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Stomata and Pollen Grains Studies of Cotton (*Gossypium hirsutum L.)* in Iraq

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Abstract

The *Gossypium hirsutum* genotypes were have been different types of stomata as anisocytic, paracytic, hemi-paracytic and tetracytic with presence in both surfaces, and found conjugated stomata. The stomata generally composed of two guard cells with ordinary epidermal cells, while some genotypes composed of one guard cell. The pollen grains were colporated, prolate, sub-prolate, and prolate-spheroidal in shaped with echinus orientation sculpture.

1.Introduction

The Malvaceae family consists of a diverse group of plants, with over 2000 species belonging to 110 genera. These plants can be found in many forms such as saplings, grasses, and trees. They are distributed across temperate and tropical regions, with some genera being more prevalent in specific areas. Multiple studies have been conducted on various species of the Malvaceae family, including *Gossypium, Hibiscus*, *Abelmoschus*, and *Malva*, due to their significant economic value. The concept of family and the categorizing of Malvaceae, especially at the tribe and generic level, are subjects of intense debate (Naggar and Sawady, 2008, Bahjat, 2019, Hasson, 2011).

Given the importance of studying the outer layers of leaves in understanding the evolutionary relationships and classification of plants, plant taxonomists have focused their emphasis on investigating the morphology of leaf epidermis to address taxonomic issues (Hameed and Hussain, 2011). The cotton leaves play a crucial role in carrying out essential physiological processes such as transpiration and photosynthesis (Bondada and Oosterhuis, 2000).

The size of pollen grain is helpful as a taxonomic characteristic, especially in the tribal level. The pollen and sculpture ornamentation is echinate. therefore, they are vary greatly and their merits able to utilize within the family to the classification and phylogeny (Naggar and Sawady, 2008).

Xiaoyan et al. (2023) analyzed the cotton samples exhibited common pollen morphological features, including spherical shapes, radial symmetry, echination, panporation, and operculation, while the pollen size, spine shape, spine density and length showed distinctive features. Pollen size varied significantly among species .The current study aims are disclose the types of stomata and morphological characters of pollen grains of *Gossypium hirsutum* genotypes.

2.Materials and methods

-Plant collections

Various *Gossypium hirsutum* genotypes (including Coker 310, Lachata (Iraqi genotypes), Cafko, Dunn 1047, Montana, Stone Ville (USA genotypes), Bakhtegon, Khdorda, Vanamin (Iranian genotypes)) were collected from Qwshtapa district, Grdmala village, located 30 km away from the center of Erbil city. The collection also included plant materials from the agriculture college fields in Bardarash, Erbil. The collected plant materials were then fixed in a solution known as FAA (formalin acetic acid alcohol).

-Pollen grains

The collected pollen grains from the flowers were prepared for observation under a light microscope (LM) according to Erdtman's method (1952).

-Stomata preparation

The leaf specimens were prepared by removing the outer layer of the leaf. The samples were examined using a light microscope equipped with a camera and analyzed using Image Analysis Software (Najmaddin and Mahmood, 2016).

3.Results and discussion

This investigation revealed that the anticlinanal walls of epidermis is straight to slightly undulate, with presence the stomata in both surface (adaxial and abaxial); they are tetracytic, hemiparacytic, paracytic and anisocytic, while in *Gossypium hirsutum* genotype Lachata consists of conjugated guard cells. In the genotypes as Montana the adaxial surface, but in Vanamin the adaxial and adaxial surfaces consists of stoma composed of 1 guard cell (usually the stomata composed of 2 guard cells), Fig. 1,2,3 and 4. The foliar of cotton are bract and capsule wall epidermis cells were differed in shape, number and configuration, but were closely sheaf; forming a consolidated layer; the periclinal outer walls of the adaxial and abaxial surface were raised and convex. The leaf and bract are amphistomatous, adaxial and abaxial stomata assorted in size; with found the stomata was rubiaceous, while the stomata on the capsule wall was ranunculaceous (Bondada and Oosterhuis, 2000). The anticlinal epidermal cell wall was slightly sinuous in *Theobroma grandiflorum* and *Theobroma speciosum*, while in *Theobroma subincanum* was straight (Garcia et al., 2014). Metcalfe and Chalk (1950) mentioned that in Malvaceae the epidermal anticlinal cell wall was straight or undulate with presence anomocytic guard cells on both surfaces.

