Scanning electron microscope of pollen grains of selected species of Iraqi Apiaceae

Microscopia electrónica de barrido de los granos de polen de especies seleccionadas de Apiacea Iraqui

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ABSTRACT

Introduction: The Apiacae is rich with species diversity in Iraq but the palynological contributions on this family are focused on some taxa on species level. Number of micromorphological characters were used in this study carried out by compound light microscope: shape of pollen grains, colpi length, exine thickness. **Material and methods:** micromorphological studies have been implemented using SEM for 12 species as three cleaning methods are done and examine the best one, on the other hand, measuring the polar axis (P), equatorial axis (E) and P/E ratio w(the ratio of the length of the polar axis to the equatorial). Moreover, ornamentation have been described also**. Results and discussion:** Ultra-sonication was found to be the best method of cleaning pollen grains. This method removed surface debris from the pollen grains but sometimes lead to fracturing of pollen grains. The pollen grains are bilateral symmetric and isopolar .The shape of pollen was both prolateor, perprolate. The ornamentation were ranged from striate-rigulose to striate in *Anisociadium* DC. Species but was striate on *Ergocarpon cryptanthum* (Rech.f.) C.C.Towns.while were striate-ruglose, striate-granulate and radiate-rugulose respectively, on the *Bunium* L species. The outline is oblong with round poles in the genera *Anisosciadium* and *Bunium*. The variation that observed in pollen shape may be caused by the different extraction methods that have been used and embedding media. Three types of apertures were observed, di-ectocolpate (*A .isosciadium Bornm.* and *A. orientale* DC., di-colporate (*A. isosciadium* and *E .cryptanthum*) and tricolporate in all species of *Bunium* . On the other hand , costae are always present , but they are clear in some pollen grains than in others . Costae are broad , band like . **Conclusion :** A scanning electron microscope study were of useful taxonomic attributes on the generic level specially, aperture type and number and sculpturing of exine.

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INTRODUCTION

Number of works on pollen of Apiaceae have included detailed description and remarked their potential value for generic level identification in this family. Erdtman [1] could construct a key to Apiaceae for France and North Africa based on light microscope. Later other author [2] made more comprehensive study on the pollen of Apiaceae , she could divide the family to five groups depending on these following characters: P/E ratio, variation of exine thickness at the poles and in the equatorial area, the length of ectocalpus, features of endoaperture, etc. Van Zeist *et al.* [3] descried nine types of pollen grains in Apiaceae from Iran. Number of micromorphological characters were used in his study carried out by compound light microscope: shape of pollen grains, colpi length, exine thickness, etc. Punt [4] divided the family Apiaceae into fifty pollen types .He constructed a key to the some species of big genera (*Eryngium* L., *Heracleum* L., etc.) based on the following characters of pollen: collumella length, P length, exine thickness, etc. On the other hand, Perveen and Qaisar [5] gave good accounts of pollen Apiaceae of Pakistan by utilizing Light and SEM microscopes. Little is known about the pollen grains of Echinophoreae, however, one paper was published by [6].He used a number of characters: P length, E length , P/E ratio, mesocolpium index , ornamentation of exine etc .His research work included one species of Echinophoreae *Echinophora spinosa* L. Since the introduction of SEM in the 1960s, it has proven to be an important scientific tool, being applied to many areas of biology [7]. The use of SEM for taxonomic studies has major advantages over the compound microscope as it gives a more accurate view of morphological surface features. It has been frequently used in higher plant taxonomic studies to examine pollen sculpturing [8]. Anisosciadium is a distinctive genus in the family is recognized by one to tri-pinnatesect leaves, its special fruit that are very unique for its species while Bunium is found widely in arid and sub-arid lands with 45 species throughout the world, however; *E. cryptanthum* is endemic species to Iraq with distinctive white large, broadly deltoid margined bracts [6, 7].

MATERIAL AND METHODS

Micromorphology study

The examined specimens

Pollen were taken from the anthers from dried herbarium sheets from the following taxa.as illustrated in table (1.1).This work has been done in the labs of Royal Botanic Gardens Edinburgh.

Table 1.1. Checklist of examined taxa

Taxa	Voucher Number	Herbaria
Anisosciadium lanatum Boiss.	7028	BUH (Baghdad University Herbarium)
Anisosciadium isosciadium Bornm.	0015409	BUH
Anisosciadium orientale DC.	0025583	BUH
Bunium brachyactis (Post) H.Wolff	1594	K (Royal Botanic Gardens Kew)
Bunium caroides (Boiss.) Haussk̃n. ex Bornm.	2084	K
*Bunium coringerum (*Boiss. & Hausskn.) Drude	0041007	BUH
Bunium cylindricum (Boiss. & Hohen.) Drude	2209	K
Bunium elegans (Fenzl) Freyn	0036957	BUH
Bunium paucifolium DC.	3159	E (Royal Botanic Gardens Edinburgh)
Bunium rectangulum Boiss. & Haussk̃n.	1355	K
Bunium verruloscum C.C.Towns.	4608	E
Ergocarpon cryptanthum (Rech.f.) C.C.Towns.	45253	K

Cleaning samples

Initial observations indicated large amounts of debry. The pollen grains needed to be cleaned before examination using the SEM. Two methods of cleaning pollen grains were tested

Method 1: Ultra-sonication

This method is modified from modified from Cao and Dong-Xu (9). Anthers from herbarium sheets were placed in a watch glass and pollen was dissected out with fine forceps and a needle under a dissecting microscope .Samples of pollen were put in 50%, ultra-sonicated for 15 minutes to separate the grains. Then pollens were dropped onto a coverslip attached to a stub using a capillary tube, and left to dry.

