JJBS

Volume 13, Number 4, December 2020
ISSN 1995-6673
Pages 499 - 508

Jordan Journal of Biological Sciences

Pollen Morphological Variations among some Cultivated *Citrus* species and its Related Genera in Egypt

Wafaa K. Taia[1], Manaser M. Ibrahim[1,*] and Mahmoud Abdel-Sattar[2,3]

[1]Botany and Microbiology Department, Faculty of Science, [2]Pomology Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt; [3]Department of plant production, College of Food Science and Agriculture, King Saud University, P. O. 2640, Riyadh, 11451, Saudi Arabia.

Received: November 8, 2019; Revised: December 16, 2019; Accepted: January 3, 2020

Abstract

The present investigation aims to study the pollen morphology and ultra-structure of pollen grain characteristics for nine *Citrus* species and three related genera cultivated in Egypt. The pollen grains were photographed by using both light (LM) and scanning electron microscopy (SEM). Twelve qualitative and quantitative pollen morphological characters were used to differentiate among the studied taxa. Statistical analysis of palynological data indicated that the pollen size, shape, colpi length, apertures number and type, ora size, amb shape, mesocolpium diameter and exine ornamentation were the most distinguished characters in the circumscription of the studied taxa and were of taxonomic value. But the characters of P/E, ora shape and exine thickness were of less taxonomic value among the closely related taxa of *Citrus*, *Fortunella margarita*, *X Citrofortunella floridana* and *Poncirus trifoliata*.

Keywords: *Citrus, Fortunella margarita, X Citrofortunella floridana*, pollen morphology, *Poncirus trifoliata*, Rutaceae

1. Introduction

According to Engler, (1931), Rutaceae is divided into seven subfamilies, primarily by gynoecium characters especially the fruit type. *Citrus* species and its related genera (i.e. *Fortunella, Poncirus, Eremocitrus, Microcitrus* and *Clymenia*) all belong to subtribe Citrinae, tribe Citreae, of the orange subfamily Aurantioideae. *Citrus* (Rutaceae), characterized by having different life forms as trees and shrubs. It contains aromatic compounds with pellucid glands on the stems, leaves and fruits. The leaves are usually opposed, compound and without stipules, sometimes with thorns (Sharma, 1993). The *Citrus* fruit is berry or hesperidium with a leathery rind or hard shell and often with pulp formed by juicy or sappy emergencies that arise on the carpellary walls. Species within the genus *Citrus* are highly economic and medicinal plants distributed all over the world (Swingle and Reece, 1967). Several taxonomists have classified various kinds of *Citrus* species into groups and given them valid names (Roxburgh, 1832; Brandis, 1874; Marcovitch, 1926; Swingle, 1943; Swingle and Reece, 1967; Hodgson, 1965 and Tanaka, 1936 and 1977). Swingle's system appears to be the most useable all over the world (Nicolosi, 2007).

Distinguishing of *Citrus* species and related genera according to morphological and geographical distribution are very difficult because *Citrus* contains an enormous degree of genetic variation, with abundant natural hybridization (Moore, 2001). The classifications of the genus *Citrus* are complex and the precise number of natural species is unclear, as many of the named species are hybrids clonally propagated through seeds (by apomixes) and there is genetic evidence that even some wild, true-breeding species are of hybrid origin (Swingle and Reece, 1967 and Chase *et al.*, 1999). Mandarins, Pomelo, Citrons, Kumquats, Papedas, Australian and New Guinean species are considered as the ancestral or original *Citrus* species and all the rest are hybrids of them (Barett and Rodes, 1976; Scora, 1975 and Nicolosi, 2007).

In Egypt, there are no wild *Citrus* species (Täckholm 1974 and Boulos, 1999). All the present species (about 7 including 9 varieties) are introduced and cultivated in a cultivation area representing about 29% of the total fruit area in Egypt (Hamza and Tate, 2017 and Abobatta, 2019).

The use of pollen morphological characters are important in plant taxonomy. As Davis and Heywood (1973) pointed out, these characters can be highly significant at the species and generic levels of taxa or among higher levels. The use of pollen morphology in solving taxonomic problems has been used since a long time ago where Erdtman, (1952) studied the pollen characters of different Angiosperm and Gymnosperm families, while both Saad and Taia, (1988) and Taia and Sheha, (2001) used pollen characters in the differentiation among *Astragalus* and *Atriplex* species, respectively. Moreover, Taia, (2004) revealed the differences among the genera of tribe Trifolieae (Leguminosae) using pollen characters. Besides, Avci *et al.*, (2013) and Inyama *et al.*, 2015 were able to differentiate among the members of *Onobrychis* (Fabaceae), and *Citrus* (Rutaceae) species, respectively, using palynological characters. Recently in (2018), Mary and Gopal studied the pollen morphological characters of the two genera (*Ehretia pubescens* and

* Corresponding author e-mail: manaser99@yahoo.com.

Cormona retusa) Ehretiaceae, and proved that it is an important tool in the identification between them. This work is considered as a step in finding the way in differentiating among nine *Citrus* species and their related genera.

Therefore, this study aims to investigate and assess the relationships among nine *Citrus* species as well as three related genera cultivated in Egypt using pollen morphological characters.

2. Materials and Methods

The present investigation was carried out on mature trees of nine *Citrus* species and three related genera; *Citrus aurantifolia* (Christm.) Swing. [Mexican lime], *Citrus aurantium* L. [Sour orange], *Citrus grandis* (L.) Osbeck. [Pummelo], *Citrus latifolia* Tanaka [Persian "Tahiti" lime], *Citrus limetta* Risso. [Sweet lime], *Citrus paradisi* Macf. [Marsh grapefruit], *Citrus reshni* Hort. ex Tanaka [Cleopatra mandarin], *Citrus reticulate*-Blanco [Clementine tangerine], *Citrus sinensis* (L.) Osbeck. [Succari orange], *Fortunella margarita* (Lour.) Swing. [Oval Kumquat], and *X Citrofortunella floridana* J. W. Ingram & H. E. Moore, which is a hybrid between *Citrus aurantifolia* (Christm.) Swing. [Mexican lime], and *Fortunella japonica* (Thunb.) Swing. [Round Kumquat], and *Poncirus trifoliata* (L.) Raf. [Trifoliate orange] grown in a private orchard. This orchard is located 120 Km away from Alexandria on Alexandria-Cairo desert road (**GPS** co-ordinates: 30°44'47.8"**N**, 30°09'15.2"**E**). These species were collected by Dr. Mahmoud Abdel-Sattar in the year 2017 and identified at Pomology Department, Faculty of Agriculture, Alexandria University, and vouchers of the studied taxa were allocated there.

Four uniform trees were selected from each *Citrus* species and related genera, from which mature anthers were taken from the upper most flowers of the branches to obtain the mature pollen grains used in this investigation.

Pollen grain samples of all studied taxa were acetolyzed according to Erdtman's technique (Erdtman, 1952). The acetolyzed samples were used for both light and scanning electron microscopy. Slides were prepared from acetolyzed portion of pollen grains for light microscope examination by mounting in glycerin jelly, examined and measured using Zeiss light microscope with a pre-calibrated eye-piece micrometer. Measurements given are the means of 40 acetolyzed well developed pollen grains from each taxa.

Pollen grains of the acetolyzed portion were dehydrated in ethanol series placed onto coverslips, left for ethanol evaporation then attached to copper stubs by double sided tape, coated with 30 nm gold using fine coat ion sputter JEOL JFC 1100E, examined and photographed at 30 KV using JEOL JSM-3500 scanning electron microscope present in the Faculty of Science, Alexandria University. The terminology used in the present study is according to Faegri, (1956) and Erdtman, (1952).