This work shows the pollen grains of *Gossypium hirsutum* genotypes are colporated, circular or spheroidal in polar view; while in equatorial view are oval, elongated, semi-oblong or semi-spheroidal. *Gossypium hirsutum* genotype Lachata has big size, while *Gossypium hirsutum* genotype Stone ville has small size, the sculpture orientation is echinus (table 1), (Fig. 5,6,7). Malvaceae pollen grains are radially symmetrical, apolar, colporate, and pantoporate and zo on the species. although the Malvaceae being a stenoplynous family; they are high variations in pollen morphological characters, which is could used in the taxonomy of Malvaceae (Hesse et al., 2009, Naggar, 2004, Bibi et al., 2010, Tonkov, 2003).

The pollen grains of *Gossypium* are spheroidal in shape, with a corporate structure. They have a varying number of apertures, often ranging from 22 to 45, and are commonly classified as having a specific spiral type. The exine is characterized by its substantial thickness, with the sexine being comparable in thickness to the nexine or slightly surpassing it. The tectum has a surface that is characterized by a combination of small wrinkles and a pattern of tiny dots. The spines had elongated, pointy structures at their tips, with a rounded, bulbous shape at the lower section. They were either found on basal cushions or sparsely distributed (Naggar, 2004, Christensen, 1986).

Abutilon, *Hibiscus* and *Waltheria* Malvaceae genera, the pollen grains of *Abutilon* was isopolar, 3zonoporate, and sub-oblate to spheroidal in shape, *Hibiscus* was apolar, pantoporate, globose to spheroidal, and isopolar, however in *Waltheria communis* are apolar, *Waltheria rotundifolia*, and *Waltheria vernonioides* sub-oblate, oblate spheroidal, and spheroidal, circular and polygonal, 5–8 zonocolporate or less often 10–14 pantobrevicolporate in *Waltheria rotundifolia* and 10–16 pantopororate in *Waltheria communis* and *Waltheria vernonioides* (Shaheen et al., 2009, Júnior et al., 2017).

Tablel: The morphological diversity of pollen in the genotypes of *Gossypium hirsutum*

genotypes / traits	Polar view	Equatorial view	Polar measurement (µm)	Equatorial measurement (µm)	P/E	shape	Sculpture ornamentation
Coker310	circular	oval	133.522±2	174.422±1.5	1.31	sub-prolate	echinus
Lachata	spheroidal	elongate	74.673±2	107.198±1.5	1.44	prolate	echinus
Cafko	circular	elongate	139.807±2	199.327±1.5	1.43	prolate	echinus
Dunn1047	circular	semi-spheroidal	145.052±2	164.818±1.5	1.14	prolate-spheroidal	echinus
Montana	circular	semi-spheroidal	117.184±2	142.059±1.5	1.21	sub-prolate	echinus
Stone ville	circular	oval	179.393±2	168.031±1.5	1.07	prolate-spheroidal	echinus
Bakhtegon	circular	Semi-oblong	138.911±2	159.297±1.5	1.15	sub-prolate	echinus
Khdorda	spheroidal	semi-spheroidal	151.699±2	140.532±1.5	1.08	prolate-spheroidal	echinus
Vanamin	spheroidal	Semi-oblong	135.871±2	174.811±1.5	1.29	sub-prolate	echinus

