

## Pollen Morphology of Some Species of Genera *Kalanchoe* and *Bryophyllum* in the Subfamily Kalanchoideae in South Western Nigeria

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### ABSTRACT

Acetolysed pollen grains of three species in the genera *Bryophyllum* and *Kalanchoe* were examined with the aim of studying the pollen characters that are of taxonomic importance among them. The three species belong to subfamily Kalanchoideae. The acetolysis procedure was carried out following standard procedures and the pollen characters were described and measured. The three species used are *Bryophyllum pinnatum* (Lam.) Oken, *Kalanchoe crenata* (Andr.) Haw and *Kalanchoe lanceolata* (Forsk.) Pers. Psilate sculpturing was observed in all the species studied. Pollen grains ranging from acolpate, monocolpate, bicolpate and tricolpate were found in all of the species investigated. Circular pollen shape was found in all of the studied species. Palynological informations about the pollen grain of the two genera were revealed.

**Keywords:** Aperture; Sculpturing; Pollen shape; Pollen diameter; Pore diameter

### Introduction

Crassulaceae comes as the third largest succulent plant family following Aizoaceae and Cactaceae with 1,500 species from 35 genera [1,2]. Members of Crassulaceae are mostly found in arid and/or rocky habitats even though they prefer warm region while they are rarely found in aquatic environment. It was affirmed by, that the family Crassulaceae is subdivided into three subfamilies namely Crassuloideae, Kalanchoideae and Sempervivoideae. Genera *Kalanchoe* Adans and *Bryophyllum* Salisb. belong to the subfamily Kalanchoideae [3,4].

Pollen morphology of Crassulaceae has been studied by [5-7]. Pollens of 20 species of Korean *Sedum* were investigated by under the scanning electron microscope to look for new features for classifying *Sedum* species located in Korea. Pollens of *Rosularia cypria*, *Rosularia pallidiflora* and *Sedum lampusae* amongst other species native to north-ern cyprus under the light and Scanning Electron Microscope (SEM) were studied by [8,9]. Palynological evidence alongside morphological evidence was used by [10] to determine the taxonomic status of *Rosularia globulariifolia* and *Rosularia pallidiflora* in the subfamily sempervivoideae. *Kalanchoe*, *Sedum* and *Rosularia* was mentioned as one of the genera in the family Crassulaceae by [3]. Pollen morphology/palynology can be used to support morphology of plants and as an important tool for contemporary taxonomist for the delimitation of species [11]. Pollen morphological characters have been found valuable and helpful in delimiting the taxa of Crassulaceae [12]. There is lack of published work on the pollen morphology of members of genera *Bryophyllum* and *Kalanchoe* especially the species found growing in Nigeria. Hence the study.

### Materials and Methods

Pollens were collected from flowers of two species of *Kalanchoe*, *Kalanchoe crenata* (Andr.) Haw and *Kalanchoe lanceolata* (Forsk.) Pers and *Bryophyllum pinnatum* (Lam.) Oken and stored in 100% ethanol. The acetolysis includes a number of centrifugation at 4000 rpm for 20 minutes at each stage. The pollens were first macerated in the centrifuge

tubes containing absolute ethanol with a stirring rod. The specimens were then centrifuged at the above mentioned speed and minute. The alcohol was poured off, 5 ml of glacial acetic acid was added to each tube and the mixture was centrifuged again at the same speed and time. The glacial acetic acid was poured off and acetolysis mixture (1 ml of H<sub>2</sub>SO<sub>4</sub> in 6 ml acetic anhydride) was added, the tubes containing the mixture was then placed in a water bath at 70°C and heated to boil while the mixture was stirred at intervals. The boiled mixture was then centrifuged again at the same speed and minute. Afterwards, 10 ml of glacial acetic acid was added, centrifuged again at the same speed and minute and was later poured off. Distilled water was finally added, the mixture shaken and centrifuged at the same speed. The water was poured off and the pollen grains were mounted in dilute glycerine for observation under the microscope as shown in Figure 1.