Statistical analysis

For all the studied taxa, the mean values of the pollen characters were separated and calculated then compared using the least significant difference (L.S.D) test at 0.05 level of probability (Snedecor and Cochran, 1990). The statistical analysis was performed using SAS (Statistical Analysis System) version 9.13, (2008).

3. Results

The results obtained from the twelve studied taxa are summarized in table 1 and illustrated in plates 1-12. The pollen grains of all taxa were monads, radially symmetrical, isopolar and were different in size. The pollen shape varied from prolate-spheroidal (P/E 1.02) to sub-prolate (P/E 1.20) except in *F. margarita* (Plate 10), where it was oblate-spheroidal (P/E 0.97). The mean polar axis length ranged from 26 µm (*F. margarita* and *X Citrofortunella floridana*) to 34.48 µm in *C. grandis*. Moreover, the mean equatorial diameter ranged from 26 µm (*F. margarita* and *X Citrofortunella floridana*) to 33.44 µm in *C. grandis*.

Table 1. Pollen morphological characters of the studied *Citrus* species and its related taxa

Characters → Taxa ↓	Common name	P. L.	E. D.	P/E	Pol. Sh.	Ap.	C. L.
C. aurantifolia (Christm.) Swing.	Mexican lime	28.00 - 34.40 **31.32**	23.20 - 32.00 **27.54**	1.17	3	2	22.40 - 30.40 **26.96**
C. aurantium L.	Sour orange	25.60 - 36.00 **30.72**	24.80 - 32.00 **28.27**	1.09	2	5	22.40 - 30.40 **25.36**
C. grandis (L.) Osbeck	Pummelo	31.20 - 37.60 **34.48**	30.40 - 36.80 **33.44**	1.03	2	4	30.21 - 34.40 **31.80**
C. latifolia Tanaka	Tahiti lime	27.20 - 36.00 **31.58**	24.00 - 31.20 **26.42**	1.20	3	5	22.40 - 31.20 **26.44**
C. limetta Risso	Sweet lime	28.80 - 40.00 **33.72**	27.20 - 37.60 **31.76**	1.07	2	1	24.00 - 35.20 **28.38**
C. paradisi Macf.	Marsh grapefruit	28.00 - 39.20 **33.14**	24.00 - 35.20 **30.64**	1.09	2	5	22.40 - 33.60 **27.85**
C. reshni Hort. ex Tanaka	Cleopatra mandarin	26.40 - 32.80 **29.80**	24.00 - 31.20 **27.46**	1.09	2	1	20.00 - 27.20 **24.48**
C. reticulata Blanco	Clementine tangerine	25.60 - 35.20 **30.46**	25.60 - 35.20 **28.14**	1.09	2	2	19.20 - 29.60 **24.84**
C. sinensis (L.) Osbeck	Succari orange	28.80 - 36.00 **32.20**	25.60 - 33.60 **29.58**	1.10	2	4	24.00 - 30.00 **27.60**
Fortunella margarita (lour.) Swing.	Oval Kumquat	23.20 - 29.60 **26.04**	24.00 - 29.60 **26.22**	0.97	1	3	18.40 - 24.00 **20.96**

X Citrofortunella floridana J. W. Ingram & H. E. Moore	Limequat	24.00 - 28.00 **26.56**	23.20 - 28.80 **26.12**	1.02	2	1	18.40 - 23.20 **21.02**
Poncirus trifoliata (L.) Raf.	Trifoliate orange	28.80 - 36.00 **32.26**	28.80 - 36.00 **31.19**	1.04	2	2	23.20 - 30.4 **26.44**
LSD$_{0.05}$		*0.93*	*0.89*	*0.03*			*0.94*

P. L. = Mean Polar Length, E. D. = Mean Equatorial Diameter, P/E = Mean Polar length/ Mean Equatorial diameter, Pol. Sh. = Pollen Shape (1. Oblate-spheroidal, 2. Prolate-spheroidal, 3. Sub-prolate), Ap. = Aperture number and type (1. Tri- and tetra-colporate, 2. Tetra- and penta-colporate, 3. Tri-, tetra- and penta- colporate, 4. tri- and tetra-colpate and tri- and tetra-colporate, 5. Tetra- and penta-colpate and tetra- and penta-colporate), C. L. = Mean Colpi Length. Bold Numbers = Mean of means, Italic numbers = Least Significant difference values.

Table 1 (Cont.) Pollen morphological characters of the studied *Citrus* species and its related taxa

Characters → Taxa ↓	Common name	Meso. D.	Ora L.	Ora W.	Amb Sh.	Ex. Th.	Ex. Or.
C. aurantifolia (Christm.) Swing.	Mexican lime	8.00 - 12.80 **9.70**	1.60 - 2.40 **2.08**	7.20 - 8.80 **7.71**	2	2.40	4
C. aurantium L.	Sour orange	8.00 - 12.00 **9.36**	2.40 - 4.00 **3.20**	8.00 - 8.80 **8.30**	2	2.70	3
C. grandis (L.) Osbeck	Pummelo	8.00 - 13.60 **11.22**	2.40 - 4.00 **3.06**	7.20 - 8.80 **7.90**	1	2.40	1
C. latifolia Tanaka	Tahiti lime	8.00 - 12.00 **9.36**	2.40 - 3.20 **2.97**	7.20 - 8.80 **7.69**	2	2.40	4
C. limetta Risso	Sweet lime	8.00 - 18.40 **12.72**	2.40 - 4.00 **3.20**	6.40 - 8.00 **7.30**	1	2.40	4
C. paradisi Macf.	Marsh grapefruit	6.40 - 15.20 **10.54**	1.60 - 4.00 **3.30**	6.40 - 8.00 **6.90**	2	2.40	1
C. reshni Hort. ex Tanaka	Cleopatra mandarin	8.00 - 12.00 **9.78**	3.20 - 4.00 **4.20**	7.20 - 9.60 **8.10**	1	2.40	2
C. reticulata Blanco	Clementine tangerine	8.00 - 12.80 **10.30**	1.60 - 3.20 **2.90**	6.40 - 7.20 **6.80**	2	2.40	2
C. sinensis (L.) Osbeck	Succari orange	6.40 - 14.40 **10.40**	2.40 - 4.00 **3.60**	7.20 - 8.00 **7.20**	1	2.40	1
Fortunella margarita (lour.) Swing.	Oval Kumquat	8.00 - 12.00 **9.82**	2.40 - 4.00 **3.50**	4.80 - 7.20 **6.08**	3	2.40	3
X Citrofortunella floridana J. W. Ingram & H. E. Moore	Limequat	8.00 - 12.80 **9.82**	2.40 - 3.20 **3.00**	5.60 - 7.20 **6.40**	1	2.40	3
Poncirus trifoliata (L.) Raf.	Trifoliate orange	8.80 - 12.00 **10.72**	3.20 - 3.40 **3.20**	4.80 - 7.20 **6.40**	2	2.40	3
LSD$_{0.05}$		*0.71*	*0.44*	*0.83*		*0.04*	

Meso. D. = Mean Mesocolpi Diameter, Ora L. = Mean Ora Length, Ora W. = Mean Ora Width, Amb Sh. = Amb Shape (1. Rounded, triangular and squared, 2. Squared and rounded, 3. Rounded-triangular, squared and rounded), Ex. Th. = Mean Exine thickness, Ex. Or. = Exine Ornamentation (1. Tectate-perforate, 2. Tectate-perforate to microreticulate, 3. Foveolate, 4. Reticulate). **Bold Numbers** = Mean of means, *Italic numbers* = Least Significant difference values.

