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Micro Morphological Leaf Epidermis Characters and Palynological Studies in four Taxa of Convolvulaceae by Scanning Electron Microscope (SEM).

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# Micro Morphological Leaf Epidermis Characters and Palynological Studies in four Taxa of Convolvulaceae by Scanning Electron Microscope (SEM).

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# Abstract

Micromorphology of leaf epidermis, and pollen grain of *Convolvulus arvensis* L., *Convolvulus pilosellifolium* Desr., *Cressa cretica* L., and *Cuscuta* spp (Convolvulaceae) were investigated under scanning electron microscope(SEM), cuticle layer appeared as crossing or parallel filament zones, epidermis cells unequal in shape and size, mostly the cells of the adaxial are larger than the abaxial epidermis. Paracytic stomata occurring in both side except genus *Cressa cretica* no stomata in adaxial side, *C. pilosellifolium* has two types of trichomes appeared on abaxial side, unicellular and peltate glandular trichomes. pollen grains of all studied taxa were monad, radial symmetry and isopolar. The pollen grains generally were tricolporate, small to medium in size, The shape were prolate, micro reticulate sculpturing.

KEY WORDS: Convolvulaceae, SEM, Micromorphological leaf characters , pollen grain.

# دراسة الصفات المظهرية الدقيقة لبشرة الورقة وحبوب اللقاح لاربعة مراتب من العائلة العليقية بواسطة المجهر الالكتروني الماسح

أسيل كاظم الانباري قسم علوم الحياة - كلية التربية للعلوم الصرفة - جامعة ديالي

DITAL A DISIPERSIT COLLEGE OF STATE

Micro Morphological Leaf Epidermis Characters and Palynological Studies in four Taxa of Convolvulaceae by Scanning Electron Microscope (SEM).

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# الخلاصة

تم فحص الصفات المظهرية الدقيقة لبشرة الورقة وحبوب اللقاح في المراتب ,... Convolvulus arvensis L. و Convolvulaceae) و Cressa cretica L. و Convolvulus pilosellifolium Desr. تحت المجهر الالكتروني الماسح ، ظهرت طبقة الكيوتكل بشكل خيوط متقاطعة او متوازية في مناطق متعددة ، خلايا البشرة الاعتيادية غير متساوية بالشكل و الحجم و غالبا" ماكانت خلايا البشرة العليا اكبر من خلايا البشرة السفلى، الثغور من النوع المتوازي ، تواجدت الثغور في كلا السطحين السفلي و العلوي عدا الجنس Cressa cretica فقد افتقر ت البشرة العليا لكبر من خلايا البشرة السفلى، الثغور من النوع المتوازي ، تواجدت الثغور في كلا السطحين السفلي و العلوي عدا الجنس Cressa cretica فقد افتقر ت البشرة العليا للثغور ، الفهر الجنس الثغور في كلا السطحين السفلي و العلوي عدا الجنس معاوية بودر عية غدية) في البشرة السفلى. البغرة من الشعيرات ( احادية الخلية ودر عية غدية) في البشرة السفلى. تميزت حبوب اللقاح لجميع المراتب بكونها احادية الشكل ذات تناظر شعاعي ومتساوية الاقطاب ، ثلاثية الاخاديد تميزت حبوب اللقاح لجميع المراتب بكونها احادية الشكل ذات تناظر شعاعي ومتساوية الاقطاب ، ثلاثية الاخاديد تميزت حبوب اللقاح لجميع المراتب بكونها احادية الشكل خات تناظر شعاعي ومتساوية الاقطاب ، ثلاثية الاخاديد تميزت حبوب اللقاح لجميع المراتب بكونها احادية الشكل خات تناظر شعاعي ومتساوية الاقطاب ، ثلاثية الاخاديد تراوحت بين الحجم الصغير و المتوسط، بيضوية الشكل عموما" اما الزخر فة السطحية فكانت شبكية دقيقة . تراوحت النها العليقية، المجهر الالكثروني الماسح، الصفات الدقيقة المظهرية للورقة، حبوب اللقاح .

# Introduction

The micro deletes of morphology are important sources of taxonomical consideration. Epidermal characters are classical source of data to identification and classification of different taxa. With this view a detailed study of the epidermal characters were taken into account for synthetic approach of taxonomy.