Photomicrographs of the acetolysed pollen grains were taken using ACCU-scope trinocular microscope(ACCU-scope 33001 led trinocular microscope with 3.2 MP CMOS digital camera). Qualitative characters such as exine pattern, type of aperture, type of pollen and shape of the pollen grains were observed. Quantitative characters that were measured or counted were pollen wall thickness, pollen diameter, depth of colpi, distance between colpi, pore diameter and number of pores. Data gotten were subjected to Single Linkage Cluster Analysis (SLCA), Principal Components Analysis (PCA) using Paleontological Statistics Software Package (PSSP) and Least Significant Difference Test (LSDT) given by Figure 2.

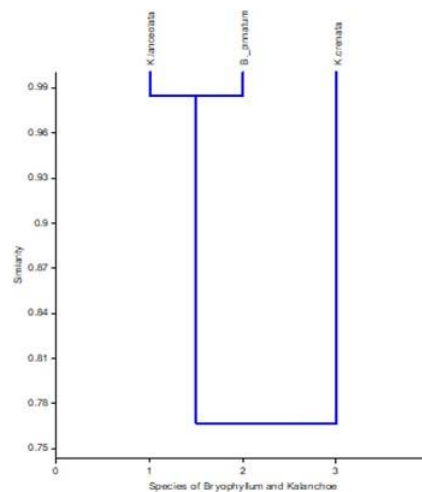


Figure 1: Dendrogram of pollen grain attributes of the species studied.

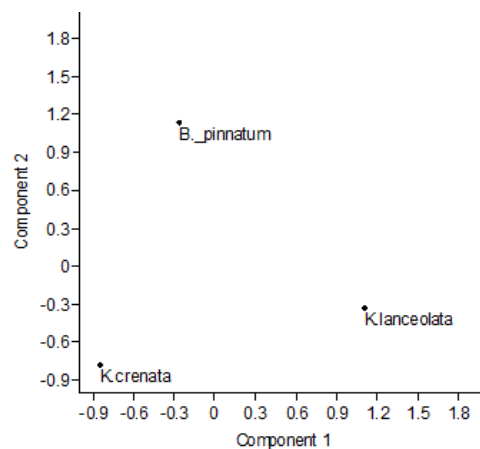


Figure 2: Principal components analysis showing the relationship of the Bryophyllum and Kalanchoe species based on pollen attributes for components 1 and 2. Legend : B.pinnatum-B.pinnatum, K.crenata-K.crenata, K.lanceolata.

**Results**

The pollen grains of these species are majorly circular to oval in shape. The two types of aperture, colpi (furrow) and pores are present in the pollen grains. The pollens are light brown in colour with psilate sculpture. Table 1 shows the qualitative characters of the pollen grains. Table 2 shows the least significant difference test of the species of Kalanchoe and Bryophyllum studied. Table 3 shows Eigenvalue and the percentage of total variation accounted for by the first two components axes of ordination of the Bryophyllum and Kalanchoe.

Species	Shapes	Pollen types	Sculpturing
<i>B. Pinnatum</i>	Circular and Oval	Acolpate, monocolpate, bicolpate, tricolpate, tetracolpate	Psilate
<i>K. Lanceolata</i>	Circular and Elliptic	Acolpate, monocolpate, bicolpate, tetraporate and tetracolpate	Psilate
<i>K. Crenata</i>	Oval and Circular	Acolpate, Monocolpate, Bicolpate, and Tricolpate	Psilate

**Table 1:** Summary of the qualitative characters of the pollen grains.

Species	Pollen diameter	Pollen wall thickness	Depth of colpi	Distance between colpi	Pore diameter	No of pores
<i>K. Crenata</i>	17.70 <sup>b</sup>	1.94 <sup>b</sup>	12.77 <sup>a</sup>	7.07 <sup>c</sup>	1.33 <sup>c</sup>	0.38 <sup>b</sup>
<i>K. Lanceolata</i>	27.28 <sup>a</sup>	1.98 <sup>b</sup>	6.33 <sup>b</sup>	15.13 <sup>a</sup>	10.75 <sup>a</sup>	3.30 <sup>a</sup>
<i>B. Pinnatum</i>	16.97 <sup>c</sup>	2.24 <sup>a</sup>	5.13 <sup>b</sup>	10.08 <sup>b</sup>	5.87 <sup>b</sup>	2.70 <sup>a</sup>