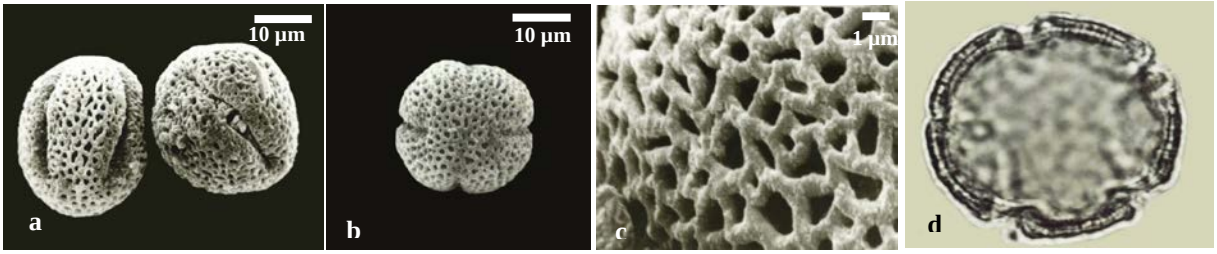

Plate 1. SEM (**a-c**) and LM (**d**) photomicrographs of *C. aurantifolia* pollen grains; **a:** Equatorial view (colporate), **b:** Polar view (aperture number), **c:** Exine ornamentation, **d:** Polar view (aperture number)**.**

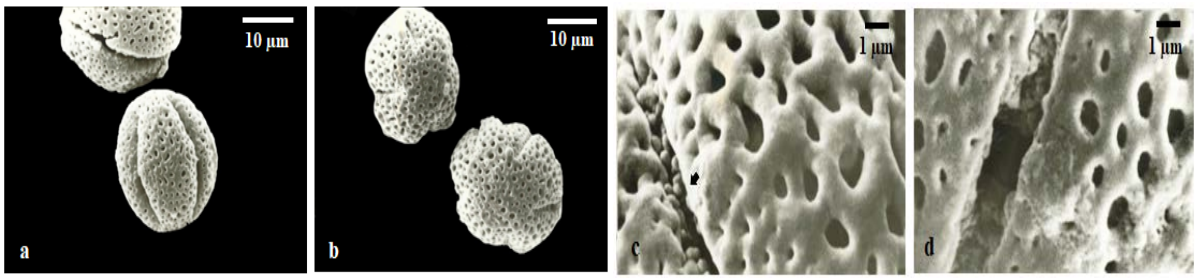

Plate 2. SEM (**a-d**) photomicrographs of *C. aurantium* pollen grains; **a:** Equatorial view (colporate), **b:** Polar view (aperture number), **c:** Exine Ornamentation (colpate - **arrow**), **d:** Exine ornamentation (colporate)**.**

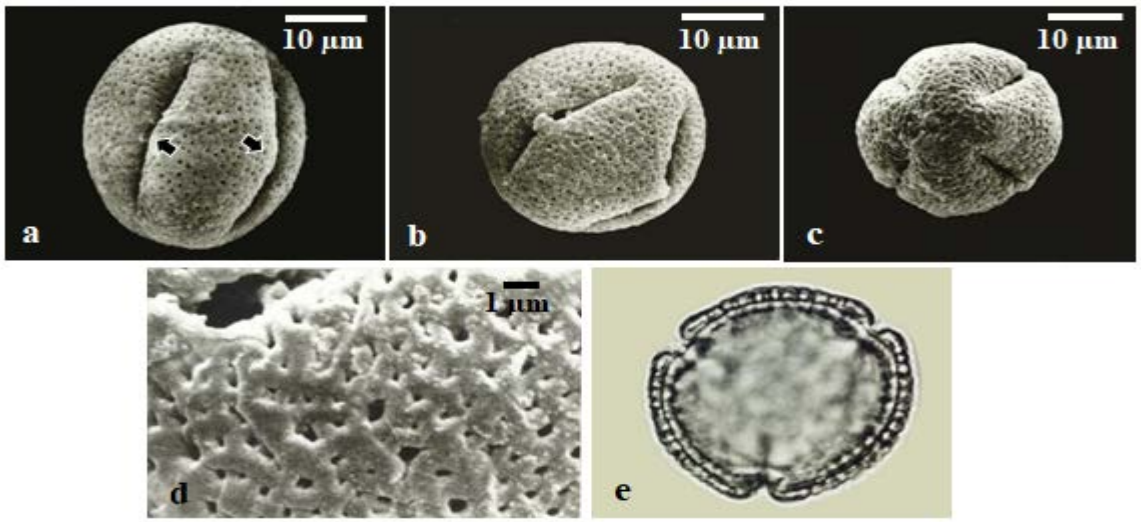

Plate 3. SEM (**a-d**) and LM (**e**) photomicrographs of *C. grandis* pollen grains; **a:** Equatorial view (colpate - **arrows**), **b:** Equatorial view (colporate), **c:** Polar view (aperture number), **d:** Exine ornamentation, **e:** Polar view (aperture number)

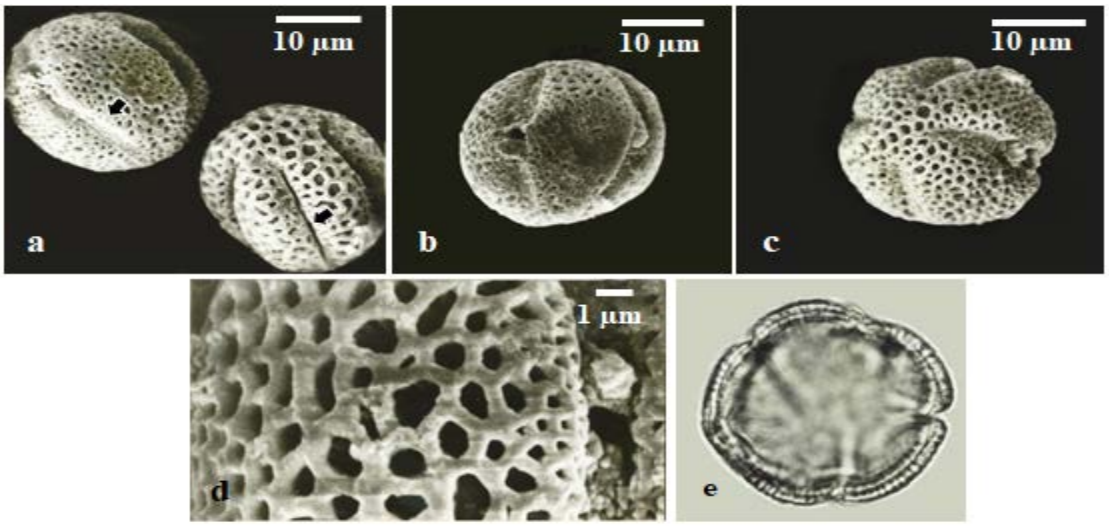

Plate 4. SEM (**a-d**) and LM (**e**) photomicrographs of *C. latifolia* pollen grains; **a:** Equatorial view (colpate - **arrows**), **b:** Equatorial view (colporate), **c:** Polar view (aperture number), **d:** Exine ornamentation, **e:** Polar view (aperture number)