During recent years, the characters of epidermis especially cuticular features, epidermal cell, shape, type of stomatal complex and its associated epidermal cells, stomatal frequency, type of epidermal trichomes and pattern of distribution were helpful in determining the phylogeny and systematic position of the taxa as suggested by Metcalfe and Chalk, (1950).

Using of modern scientific methods like scanning electron microscope(SEM) and transmission electron microscope (TEM) helped to know the smallest details about the epidermal layers and pollen grain wall, which has proved its importance.

These characters, may be used as a major criteria in the separation at generic and species levels. (Solereder, 1908; Inamdar, 1969).



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There were only a few reports on the epidermal studies in Convolvulaceae family, which is too confined to the development of stomata (Pant and Banerji, 1965; Shah, 1967; and Kant, 1983). Jamil et al (2014) also have done stem anatomical investigation of Cuscuta L. (Convolvulaceae) species in Khorassan provinces(Iran), But the leaf characters were most commonly used among all the non-reproductive organs in taxonomy it can making primary taxonomic division (Stace, 1984). There were a few attempts on this line covering various aspects of leaf epidermal characters and anatomy (Pant and Banerji 1965; Shah 1967; Inamdar and Patel 1971; Jain and Sharma 1974; Karatela and Gill 1985; Leela and Rao 1994; Tayade and Patil 2003; and Tayade and Patil, 2012).

Pollen morphology of Convolvulaceae genera have been some attempts to use pollen features in delineation as Hallier (1893) was the first to divide the family on the basis of pollen features into two groups, Echinoconiae and Psiloconiae. In the classification of Gamble (1923), the family was divided into two groups on the basis of echinate and non-echinate pollen grains with the genus Convolvulus included in the latter one. Erdtman (1952) separated the Convolvulaceae pollen grains into two groups, namely *Ipomoea* species and their species, another group which included the genus Convolvulus. The Ipomoea species pollen grains which were polyporate, with a thick nexine and echinate, whereas the Convolvulus type were distinctly perforate. O'Donell (1959) separated the genera Convolvulus and Calystegia R.Br. on the basis of Hallier's aperture descriptions, but he reported that in Convolvulus the pollen is 3-colporate. Lewis & Oliver (1965) stated that their findings agree those with Hallier, but O'Donell(1959) and Sengupta (1972) not carried out a comprehensive study in the family and divided the Convolvulaceae into four main pollen types based on the number and distribution of apertures. Cronk & Clarke (1981) not pursued previously systems, So, The present author extended epidermal leaf features observations, it was undertaken to highlight the detailed morphological characters with regard to the dermal appendages on four species of the family, there are being presented in this communication. On the other hand there are no information on pollen morphology of Convolvulaceae taxa studied under SEM in Iraq. Therefore the



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present study aimed to investigate morphological patterns of the pollen grains of some taxa by SEM.

# **Material and Methods**

Four taxa of Convolvulaceae family were collected from Dyiala province during different developing stages, collected during the Survies trips. The samples of *Cressa cretica* L. were dried. The following anatomical aspects were studied

## **A-Leaf surface**

Fresh leave, except genus *Cressa cretica* L. was dried, studies of epidermal characters such as type and number of stomata, number of epidermal cells, stomatal frequency, stomatal index, length and width of stomata in abaxial and adaxial side, nature, type, and trichome shape were observed (Table 1). The method adopted by Kant (1983), for stomata analysis was followed Ahmad (1964), epidermal peeling were taken mechanically from upper and lower epidermises using a razor blade. They were first washed in distilled water then suspended in 100% alcohol and put on stub coated with gold, the samples were investigated under x500 magnification power by LEO 1450VP Scanning Electron Microscope in Baghdad University, college of education for pure sciences Ibn-AL-Hatiam (central lab).

For estimating the size of stomata, ocular micrometer was used. Throughout the study, objective lens of x40 have been used. Type, frequency and index of stomata according formulas, the frequency of stomata=  $S/E \times 100$ , Index of stomata =  $S/E+S \times 100$  (where S= Stomata, E= epidermal cells in the same unit area) for the abaxial leaf epidermises were calculated using laboratory microscope having 10x magnifications. Photomicrographs were taken for all the observations by scanning electron microscope was used for micro photographing. All measurement was taken from an average of twenty readings.