*P=Polar view

*E=Equatorial view

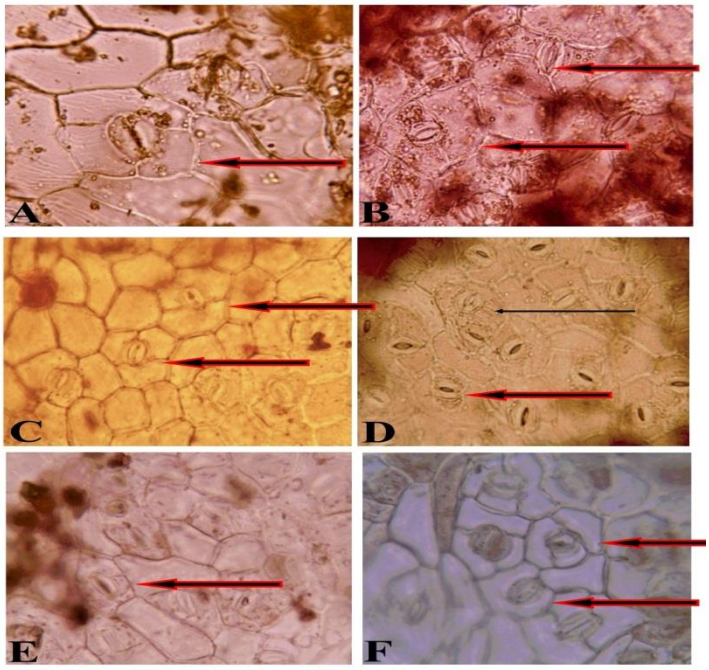

Figure 1: Epidemis of *Gossypium hirsutum* genotypes: A. adaxial of Coker310, B. abaxial of Coker310, C. adaxial of Lachata, D. abaxial of Lachata, E. adaxial of Cafko, F. abaxial of Cafko. Stomata (large black arrow), conjugated stomata (small black arrow). A, B, C, D, E, F=40X.

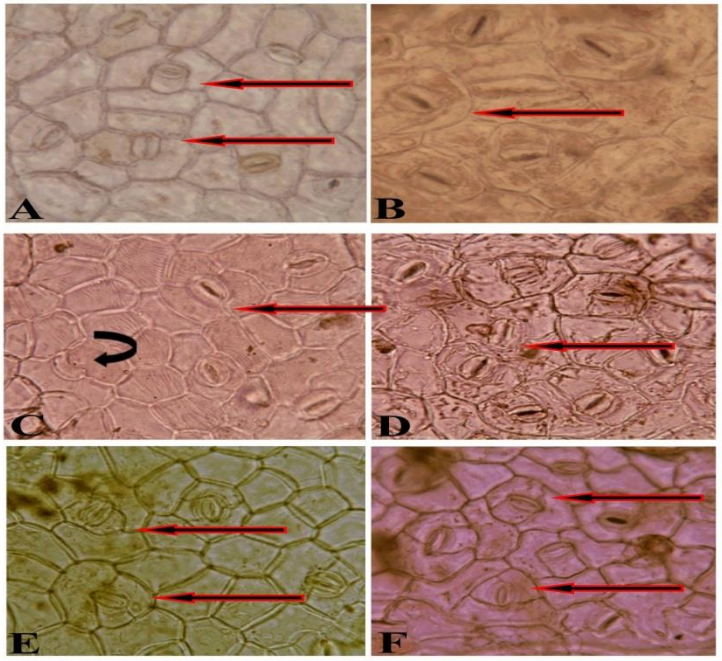

Figure 2: Epidemis of *Gossypium hirsutum* genotypes: A. adaxial of Dunn1047, B. abaxial of Dunn1047, C. adaxial of Montana, D. abaxial of Montana, E. adaxial of Stone ville, F. abaxial of Stone ville. Stomata (large black arrow), single guard cell (curved black arrow). A, B, C, D, E, F=40X.