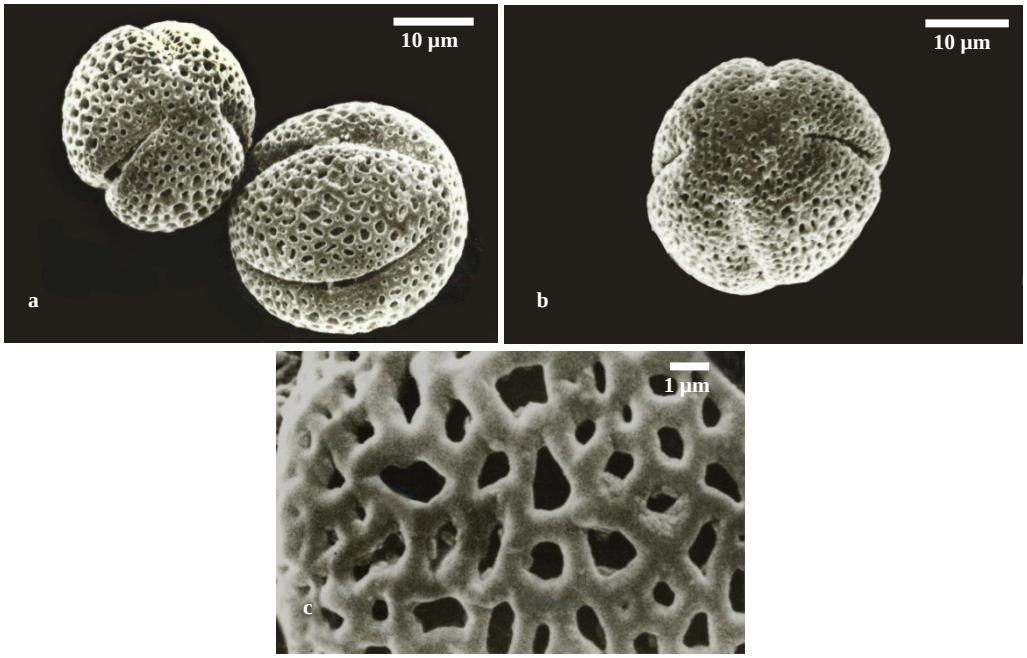

Plate 5. SEM (**a-c**) photomicrographs of *C. limetta* pollen grains; **a:** Equatorial and polar views (colporate, aperture number), **b:** Polar view (aperture number), **c:** Exine ornamentation

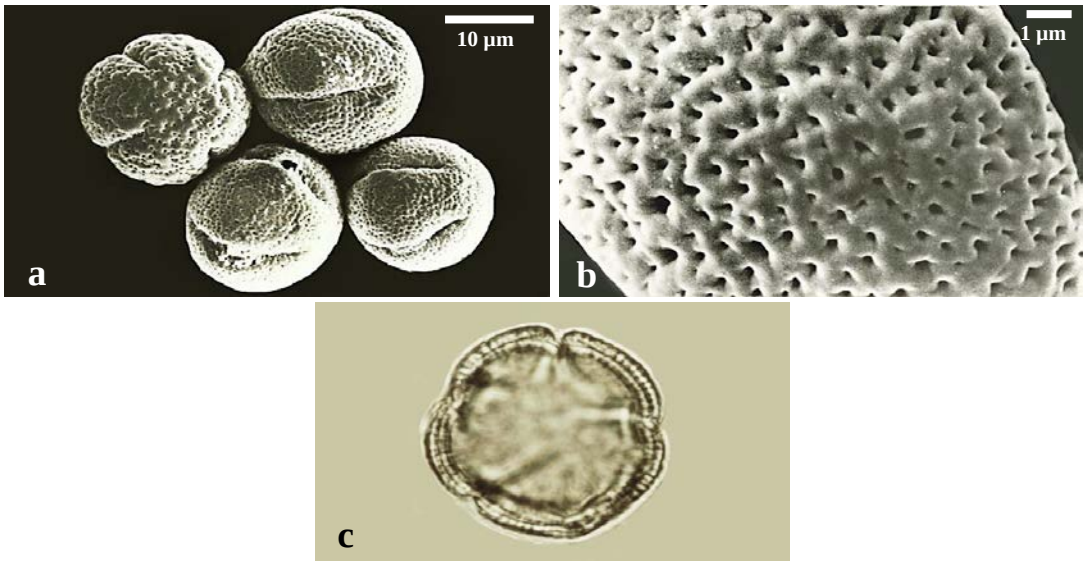

Plate 6. SEM (**a-b**) and LM (**c**) photomicrographs of *C. paradisi* pollen grains; **a:** Equatorial and polar views (colpate - **arrow**, colporate, aperture number), **b:** Exine ornamentation, **c:** Polar view (aperture number)

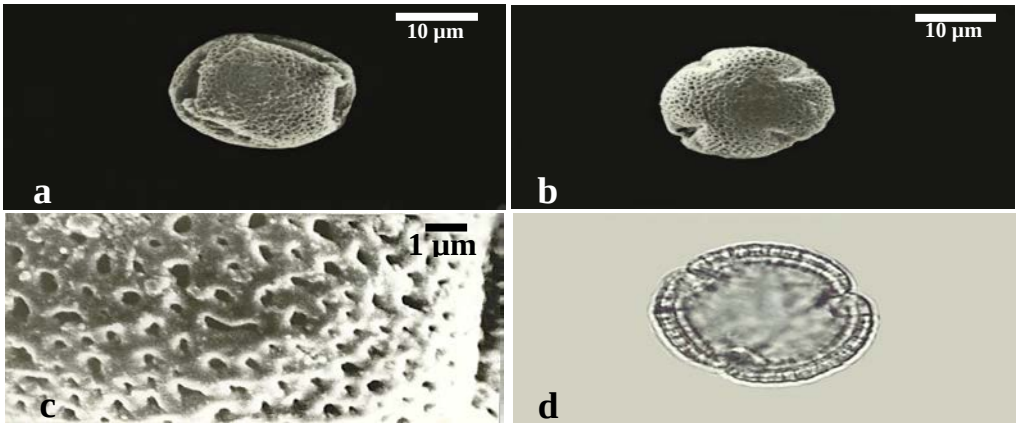

Plate 7. SEM (**a-c**) and LM (**d**) photomicrographs of *C. reshni* pollen grains; **a:** Equatorial view (colporate), **b:** Polar view (aperture number), **c:** Exine ornamentation, **d:** Polar view (aperture number)**.**

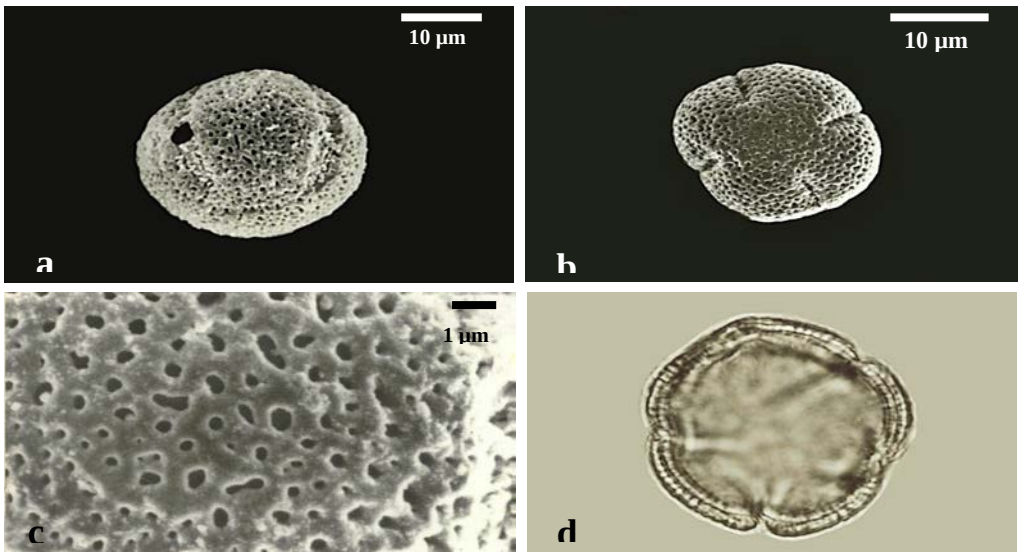

Plate 8. SEM (**a-c**) and LM (**d**) photomicrographs of *C. reticulata* pollen grains; **a:** Equatorial view (colporate), **b:** Polar view (aperture number), **c:** Exine ornamentation, **d:** Polar view (aperture number)**.**