#### **B-** Pollen grains

The pollen material was obtained from fresh specimens (Table 2). were collected from flowering buds just before anthesis then stored in the ethanol 70%. Placed in a watch glass and squashed with the addition of a few drops of distil water, Then the floral fragments were



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drawn to the side of the watch glass with fine forceps and a mounted needle under a dissecting microscope, leaving just the pollen grains to dry. Pollen samples were acetolysed according to Erdtman (1960). For preparing light microscope slides, mounted the remaining pollen grains in the watch glasses were transferred onto the slides on a small block of in silicone oil and sealed with paraffin. For the SEM study, acetolysed pollen grains were suspended in 100% alcohol and put on stubs that were coated with gold for 5-6 minutes. The measurements were carried out using light microscopy and based on 20 readings for each specimen. Then were observed and photographed with an LEO 1450VP SEM and using Olympus BH<sub>2</sub> LM. The terminology used in the present study is according to Walker and Doyle (1975).

**Result and Discussion** 

#### **A-Leaf surface**

#### 1- Convolvulus arvensis

**Abaxial:** cuticle layer almost interlocked fibers, zigzag walls of normal epidermal cells attached with stomata, the edegs epidermal cells is not clear, crossing as intertwined fibers of cuticle, the epidermal cells have been multiple shape often triangular, with the not sharp ends, or variable shape, Stomata were paracytic, Numbers of stomata in the abaxial side were 18 - 22 respectively. Length and breadth of the stomata were 29- 40, 20- 24  $\mu$ m. Stomatal frequencies had an average of 41.66 %, Stomatal index were 29.41%. Number of epidermal cells in the abaxial surface were 45- 52, as shown in the table 1,and figure 1

Adaxial: the adaxial surface cuticle has tiny strands intertwined and additions was not equal either ends of the cells or walls cells appear in a clear and bold cuticle additions compared to the cells, the cells forms may be rectangular, pentagonal, hexagonal ,or irregular shape in dimensions, stomata in this surface were paracytic, Numbers of stomata were 1-4. Length and width stomata were 20- 27, 10- 15  $\mu$ m. frequencies Stomata had an average 4.34%, Stomatal index were 4.16%. Number of epidermal cells were 42-50. No unicellular trichomes. Table 2,



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and figure 2, While peltate glandular trichomes have been observed on this genus with diameter was arranged between  $20-35\mu m$ . Figure 1.

#### 2- C. pilosellifolium Desr.

**Abaxial:** cuticle layer appeared of high-filament crossing in some zones or parallel in other area, but the ends of the cells or cell walls look grooves, changing epidermal cells including peltate glandular trichomes and unicellular trichomes, Stomata were paracytic, Numbers of stomata in the abaxial surface were 67- 75 succession. Length and width stomata were 19 - 26, 20 - 27  $\mu$ m. frequencies Stomata had rating 49.30%, Stomatal index were 33.02%. Number of epidermal cells were 137- 150, trichomes appear to be a straight line and that epidermis cells including a rectangular shape, peltate glandular trichomes diameter between 20-35  $\mu$ m, unicellular non-glandular trichomes( figure 3), length have been an average between75- 225 $\mu$ m, width 3-10 $\mu$ m. Data shown in the table 1, and figure 1

**Adaxial:** cuticle layer showed tiny filaments intertwined either ends of the cells or walls cells clear appearing, rectangular, pentagonal, or irregular epidermal cells shapes, stomata does shown in adaxial surface as paracytic type, have been numbers were (1-3) as maximum. Length and width stomata were (19 - 26, 10 - 17) µm. Stomata frequency had rating (3.63%), Stomatal index were (3.5%). Number of epidermal cells were (50-67), while trichomes peltate glandular diameters has an average between (23-32) µm stabled among epidermal cells, No trichomes have been exist on this surface. Figure 2, and table 2

#### 3- Cressa cretica L.

**Abaxial:** Meandrous lines showed up the cuticle filaments crossing or parallel in different regions, but the edegs of the cells or cell walls were not distinguished, diffent epidermal cells, stomata complex were so clear, Stomata were paracytic, Numbers of stomata in this surface were (8- 13) maximum rate. Length and width stomata were (20- 26, 17- 22)  $\mu$ m. frequencies Stomata had average (50), Stomatal index were (33.33). Number of epidermal