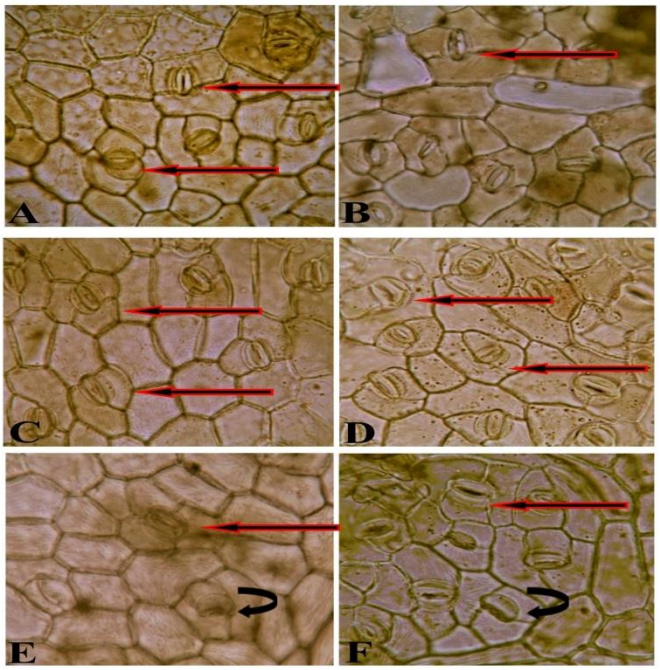

Figure 3: Epidemis of *Gossypium hirsutum* genotypes: A. adaxial of Bakhtegon, B. abaxial Bakhtegon, C. adaxial of Khdorda, D. abaxial of Khdorda, E. adaxial of Vanamin, F. abaxial of Vanamin. Stomata (large black arrow), single guard cell (curved black arrow). A, B, C, D, E, F=40X.

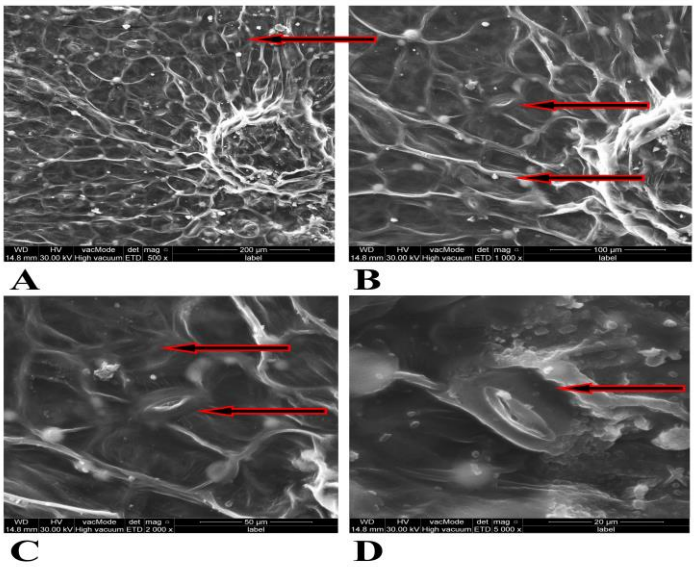

Figure 4: SEM of epidermis of *Gossypium hirsutum* genotypes

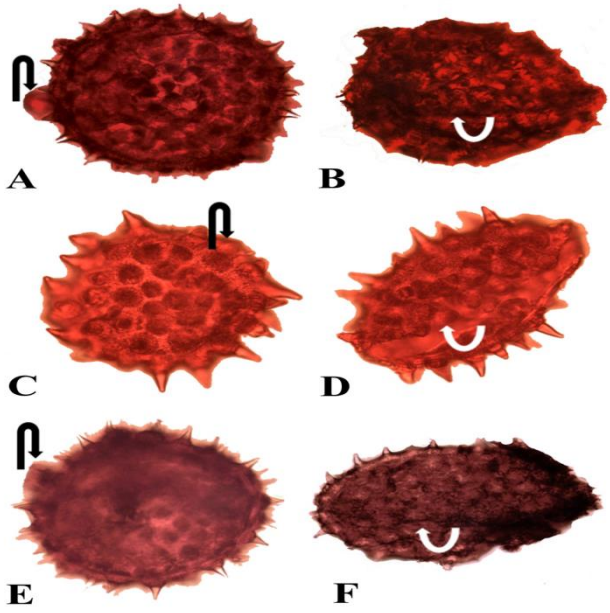

Figure 5: *Gossypium hirsutum* genotypes pollen grains: A. polar view of Coker310, B. equatorial view of Coker310, C. polar view of Lachata, D. equatorial view of Lachata, E. polar view of Cafko, F. equatorial view of Cafko. Pore: (circular white arrow), colpus (U-turn arrow). A, B, C, D, E, F= 40X.