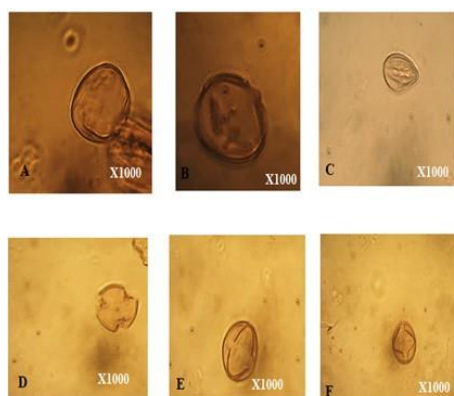
**Table 2:** The mean values of the quantitative palynological characters of the species studied with LSD (Means with the same alphabet along the same column are not significantly different).

Principal components axis	Eigenvalue	Percentage of total variation	Cummulative percentage
I	4.0739	67.898	67.898
II	1.9261	32.102	100

**Table 3:** Eigenvalue and the percentage of total variation accounted for by the first two components axes of ordination of the Bryophyllum and Kalanchoe species based on palynological data.

***K. Lanceolata***

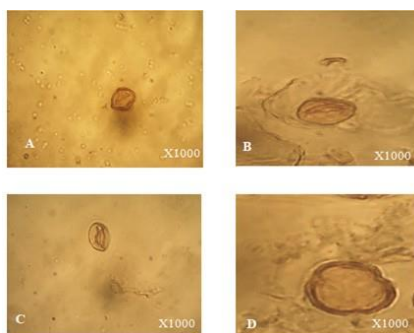
The types of pollen grains present are acolpate, monocolpate, bicolpate, tricolpate, tetraporate and tetracolpate. Shape is majorly circular and occasionally elliptic. Sculpturing is psilate. The mean diameter of the pollen grains is  $27.28 \pm 0.27 \mu\text{m}$ , mean pollen wall thickness is  $1.98 \pm 0.05 \mu\text{m}$ , mean depth of colpi is  $6.33 \pm 0.34 \mu\text{m}$ , mean distance between colpi is  $15.12 \pm 0.49 \mu\text{m}$ , mean pore diameter is  $10.75 \pm 0.35 \mu\text{m}$  and number of pores,  $9.5 \pm 0.21 \mu\text{m}$ . The apertures were situated in both the polar and equatorial regions of the pollen grains (Figure 3A-F; Tables 1 and 2).



**Figure 3:** Pollen grains of Kalanchoe lanceolata, A. Acolpate , B. Monocolpate, C. Bicolpate, D. Tricolpate, E. Tetraporate, F. Tetracolpate.

**K. Crenata**

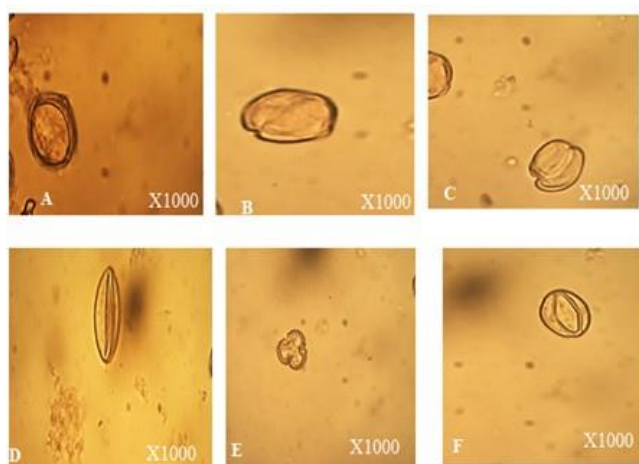
The types of pollen grains present are acolpate, monocolpate, bicolpate and tricolpate. Few pollens were encountered. Shape is majorly circular. Sculpturing is psilate. The mean diameter of pollen is  $17.7 \pm 0.24 \mu\text{m}$ , mean pollen wall thickness is  $1.94 \pm 0.06 \mu\text{m}$ , mean depth of colpi is  $12.77 \pm 0.52 \mu\text{m}$  and mean distance between colpi is  $7.07 \pm 0.3 \mu\text{m}$ , mean pore diameter is  $6.5 \pm 0.54 \mu\text{m}$  and mean number of pores,  $6.39 \pm 0.94 \mu\text{m}$ . The apertures were situated in both the polar and equatorial regions of the pollen grains (Figure 4: A-D; Tables 1 and 2).