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cells were (18- 22), No peltate glandular trichomes or unicellular trichomes. Table 1, and figure 1

Adaxial: Winding filaments cuticle linked with ends of walls cells clear appearing, dimensions of epidermal cells look rectangular or pentagonal shapes, stomata does not exist in adaxial surface, just peltate glandular trichomes rating between (20-37)  $\mu$ m diameter, No stomata, and no unicellular trichomes have been observed on this surface. Table 2, and figure 2,3

#### 4- Cuscuta sp.

**Abaxial:** cuticle layer appeared winding filaments form crossing in some area or parallel in other zones, but the ends of the cells or cell walls look sink down, epidermal cells changing among rectangular, pentagonal or hexagonal shape, no peltate glandular trichomes, or unicellular trichomes containing in this side, Stomata were paracytic, Numbers of stomata in the abaxial surface were (12-16) at maximum. length and width stomata were (18 - 22, 16 – 18)  $\mu$ m. frequencies Stomata have been rating (34.14), Stomatal index were (25.45). Number of epidermal cells were (38-43), Data shown in the table 1, and figure 1

Adaxial: cuticle layer rised as tiny meandrous filaments crossing with ends of cells, walls cells looks holes, rectangular or pentagonal dimensions epidermal cells forms, stomata that shown in adaxial surface were paracytic, have been numbers (1- 2) at maximum. Length and width stomata were (19 - 25, 10 – 19)  $\mu$ m. Stomata frequency had rating (1.72%), Stomatal index were (1.69%). Number of epidermal cells were (55- 62), while no peltate glandular trichomes or unicellular trichomes have been existed on this surface as shown in table 2, and figure 2.

#### **B-** Pollen grain

The pollen grains of all studied taxa were monad, radial symmetry and isopolar. The pollen grains generally were tricolporate small to medium in size, The shape were prolate, micro



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reticulate sculpturing, polar axis rating between (22.3- 25. 4)  $\mu$ m in*C. pilosellifolium*, and *C. arvensis*, while the equatorial axis in *Cressa cretica* has been maximum rate was (16.6)  $\mu$ m, but in *Cuscuta* spp\_the lower rate was (14.2)  $\mu$ m, P/E have been arranged between (1.43- 1.45, 1.82- 187)  $\mu$ m in\_*Cressa cretica* and *Cuscuta* sp., nexine estimated results observed the same rates in *C. arvensis*, and *Cuscuta* sp. was (1.0)  $\mu$ m, while in *C. pilosellifolium*, and *Cressa cretica* was (1.1)  $\mu$ m, according to sexine layer measured the genus *Cressa cretica* have a lower rate was (1.9)  $\mu$ m, but genus *Cuscuta* sp. have been a maximum rate was (2.3)  $\mu$ m. table 3, and figure 4.

		No. of	No. of epiderma l cells	S. F	S.I	Size of sto	mata µm	trichomes		
ха		stomata				length	Width	Uni cellular		Glandular (peltate)
Таха			DI	VΔT	ΔΤ	NUM	FRST	length	width	diameter
Convolvulu	s arvensis L	18- 22 (20)*	45- 52 (48)	41.66 <u>+</u> 1	29.41	29- 40 (37)	20- 24 (22)	CE	0 <u>-</u>	-
c.	pilosellifoli um Desr.	67-75(71)	137-150 (144)	<u>49.30+</u> 2	33.02	19-26 (21)	20- 27 (24)	75-225 (190)		20-35 (30)
Cressa	cretica L.	8-13(10)	18-22 (20)	50 <u>+</u> 1	33.33	20-26 (23)	17-22 (19)			
Cuscuta sp.		12-16 (14)	38-43 (41)	34.14 <u>+</u> 2	25.45	18-22 (20)	16-18 (17)			