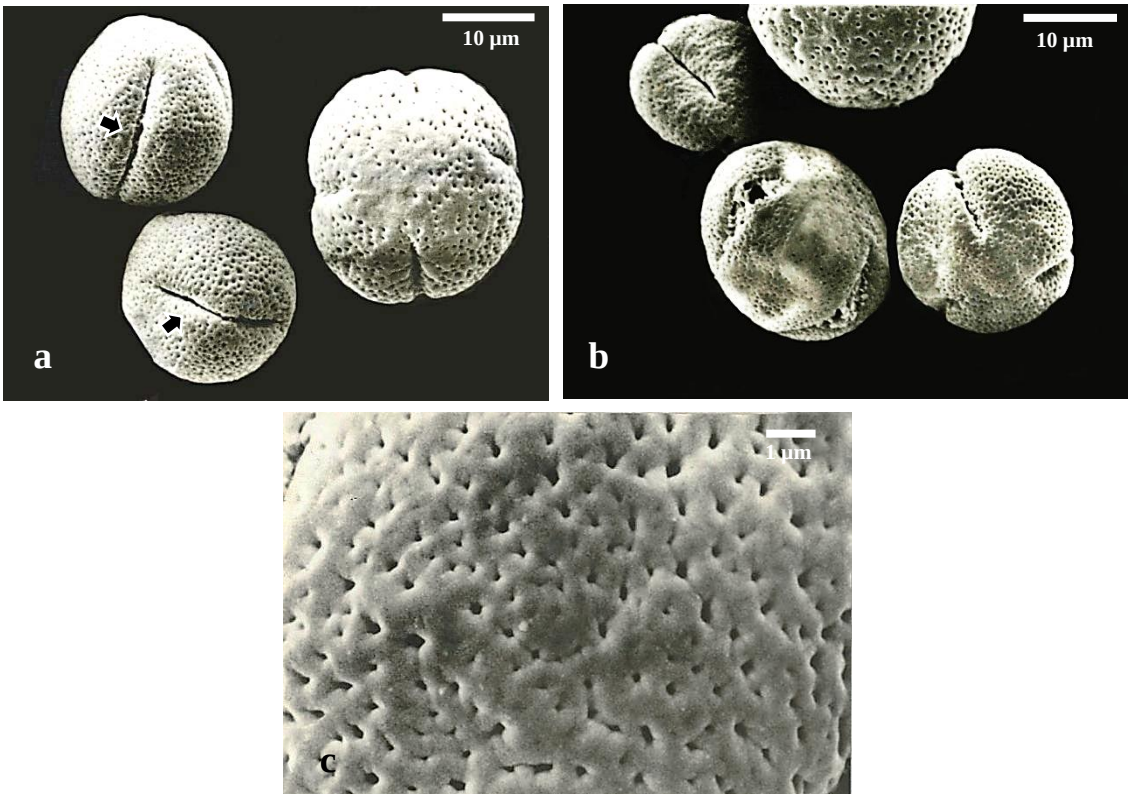

Plate 9. SEM (**a-c**) photomicrographs of *C. sinensis* pollen grains; **a:** Equatorial and polar views (colpate - **arrows**, aperture number), **b:** Equatorial and polar views (colporate, aperture number), **c:** Exine ornamentation

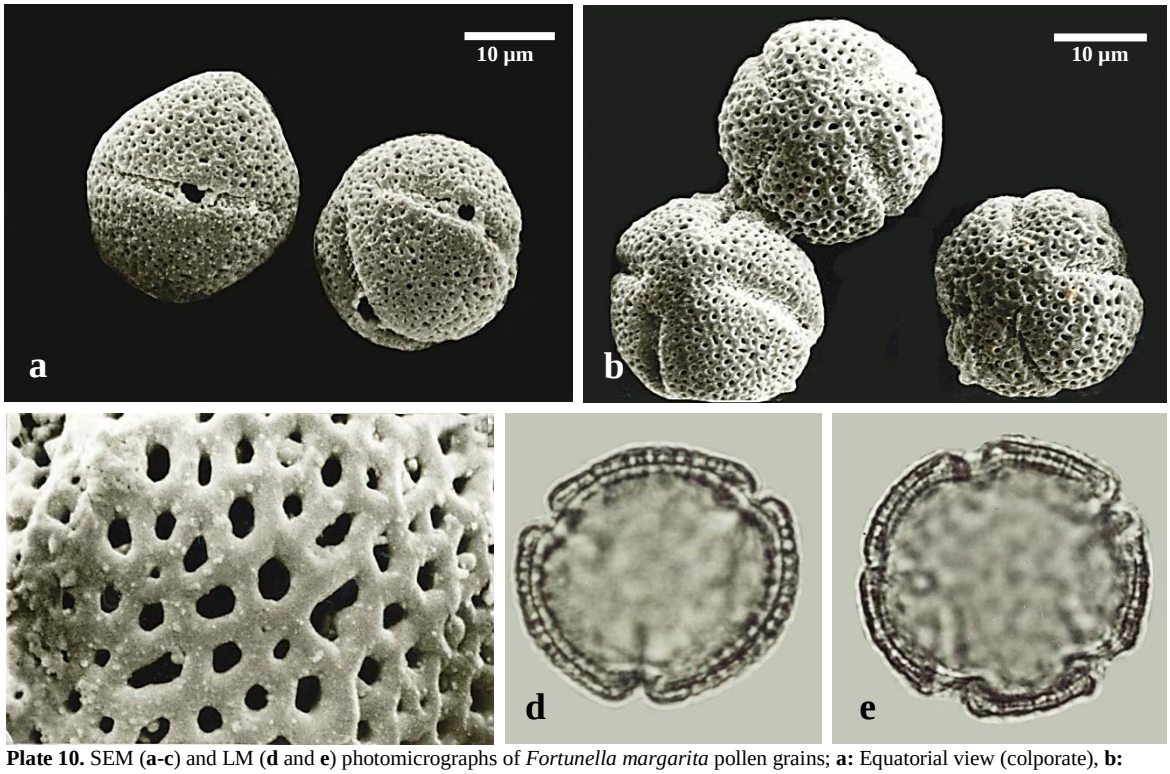

Plate 10. SEM (**a-c**) and LM (**d** and **e**) photomicrographs of *Fortunella margarita* pollen grains; **a:** Equatorial view (colporate), **b:** Equatorial and polar views (aperture number), **c:** Exine ornamentation, **d:** Polar view (aperture number)**, e:** Polar view (aperture number)