**Figure 4:** Pollen grains of *Kalanchoe crenata*, A. Acolpate, B. Monocolpate, C. Bicolpate, D. Tricolpate.

**Bryophyllum pinnatum**

The types of pollen grains present are acolpate, monocolpate, bicolpate, tricolpate, tetracolpate. Shape is majorly circular and oval, sculpturing is psilate. The mean diameter of pollen is  $16.97 \pm 0.16 \mu\text{m}$ , mean pollen wall thickness is  $2.24 \pm 0.04 \mu\text{m}$ , mean depth of colpi is  $5.13 \pm 0.47 \mu\text{m}$ , mean distance between colpi is  $10.08 \pm 0.19 \mu\text{m}$ , mean pore diameter is  $5.87 \pm 0.22 \mu\text{m}$  and mean number of pores is  $7.9 \pm 0.33 \mu\text{m}$ . The apertures were situated in both the polar and equatorial regions of the pollen grain (Figure 5: A-F; Table 1 and 2).



**Figure 5:** Pollen grains of *Bryophyllum pinnatum* A. Acolpate, B. Monocolpate, C. Bicolpate (polar), D. Bicolpate (equatorial), E. Tricolpate, F. Tetracolpate.

**Discussion**

Palynological evidences have been found useful at all taxonomic levels due to the fact that the pollen wall structure is not influenced by environmental factors [13]. Differences in pollen shape, aperture type, grains arrangement and exine surface pattern has been found useful in the delimitation of taxa [14].

Psilate sculpturing observed in all the species studied is in accordance with the result of the work of [12]. Who ob-

served psilate type of ornamentation within the family Crassulaceae and also [10]. Who observed psilate ornamentation in species of *Rosularia* (Crassulaceae).

It was reported by that pollen aperture was a good classification character in the species of Cassia and Senna. It was noted by that the first features for the identification of any fossil grain is the aperture [15,16]. Pollen apertures, according to [17], are one of the most essential characteristics of pollen that provide informative qualities for systematic study. Pollen grains from acolpate, monocolpate, bicolpate and tricolpate were found in all of the species investigated. Tetracolpate pollen was found in *K. Lanceolata* and *B. Pinnatum* which delimits them from *K. Crenata*.

Tetracolpate pollen in *K. Lanceolata* and *B. Pinnatum* affirms that they are more advanced than *K. Crenata*. Number of colpi on pollen grains has been a useful tool in tracing evolutionary relationship among the species of a genus. The advanced dicotyledons have more colpi than the primitive ones with a colpus, monocolpate, or none at all acolpate [18,19].

Pollen shape has been observed as an important diagnostic character of taxonomic value [20]. Pollen with a circular shape was found in all of the species investigated, indicating that there were no major differences in pollen shapes among the species. Oval shaped pollen was however observed in *B. Pinnatum* and *K. Crenata*, while elliptic shaped pollens were observed in *K. Lanceolata*. Pollen shape has been variously employed in the separation of plant species [16-21].

Quantitative pollen characters have been used as important taxonomic characters, an example is [22] who used spine length as a diagnostic character to distinguish species in the family dipsacaceae. The pollen diameter and the distance between colpi of the three species of the genera *Bryophyllum* and *Kalanchoe* studied varied and thus can be used as a diagnostic character. From the cluster analysis, *K. Crenata* was clearly separated from the other two species that were clustered together at 98% similarity index [23].

The first two components of the Principal Components Analysis (PCA) accounted for 100% of the variation among the species studied. Based on component one, pollen diameter, depth of colpi, distance between colpi, pore diameter, number of pores are responsible for the variation observed among the species. Based on component two, only pollen wall thickness is responsible for the variation observed among the species.

## Conclusion

Unifying and diagnostic characters of taxonomic importance were revealed.

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