Table 1. The abaxial characters in genera under study

\* Rate of 20 replicates



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Table 2. The adaxial characters in	genera under study
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Таха	No. of stomata	No. of epiderma	S. F %	S.I %	Size of stomata µm		Glandular trichomes	
		l cells			length	Width	Diameter µm	
Convolvulus arvensis L.	1-4(2)	42-50(46)	4.34 <u>+</u> 1	4.16	20-27(23)	10-15(13)	20-35(30)	
C. pilosellifolium Desr.	1-3(2)	50-67(55)	3.63 <u>+</u> 1	3.50	19-26(25)	10-17(16)	23-32(27)	
Cressa cretica L.	-07	-	-	-	- 0	-	20-37(34)	
Cuscuta sp.	1-2(1)	55-62(58)	1.72 <u>+</u> 1	1.69	19-25(23)	10-19(16)	-	

Table 3. SEM micrographs of the pollen grains in genera under study  $(\mu m)$ 

Taxa	Polar axis (P)	Equatorial axis (E)	P/E A UN	Shape	Nexine (N)	Sexine (S)	N/S
<b>C.arvensis</b>	18.3-29.2 (25.4)	13.7- 17.2 (15.2)	1.60-1.64	prolate	0.9- 1.1 (1.0)	1.8- 2.3 (2.1)	0.50-0.48
C. ilosellifolium	20.7- 26.9 (22.3)	12.4- 16.0 (15.1)	1.47-1.54	prolate	0.9- 1.2 (1.1)	1.8- 2.1 (2.0)	0.50-0.57
Cressa cretica	17.7- 29.1 (23.8)	12.7-20.8 (16.6)	1.43-1.45	prolate	0.9- 1.3 (1.1)	1.7- 2.1 (1.9)	0.53-0.62



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	20.3- 29.2	12.3-17.3 (14.2)	1.82-1.87	prolate	0.9-1.1 (1.0)	2.0- 2.5 (2.3)	0.44-0.45
dds	(25.7)						
cuta							
Cusc							
0							

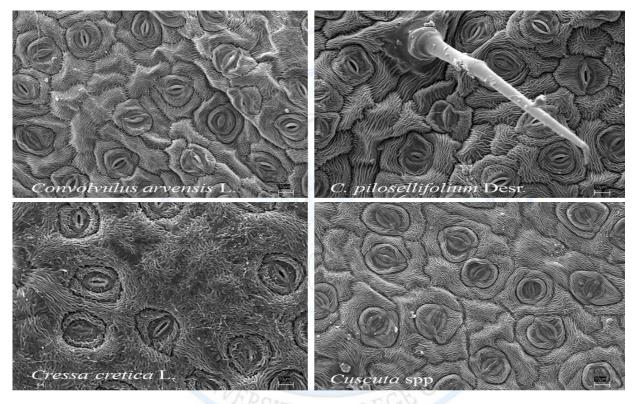


Figure 1. SEM micrographs of the abaxial surface in genera Scale bar= 10 µm



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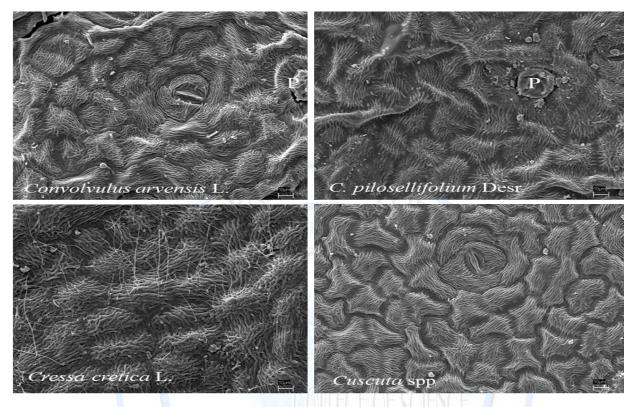


Figure 2. SEM micrographs of the adaxial surface in genera. Scale bar= 10 µm



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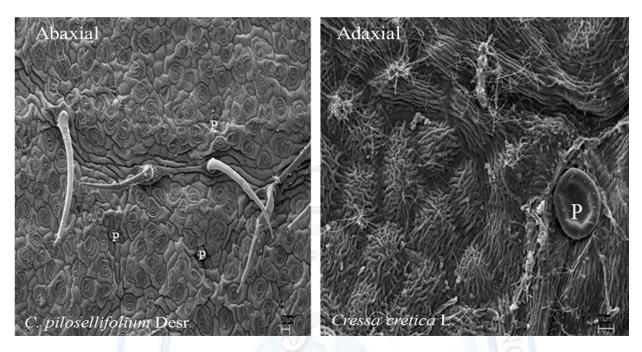