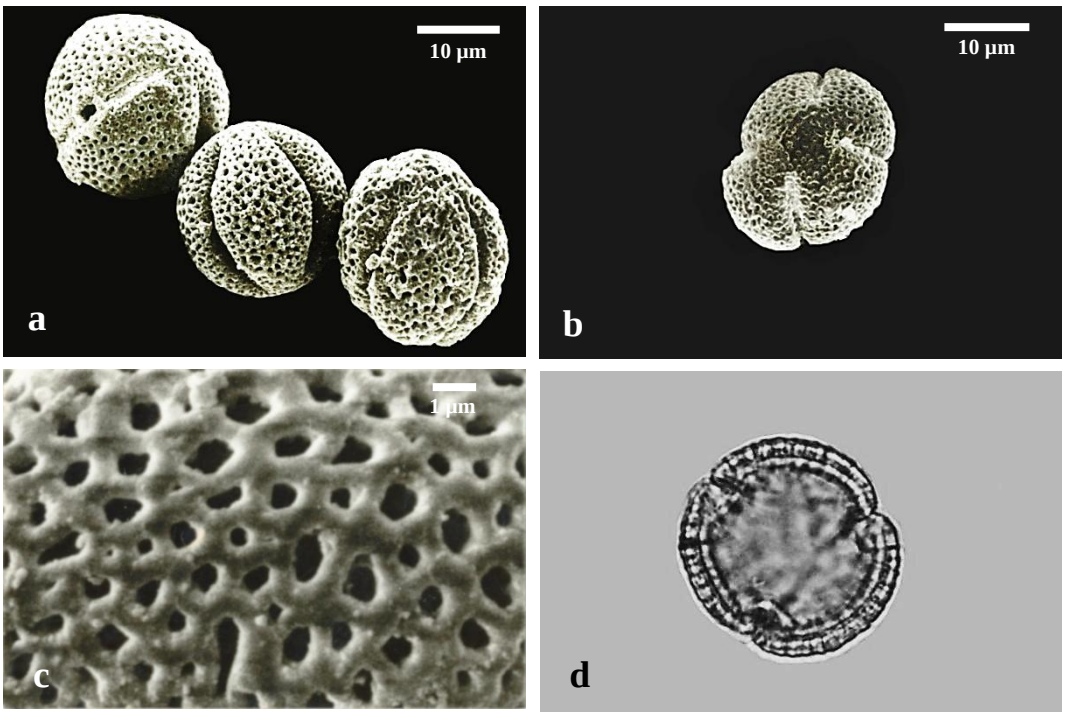

Plate 11. SEM (**a-c**) and LM (**d**) photomicrographs of *X Citrofortunella floridana* pollen grains; **a:** Equatorial view (colporate), **b:** Polar view (aperture number), **c:** Exine ornamentation, **d:** Polar view (aperture number)

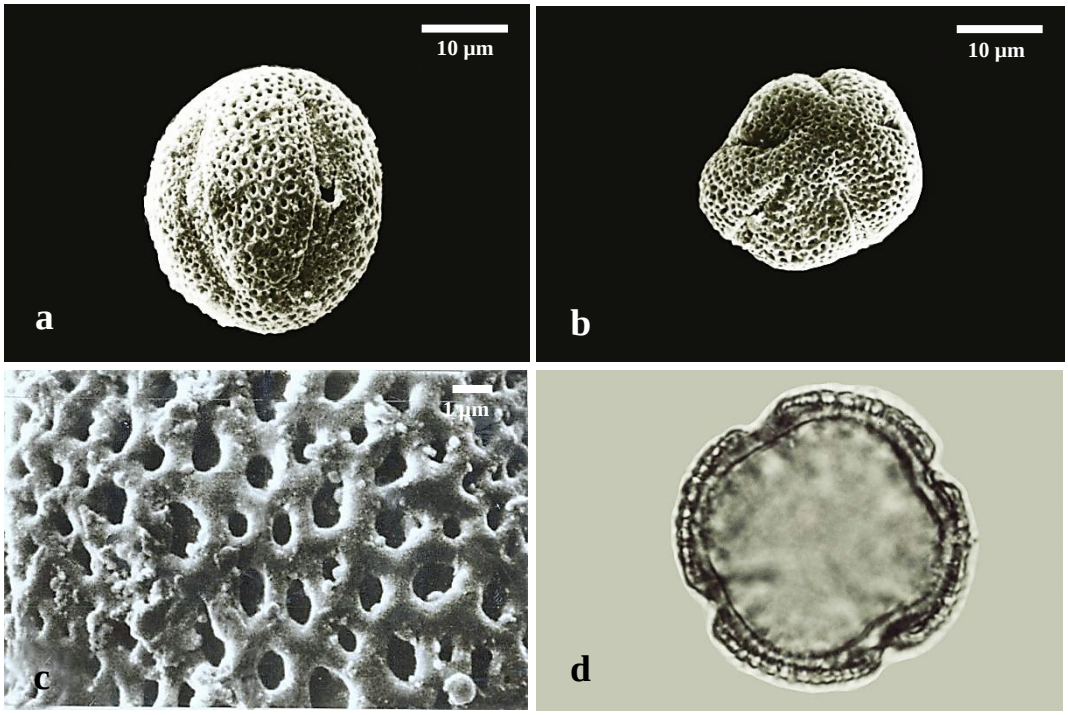

Plate 12. SEM (**a-c**) and LM (**d**) photomicrographs of *Poncirus trifoliata* pollen grains; **a:** Equatorial view (colporate), **b:** Polar view (aperture number), **c:** Exine ornamentation, **d:** Polar view (aperture number)

Apertures types

The types of apertures were either colpate or colporate and ranged from three to five in number. The variations in the type and number of apertures were found to be within the same taxa and the same anther as well. Five groups of aperture types were found; 1) the first group included three taxa characterized by tri-tetra-colporate aperture types, *C. limetta* (Plate 5a and b), *C. reshni* (Plate 7a, b and d) and *X Citrofortunella floridana* (Plate 11a, b and d). 2). The second group also included three taxa characterized by tetra-penta-colporate aperture types in *C. aurantifolia* (Plate 1a, b and d), *C. reticulata* (Plate 8 a, b and d) and *P. trifoliata* (Plate 12a, b and c). 3). The third group has only *F. margarita* which included the tetra-penta-colporate types, in addition to the tri-colporate ones (Plate 10a, b, d and e). 4) The taxa in the fourth group were characterized by tri-tetra-colpate and tri-tetra-colporate aperture types in *C. grandis* (Plate 3a, b, c and e) and *C. sinensis* (Plate 9a and b). 5) Finally, group five comprised *C. aurantium*, *C. latifolia* and *C. paradisi* with pollen grains that have tetra-

penta-colpate and tetra-penta-colporate types of apertures (Plate 2a, b, c and d; Plate 4a, b, c and e and Plate 6a and c), respectively.

The ecto-aperture colpi, in all the studied taxa were long, wide, with rounded or pointed ends, equally spaced around the equator. They were characterized by uneven margins and covered with granular membranes. The mean colpi length varied within the studied taxa from a minimum of 20.00 µm in both *F. margarita* and *X Citrofortunella floridana* to a maximum of 31.80 µm in *C. grandis*. Moreover, the mean mesocolpium diameter varied from 9.36 µm in *C. aurantium* and *C. latifolia* to 12.72 µm *in C. limetta*. The endo-apertures pori were lalongate in all the studied taxa, where the ora width ranged from 6.08 to 6.40 µm in *F. margarita, X Citrofortunella floridana and P. trifoliata*; slightly wider from 6.80 to 7.30 µm in *C. reticulata a, C. paradisi, C. sinensis* and *C. limetta* and more than 7.30 µm in the rest of the taxa. The amb shapes are mostly rounded- triangular or square, and sometimes both shapes are found in the same taxon. The pollen amb was of two shapes; rounded-triangular and square in *C. grandis* (Plate 3c and e), *C. limetta* (Plate 5a and b), *C. reshni* (Plate 7b and d), *C. sinensis* (Plate 9a and b), and *X Citrofortunella floridana* (Plate 11b and d), while it was also of another two shapes; rounded and square in *C. aurantifolia* (Plate 1b and d), *C. aurantium* (Plate 2b), *C. latifolia* (Plate 4c and e), *C. paradisi* (Plate 6a and c), *C. reticulata* (Plate 8b and d) and *P. trifoliata* (Plate 12b and d). Besides, the amb was of three shapes; rounded-triangular, rounded and square in *F. margarita* (Plate 10b, d and e).

Exine ornamentations

The exine is considerably thin; it was 2.40 µm thick in all the studied taxa, except in *C. aurantium* as it was 2.70 µm. The exine ornamentation of the pollen grains of the studied taxa, as observed by the scanning electron microscope, appeared in four different types. The first type was tectate perforate with smooth tectum, which is provided by more or less rounded pores in *C. grandis* (Plate 3d), *C. paradisi* (Plate 6b) and *C. sinensis* (Plate 9c). The second type was tectate perforate to microreticulate with latimurate reticulum, which is characterized by more or less straight and smooth muri and rounded to oval small sized lumina in *C. reshni* (Plate 7c), and *C. reticulata* (Plate 8c). The third type was foveolate with latimurate reticulum, which is characterized by more or less straight and smooth muri and nearly rounded large-sized lumina in *C. aurantium* (Plate 2c and d), *F. margarita* (Plate 10c), *X Citrofortunella floridana* (Plate 11c) and *P. trifoliata* (Plate 12c). The fourth type was reticulate with angustimurate reticulum, which is characterized by straight and rough muri and the lumina were different in size and shape in *C. aurantifolia* (Plate 1c), *C. latifolia* (Plate 4d) and *C. limetta* (Plate 5c).