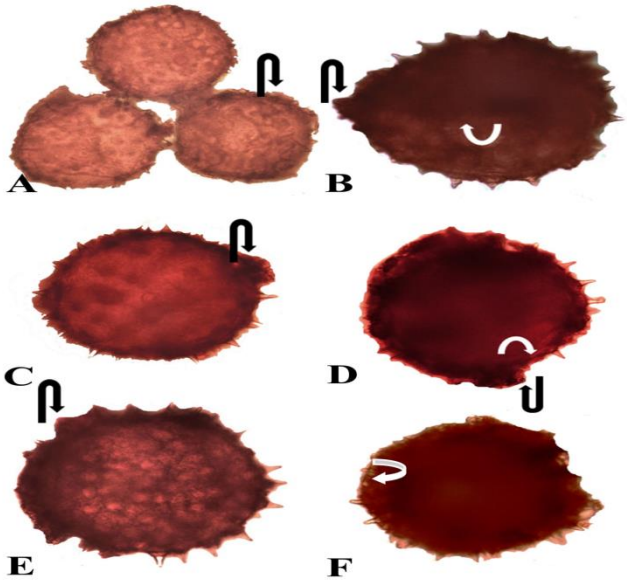

Figure 6: *Gossypium hirsutum* genotypes pollen grains: A. polar view of Dunn1047, B. equatorial view of Dunn1047, C. polar view of Montana, D. equatorial view of Montana, E. polar view of Stone ville, F. equatorial view of Stone ville. Pore: (circular white arrow), colpus (U-turn arrow). A,B,C,D,E,F= 40X.

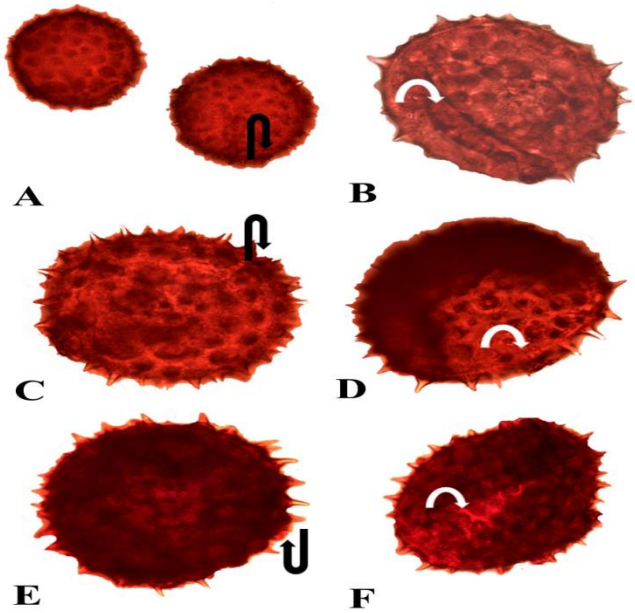

Figure 7: *Gossypium hirsutum* genotypes pollen grains: A. polar view of Bakhtegon, B. equatorial view of Bakhtegon, C. polar view of Khdorda, D. equatorial view of Khdorda, E. polar view of Vanamin, F. equatorial view of Vanamin. Pore: (circular white arrow), colpus (U-turn arrow). A, B, C, D, E, F= 40X.

4.Conclusion

Although pollen characteristics alone are not enough to resolve taxonomic and systematic relationships within the genotypes of *Gossypium*, our results add to knowledge on palynomorphology (The pollen grains were colporated, prolate, sub-prolate or prolate-spheroidal shaped and echinate sculpture; and contribute to stomata information on these genotypes the *Gossypium hirsutum* genotypes that the possess.

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