector), (which coollect secondary electrons with low energy emitted from the specemen; spot: electron beam diameter).

a. *K. blossfeldiana* Calandiva Monro,
b. *K. blossfeldiana* Calandiva Bardot,
c. *K. blossfeldiana* Calandiva Fonda,
d. *K. blossfeldiana* Calandiva Hayworth.

mag: Magnification power (% image aerea; scanned area); HV: High voltage; WD: The working distance between the sample and the objective lens; det ETD (Everhart –Thornly Detector), (which coollect secondary electrons with low energy emitted from the specemen; spot: electron beam diameter)

a. *K. blossfeldiana* Calandiva Rosalina,
b. *K. blossfeldiana* Calandiva Leonardo,
c. *K. blossfeldiana* Calandiva Middler

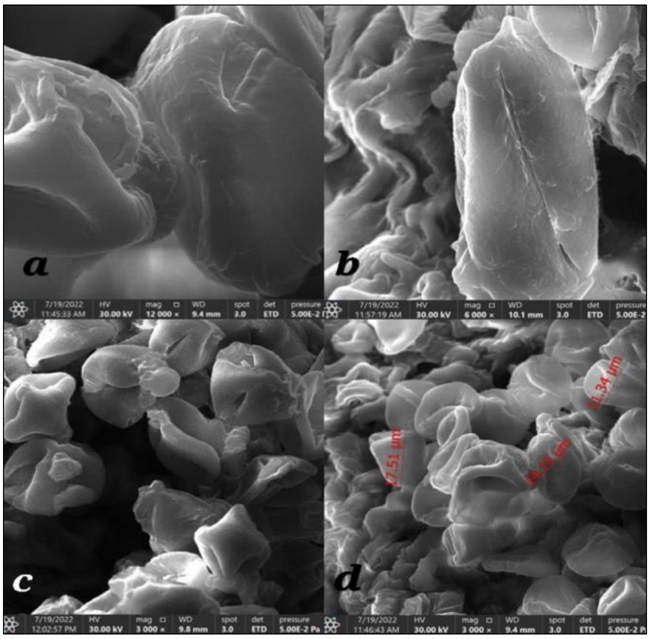

Fig 6: Scanning electron (S.E.M) of pollen grain micrographs of studied cultivars

Discussion

The present results showed that the pollen grains of the studied *K. Blossfeldiana* cultivars were monads and ranged from small to medium in size, with shapes varying from subprolate to prolate-spheroidal. These findings are generally consistent with previous reports on species of the genus Kalanchoe. For example, K. densiflora was reported to have monad, medium-sized, colporate, isopolar pollen grains with an oblate shape based on the P/E ratio [10,12]. Similarly, Pereira and Dubet [6] reported that the pollen grains of Kalanchoe humilis are medium-sized, monad, isopolar, radially symmetrical, prolate-spheroidal, and tricolporate.

The present findings also showed some similarity to the genus Sempervivum, in which pollen grains observed under SEM and light microscopy were reported as tricolporate and oblate-spheroidal [20]. However, the studied cultivars differed by having smaller polar and equatorial axis lengths and, in some cases, greater exine thickness. Variation in exine thickness among the studied cultivars may have taxonomic value. From the current study, exine thickness diverge from (0.4 µm to 1.30 µm), which is in general agreement with the range reported for members of the family Crassulaceae by Shehata *et al*. [16]. This variation, together with differences in polar and equatorial dimensions, supports the usefulness of pollen characters in distinguishing among cultivars.

The inverse relationship observed between pollen size and stamen number may also be of diagnostic significance. Cultivars with fewer stamens tended to have larger pollen grains, whereas those with more stamens showed smaller pollen dimensions. This pattern may reflect developmental or genetic differences among the cultivars and could be useful in comparative taxonomic studies. Pollen wall structure plays an important role in the interpretation of ornamentation patterns. The pollen wall consists of exine and intine, and the exine includes ectexine and endexine. The ectexine is composed of the tectum and columella, which together form the sexine, while the nexine lies beneath it and above the intine [21]. Because ornamentation is mainly expressed on the tectum, SEM is particularly useful for identifying fine surface patterns and for distinguishing closely related cultivars.

In the present study, striate ornamentation was found in all cultivars and therefore represents a common feature among them. In contrast, verrucate ornamentation was mainly observed in *K. blossfeldiana* Calandiva Bardot, *K. blossfeldiana* Calandiva Monro, and *K. blossfeldiana* Calandiva Leonardo. This feature may therefore be used as a diagnostic character for these cultivars. The shared pollen characters among the studied cultivars may be explained by their genetic relatedness as members of the same species. At the same time, the observed differences in pollen size, exine thickness, and ornamentation may reflect divergence resulting from cultivar selection, interspecific hybridization, or inbreeding. This interpretation is supported by Al-Naib and Al-Wattar [22], who suggested that the studied cultivars are not monophyletic.

Conclusions

In conclusion, the average of polar and equatorial length can be used- to classify *Kalanchoe blossfeldiana* cultivars. Thickness of exine average; number of stamens, number of petals, and can be used in distinguishing *K. blossfeldiana* cultivars. The type of ornamentation on the exine can be used- as a diagnostic feature for studied cultivars.

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NA

Conflict of Interests

The author declares no conflicts of interest.

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