Figure 3. SEM micrographs of trichomes in genera. Abaxial scale bar= 20 µm, adaxial scale bar= 10 µm, P= peltate glandular trichomes

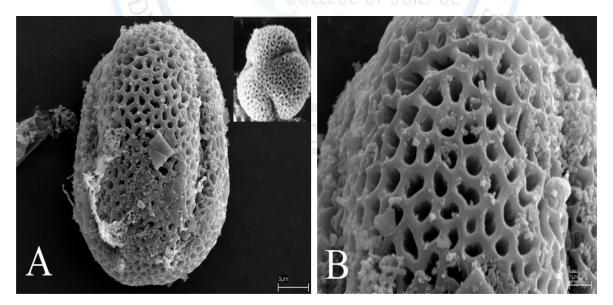


Figure 4. SEM micrographs of pollen grain in genera. A= polar axis, Equatorial axis, B= micro reticulate sculpturing

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## **Discussion**

#### **A-Leaf surface**

Due the not equal supplement of cuticle layer appearing as filaments, the epidermis in all studies genera were usually irregular in shape and unequal in size, mostly the cells of the adaxial were larger than the abaxial epidermis but mostly in midrib region were regularly arranged and equal in size compared with other epidermis cells, Stomata parcytic in all genera, this results agree with Ugborogho(1992),but not agree with Essiett and Okono (2014) who reported that convolvulace have been many types of stomata complexes, the results confirmed stomata occur on abaxial and adaxial sides, except genus *Cressa cretica* didn't have stomata on the adxial surface, results agree with Purushoth et al (2014) when he was studied *Merremia emarginata* reached to Stomata occur on both the adaxial and abaxial sides.

Numbers of stomata were in *C. pilosellifolium* Desr. (71) as a maximum but in *Cressa cretica* L. were (10), but they have almost the same stomata index (33.02, 33.33), distinguished genus *C. pilosellifolium* has two types of trichomes appeared on abaxial side of leaf surface, but on adaxial has peltate type of glandular trichomes, this agree with Werker (2000) results that he reported the leaves were of many species of Convolvulaceae densely covered with glandular and non-glandular trichomes, which rised from epidermal cells when he worked on *Ipomoea* taxa, this may be refer to there was genetics relationship between *C. pilosellifolium* and *Ipomoea*, but Short stalked, peltate type of glandular trichomes have been occasionally seen on the abaxial *Convolvulus arvensis* L., *C. pilosellifolium* Desr., *Cressa cretica* L. this result confirmed with the result obtained by Purushoth *et al* (2014) of *Cuscuta* sp. has stomata on the both surfaces but no trichomes on both surfaces.

#### **B-** Pollen grain

The pollen grains of Convolvulus genus has been studied by Lewis & Oliver (1965). They described the Convolvulus pollen grains as 3- or rarely 4-zonocolpate and prolate to subspheroidal, but El Ghazali (1993) confirmed that *C. arvensis* 

pollen grains were tricolpate with a perforate tectum, The present study on four taxa of Convolvulaceae agree with Lewis & Oliver (1965) and El Ghazali (1993) that pollen grain





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have tricolpate, but have microreticulate sculpturing not perforate, and does not agree with O`Donell (1955), who referred to that the Convolvulus pollen grains were tricolporate. The pollen grains of the genus Convolvulus from Morocco showed variation between the species that sometimes corresponds with the morphologic features.

Therefore, One of the major peculiarity found in the pollen grain of all taxa have been the same shape, so, the pollen characteristics cannot be used to distinguish the taxa under study.

## Conclusion

The SEM micro morphological and palynological features can be considered as reliable and magnify characters for botanical diagnosis, moreover can also may be use the pollen characteristics effectively in palynological study.

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The author thank Mrs. Nisreen Sabar Hashim Ms. Sci (plant taxonomy) in Department of Biology/ College of education of pure sciences/ Dyiala university to provided *C*. *pilosellifolium*, and *Cressa cretica*, plants, and her help to identify genera under study.

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