Method 2: Acetolysis

It is modified from Erdtman (10). Anthers from herbarium sheets were dissected out as described above. The pollen was transferred to labeled glass tubes, 1-2 ml acetolysis mixture (9:1 acetic anhydride, concentrated sulphuric acid) was added into each .These were then placed in a preheated block at 90 C. The tubes were heated for 8 minutes; a glass rod was used to macerate the material during heating. The pollen solution was centrifuged for five minutes at 3500 rpm. The acetolysis mixture was decanted, leaving the pollen pellet intact, then. 2 ml of distilled water was added to glass tubes .This was centrifuged for 5 minutes at 3500 rpm and the supernatant was decanted. This step was repeated. Each sample was sieved using an Endecott sieve in a funnel over a clean, labeled tube, the tube was rinsed. Centrifuging was repeated for 10 minutes at 3500 rpm and supernatant was decanted. The tube of water-washed grains was covered with a tissue labeled SEM and left overnight to dry.

Method 3: KOH clean-up

It is modified from Hanks and Fairbrothers (11). Anthers from herbarium sheets were dissected out as described above .The pollen grains were transferred to labeled slides, these were then boiled in 10% KOH for 1-2 minutes.

Specimens coating and scanning:

All pollen materials were attached to a sticky carbon disc mounted on a 0.5 aluminum SEM pin stub and sputter coated with platinum for a period one minute using EMITECH K575X, samples were observed using Leo SupraA55VP scanning electron microscope at a working distance of 6-7mm at and 5kv under varying magnification.

Characters measurement

The following characters that have been measured in µm polar axis (P) and equatorial axis (E). P/E ratio was calculated from these measurements (the ratio of the length of the polar axis to the equatorial. Ten pollen grains of each taxa were measured except when there are insuffient materials: *Anisosciadium isosciadium* .

RESULTS

Cleaning methods:

Ultra-sonication was found to be the best method of cleaning pollen grains. This method removed surface debris from the pollen grains but sometimes lead to fracturing of pollen grains. This seemed to be related to the age of the specimen, with older sample being more easily fractured. KOH cleanup methods were also attempted but were unsuccessful in removing debris.

Polarity, symmetry and shape:

The pollen grains are bilateral symmetric and isopolar .The shape of pollen was both prolateor, perprolate (table.2.2) and fig.1**.** The shape classification follows Erdtman (1952), with polar axes ranged from 23.20 to 32.2 micrometer and equatorial axes ranged from 10.02 to 15.61 micrometer (table 3.2). The outline is oblong with round poles in the genera *Anisosciadium and Bunium* .The variation that observed in pollen shape may be caused by the different extraction methods that have been used and embedding media. Three types of apertures were observed, di-ectocolpate (*A .isosciadium* and *A. orientale*), di-colporate (*A. isosciadium* and *E .cryptanthum*) and tricolporate in all species of *Bunium*. On the other hand, costae are always present, but they are clear in some pollen grains than in others. Costae are broad, band like.

Table: 2.2. Measurements of pollen grains for studied species

Speciess	P	E	P/E ratio	Ornamentation
Anisosciadium isosciadium	30.79±0.57	11.87±0.91	2.59 perprolate	**Striate - granulate**
Anisosciadium lanatum	29.68±2.43	13.45±0.94	2.92 perprolate	**Striate - rugulose**
Anisosciadium orientale	30.02±2.64	14.13±1.23	2.12 perprolate	**Striate - granulate**
Bunium brachyactis	23.20±1.62	14.08±1. 19	1.64 prolate	**Striate-rugulose with some pore at polar areas**
Bunium caroides	24.66±1.68	15.61±1.64	1.57 prolate	**Radiate-rugulose with prominent ridges**
Bunium cornigerum	25.28±1.90	10.20±0.33	2.47 perprolate	**Striate-rugulose with perforations between the**
Bunium rectangulum	23.51±1.70	13.60±0.40	1.73 prolate	**Radiate-rugulose with perforations at polar area**
Ergocarpon cryptanthum	**32.2±02.35**	**15.21±1.92**	**2.01 prolate**	**Striate**

Figure 1. Ornamentation of pollen grains.

DISCUSSION

Three methods have been done to process pollen grains, in spite of the Ultra-sonication was the superlative method of cleaning pollen grains but this method lead sometimes to fracture of pollen grains besides, removing surface debris from the pollen grains. This seemed to be related to the age of the specimen, with older sample being more easily fractured. While KOH cleanup methods were also ineffective in eliminating debris. In relation to polar axes of pollen grains there were wide range in measurements. This variation in pollen shape may be caused by the different extraction methods that have been used and embedding media. Three patterns of apertures have been observed in this research as supported by De Leonardis *et al* [6, 12] and Punt [4], who stated that ect-ocolpi are distinctive character of Umbelliferae and divided the family to fifty types. In the analysis of the mean P and E values, the largest P was found in the species *Anisosciadium isosciadium* and the smallest E was found in species *Ergocarpon cryptanthum*, the sizes of pollen grains can vary within one taxon, variation is caused by different treatment and also influenced by the position of the flowers and the level of umbel [4, 12]. The pollen microphology characters (shape of pollen grains and the ratio P/E) seem to have the potential for evaluation of generic relationships in Echinophoraea.

CONCLUSIONS

A scanning electron microscope study were of useful taxonomic attributes on the generic level specially, aperture type and number and sculpturing of exine for Echinophoraea as little studies are observed about this tribe.

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