Pollen types

Accordingly, the studied taxa can be classified into three different groups according to their pollen characters. The first group included five taxa; *C. grandis*, *C. limetta*, *C. paradise*, *C. sinensis* and *P. trifoliata*. These five taxa were characterized by the biggest pollen size, where the polar axis length was more than 32.20 µm, with prolate-spheroidal pollen, colpi length more than 26.44 µm and mesocolpium diameter exceed 10.30 µm. The second group included five taxa viz. *C. aurantifolia*, *C. aurantium*, *C. latifolia*, *C. reshni* and *C. reticulata*. These taxa have medium polar axis length, ranged from 29.80 to 31.58 µm with prolate-spheroidal and sub-prolate pollen, colpi length ranged from 24.48 to 26.44 µm and mesocolpium diameter ranged from 9.36 to 10.30 µm. Meanwhile, the third group included *F. margarita* and *X Citrofortunella floridana*. Both species have the polar axis length ranging from 26.00 to 26.60 µm, oblate-spheroidal or prolate-spheroidal pollen, with shorter colpi ranging from 20.96 to 21.02 µm and mesocolpium diameter of about 9.82 µm.

In summary, by using pollen morphological characters, it is very difficult to construct pollen key for the studied *Citrus* species, and its related genera, because of the great similarities among them in shape, aperture, and exine ornamentation

4. Discussion

The pollen morphological characters and ultrastructure have been used to identify and distinguish between species and cultivars of fruit trees (Asma, 2008; Gilani *et al.,* 2010 and Nikolića and Milatović, 2016). *Citrus* and its related two genera, *Fortunella* and *Poncirus*, are considered one of the important economic and medicinal fruits in the world; they are rich plants in vitamin C and volatile oils (Scora, 1988). However, there are no reports on pollen morphology of *Citrus* species in Egypt. Inyama *et al.,* (2015) found that palynological characters were useful in delimiting six studied *Citrus* species. They could be exploited in conjunction with other evidence in species identification and characterization, while they were insignificant in the reclassification of the investigated taxa.

In the present study, palynological investigations indicated that variations in pollen morphological characters were of taxonomic significance. In particular, the twelve studied taxa were found to be significantly different from each other in six quantitative pollen characters; this includes polar length, equatorial diameter, colpi length, ora length, ora diameter, mesocolpi diameter. While the mean ratio of the polar length and the equatorial diameter (P/E) and the exine thickness were insignificantly different from each other. These results were in agreement with those reported by Breis *et al.,* (1993) and Mohammad *et al.,* (1999). The pollen shape varies from oblate-spheriodal, prolate-spheriodal to subprolate in all the studied taxa. This finding agrees with that found by Ye *et al.,* (1981) and Mohammad *et al.,* (1999). The variations of pollen size were suggested by Kozaki and Hirai, (1986) and Mohammad *et al.,* (1999) where they reported that pollen grain of *C. grandis* and *P. trifoliata* had larger pollen than *C. latifolia*, *C. limetta* and *F. margarita*, while those of *C. aurantium, C. sinensis* and *C. reshni* were intermediate in size. These suggestions were in agreement with the results of the present study where the studied taxa were classified into three different groups according to their pollen size. The first group included *C. grandis*, *C. limetta*, *C. paradise*, *C. sinensis* and *P. trifoliata*, which have the largest pollen grains, where the polar axis length ranged from 32.20 to 34.48 µm, while the equatorial

diameter ranged from 29.58 to 33.44 µm. The second group which included *C. aurantifolia*, *C. aurantium*, *C. latifolia*, *C. reshni* and *C. reticulata* had medium sized pollen grains, where the polar axis length ranged from 29.80 to 31.58 µm, while the equatorial diameter ranged from 26.42 to 28.27 µm. Moreover, the third group included two taxa *F. margarita* and *X Citrofortunella floridana* with the smallest pollen grains, where the polar axis length ranged from 26.04 to 26.56 µm, while the equatorial diameter ranged from 26.12 to 26.22 µm. On the contrary, these groups did not coordinate with Al-Anbari *et al.*, (2015), who recognized four groups in the Iraqi pollen grains based on pollen size and exine ornamentation.

Meanwhile, the most variable characters found in the present investigation were within the number and type of apertures, exine ornamentations, ora width as well as mesocolpium diameters. This was in line with Ye *et al.*, (1981) and Mohammad *et al.*, (1999).

Grant *et al.*, (2000) found considerable variation in pollen morphology of subfamily Aurantioideae, which divided the studied taxa into five pollen types. The differences include aperture number, ecto-colpus shape and size, exine ornamentation and wall structure. When designating pollen types for the subfamily Aurantioideae, the principal characters used were the aperture number and exine ornamentation. These characters were in harmony with the obtained results and as a conclusion, the aperture type and ora size were the most distinguished characters in the circumscription of the studied taxa. According to the type and number of apertures, five types were observed in the studied taxa. Type (1) Tri-tetra-colporate was found in *C. limetta, C. reshni* and *X Citrofortunella floridana*. Type (2) Tetra-penta-colporate was found in *C. aurantifolia, C. reticulata and P. trifoliata*. Type (3) Tri-tetra-penta-colporate was found in *F. margarita*. Type (4) included both "tri-tetra-colpate and tri-tetra-colporate" and was found in *C. grandis and C. sinensis*. Finally, type (5) included both "tetra-penta-colpate and tetra-penta-colporate" and was found in *C. aurantium, C. latifolia* and *C. paradisi*. These multi types of pollen apertures were found in the studied species from the same anther which may be due to chromosomal abnormalities as mentioned by Stace *et al.*, (1993).

Moreover, the exine thickness was the same in all the studied taxa and considered as an insignificant character, while the exine ornamentations showed great variations in the sculpturing types and have taxonomic value in the classification of the studied taxa, where it was diversified from tectate-perforate, tectate-perforate to microreticulate, foveolate or reticulate. According to the exine ornamentations, four different types were observed. Type (1) was tectate-perforate in *C. grandis, C. paradisi* and *C. sinensis*. Type (2) was tectate-perforate to microreticulate in *C. reshni* and *C. reticulate*, while Type (3) was foveolate with latimurate reticulum in *C. aurantium, F. margarita, X Citrofortunella floridana* and *P. trifoliata*. Type (4) was reticulate with angustimurate reticulum, in *C. aurantifolia, C. latifolia* and *C. limetta*. These findings agree with those found by Ye *et al.*, (1981) and Mohammad *et al.*, (1999), while disagreeing with the results of Kozaki and Hirai (1986) who stated that the exine patterns were sub-reticulate in the species of *Citrus, Poncirus, X Citrofortunella floridana* and *Fortunella*.

5. Conclusions

In the present investigation, the pollen size, pollen shape, colpi length, the apertures number and type, ora size, amb shape, mesocolpium diameter, and exine ornamentation were the most distinguished characters in the circumscription of the studied taxa. All the studied pollen grain characters except ora shape and exine thickness could be considered as of taxonomic value in the differentiation among the closely related taxa of *Citrus, Fortunella, X Citrofortunella floridana* and *Poncirus* in the present study.

References

Abobatta WF. 2019. *Citrus* Varieties in Egypt: An Impression. *Int. Res. J. Applied Sci.*, 1:63-66.

Al-Anbari AK, Barusrux S, Pornpongrungrueng P and Theerakulpisut P. 2015. Pollen grain morphology of *Citrus* (Rutaceae) In: Iraq. International Conference on Plant, Marine and Environmental Sciences (PMES-2015) Jan. 1-2, 2015 Kuala Lumpur (Malaysia), pp. 8-13.

Asma BM. 2008. Determination of pollen viability, germination ratios and morphology of eight apricot genotypes. *Afr. J. Biotechnol*., **7**:4269-4273.

Avci S, Sancak C, Can A, Acar A and Pinar N. 2013. Pollen morphology of the genus *Onobrychis* (Fabaceae) in Turkey. Turk J Bot. , **37**(4):669-681. DOI: 10.3906/bot-1207-52.

Barrett HC and Rhodes AM. 1976. A numerical taxonomic study of affinity relationships in cultivated *Citrus* and its close relatives, *Syst Bot*, **1**:105-136. http://dx.doi.org/10.2307/2418763.

Boulos L. 1999. **Flora of Egypt.** Vol. 2, Al Hadara Publishing, Cairo, Egypt.

Brandis D. 1874. **Rutaceae: The forest flora of north-west and central India**. A. Constable St Company, Limited edition, London, pp. 50-56.

Breis FB, Sanchenz CP, Gilabert CE and Castillo MEC. 1993. The pollen morphology of *Citrus* limon cv. "Verna" from the Murcia region. *S.E. Anales de Biologia*, **19**:63-69.

Chase MW, Morton CM and Kallunki JA. 1999. Phylogenetic relationships of Rutaceae: A cladistic analysis of the subfamilies using evidence from RBC and ATP sequence variation. *Am. J. Bot.*, **86**(8):1191-1199.

Davis PH and Heywood VH. 1973. **Principles of Angiosperm Taxonomy**. Robert E. Krieger Publishing Company, Huntington, New York.

Engler A. 1931. Rutaceae. In: Engler A, and Prantl k, (editors). **Die Natürlichen Pflanzenfamilien**, Second edition, Engelmann, Leipzig, **19a**:187-359.

Erdtman G. 1952. **Pollen morphology and plant taxonomy of angiosperms**. Almqvist and Wiksell, Stockholm.

Faegri K. 1956. Recent trends in Palynology. *Bot Rev*, **22**:639-664.

Gilani SA, Qureshi RA, Khan AM and Potter D. 2010. Morphological characterization of the pollens of the selected species of genus Prunus Linn. from northern Pakistan. *Afr. J. Biotechnol*., **9**:2872-2879.

Grant M, Blackmore S and Morton C. 2000. Pollen morphology of the sub-family Aurantioideae (Rutaceae). *Grana*, **39**:8-26.

Hamza M and Tate B. 2017. Egyptian Orange Exports Thrives Thanks to Currency Devaluation. United States Department of Agriculture, Foreign Agricultural Service. Gain Report, Global Agricultural Information Network.

Hodgson RW. 1965. Taxonomy and Nomenclature in *Citrus* fruits. In: Advances in Agricultural Science and their application. *The Madras Agr. J.*, Coimbatore, 317-333.

Inyama CN, Osuoha VUN, Mbagwu FN and Duru CM. 2015. Systematic Significance of Pollen Morphology of *Citrus* (Rutaceae) from Owerri. *Med Aromat Plants*, 4-191 DOI:10.4172/2167-0412.1000191.

Kozaki I and Hirai M. 1986. **Pollen ultrastructure of *Citrus* for taxonomic identification.** In: Development of new technology for identification and classification of tree crops and ornamentals (edited by Kitaura K Akihama T; Kukimura H; Nakajima K; Rori M and Kozaki I). Fruit tree Research Station, Ministry of Agriculture, Forestry and Fisheries, Japan pp 11-17.

Mohammad P, Shiraishi M, Toda J, Aguja SE and Ohmine Y. 1999. Characterization with scanning electron microscope of the pollen of *Citrus* plant. *Sarhad Journal of Agriculture*, **15**(1):29-35 [Cited from Horticultural Science Abstracts **69**(8)].

Marcovitch VV. 1926. Indeeling van het geslacht *Citrus*, Landbouw, **2**(4).

Mary A and Gopal GV. 2018. Pollen Morphology of selected taxa of Ehretiaceae from Western Ghats, India. *Ann. Plant Sci.*, **7**(11):2446-2450 http://dx.doi.org/10.21746/aps.2018.7.11.1.

Moore GA. 2001. Oranges and lemons: Clues to the taxonomy of *Citrus* from molecular markers. *Trends Genet.*, **17**(9):536-40.

Nicolosi E. 2007. **Origin and taxonomy**. In: Khan IA (editor). *Citrus* Genetics, Breeding and Biotechnology. CAB International, Wallingford, United Kingdom, pp. 19-44.

Nikolić D, and Milatović D. 2016. Pollen morphology of some sweet cherry cultivars observed by scanning electron microscopy. *Acta Hortic*., **1139**:369-374 DOI:10.17660/ActaHortic.2016.1139.64.

Roxburgh W. 1832. **Flora Indica.** 2nd ed. by Carey W 3. Thacker and Company, Calcutta and Parbury, Allen and Company, London.

Saad SI and Taia WK. 1988. Palynological studies of some species in the genus *Astragalus* L. (Leguminosae) in *Egypt. Arab Gulf J Sci Res*, **B6**(2):227-243.

Singh Z and Singh L. 2008. **The SAS System for Windows**, Version 9.13, SAS Institute Inc., Cary, NC, USA.

Scora RW. 1975. On the history and origin of *Citrus*. *Bulletin of the Torrey Botanical Club.*, **102**:369-375. http://dx.doi.org/10.2307/2484763.

Scora RW. 1988. Biochemistry. Taxonomy and evolution of modern cultivated *Citrus*. Proceeding of International Society of Citriculture, **1**:277-289.

Sharma OP. 1993. **Plant Taxonomy**. Tata McGraw-Hill. Publishing Company. New Delhi-New York, pp. 244-247.

Snedecor GW and Cochran WG. 1990. **Statistical methods** 7th Ed. The Iowa State Univ. Press Ames Iowa U.S.A., pp. 593.

Stace HM, Armstrong JA and James SH. 1993. Cytoevolutionary patterns in Rutaceae. *Plant Syst Evol***, 187**:1-28.

Swingle WT. 1943. The botany of *Citrus* and its wild relatives in the orange subfamily. In: **The *Citrus* industry**. Webber, HJ and Batchelor, LD (editors), University of California Press, Berkeley **1**:128-474.

Swingle WT and Reece PC. 1967. The botany of *Citrus* and its wild relatives. In: **The *Citrus* industry**. Reuther W, Webber HJ and Batchelor LD (editors). University of California Press, Berkeley, **1**:190-430.

Taia WK. 2004. Palynological Study within Tribe *Trifolieae (*Leguminosae). *Pak J Biol Sci*, **7**(7):1303-1315.

Taia WK and Sheha MA. 2001. Palynological study within some *Atriplex* species. *Bio Sci. Res. Bull.*, **17**(2):91-97.

Täckholm V. 1974. **Students Flora of Egypt**. Cairo University, Beirut, **2**:334-335.

Tanaka T. 1936. The taxonomy and nomenclature of Rutaceae-Aurantioideae. *Blumea - Biodiversity, Evolution and Biogeography of Plants*, **2**(2):101-110.

Tanaka T. 1977. Fundamental discussion of *Citrus* classification. *Stud Citrol*, **14**:1-6.

Ye YM; Kong Y; Zheng XH. 1981. Studies on Pollen Morphology of *Citrus* Plants. In: Matsumoto K. (ed.) Proc. Int. Soc., Citriculture, Vol. 1. I.S.C. Printing Co., Misaki-cho, Japan, pp. 23-25.