GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(Research Article)

Check for updates

Morphological diversity of pollen grains within the flowering stages of *Tecoma stans* (L.) Juss. ex Kunth

Wafaa Kamal Taia [1], Rim Samir Hamdy [2], Amany Mohamed Abd El-Maged [3,*]

[1] *Department of Botany and Microbiology, Alexandria University, Faculty of Science, Egypt.*

[2] *Department of Botany and Microbiology, Cairo University, Faculty of Science, Egypt.*

[3] *Department of Biology and Geology, Alexandria University, Faculty of Education, Egypt.*

GSC Advanced Research and Reviews, 2023, 16(01), 280–287

Publication history: Received on 13 June 2023; revised on 21 July 2023; accepted on 24 July 2023

Article DOI: https://doi.org/10.30574/gscarr.2023.16.1.0320

Abstract

The study of the morphology of pollen grains is essential to understand plant evolution, adaptation, diversity and discrimination of taxa. This study aims to determine the importance of choosing the perfect stage of flower development in the study of pollen morphological characters as a taxonomical tool. In this work a woody, perennial street tree *Tecoma stans* was chosen as a model species belonging to family Bignoniaceae. *Tecoma stans* has many polymorphic forms native to North America and flourished in hot climates. This tree is commonly planted in both Cairo and Alexandria streets. Samples of flower buds in nine different stages were gathered from five to six different trees from March to June 2023. Non-acetolysed pollen grains were investigated using both light and Scanning electron microscopes. The results showed that pollen density, size, shape beside colpi length as well as their state varied between the different developmental flowering stages and cannot be relied on taxonomical decisions, while exine ornamentation slightly differed between the pollen grains from stage two to nine, but it was faint in immature flower bud (stage one). This study confirms that we must be precise in choosing the correct flower stage in pollen morphological studies to obtain significant morphological description. The results obtained revealed that the aperture type is more stable than pollen shape and size.

Keywords: Bignoniaceae; Flowering stages; Pollen diversity; Taxonomy; *Tecoma*

1. Introduction

Pollen grains are the male gametes in both Gymnosperms and Angiosperms played a comprehensive role in the taxa classification and discrimination since the last century [1]. Pollen grains of the different taxa are diverse in their morphological characters that can be consistently inherited from generation to generation. Pollen morphology considered an important tool for taxa ranking, grouping and identification. Despite the importance of pollen morphology in plant taxonomy, many severe faults happened which led to misinterpretations of the data obtained. [2] cited three parameters as the source of these erroneous inferences (1) the interpretations of the palynologist (2) the pollen terminology applied, and (3) the magnification, resolution, and methods used. Many other factors give wrong descriptions, such as the hydration state and the harmomegathic effect resulting from the acetolysis mixture and chemical treatments beside many other environmental factors. [3] noted that the incorrect interpretation resulted from the appearance of the sulcus in some monocot families, which may be related to their hydrostatic condition.

Tecoma stans (L.) Juss. ex Kunth (yellow bells) or *Bignonia stans* L. is a perennial woody long tree, commonly cultivated in Cairo and Alexandria streets for its yellow fragrant showy flowers clustering at the end of the branches. It is the type species of the genus *Tecoma* which included 16 species, belonging to family Bignoniaceae order Lamiales [4]. Many

* Corresponding author: Amany Mohamed Abd El-Maged

polymorphic complex forms have been recorded which have been segregated into many subspecies and varieties [5]. The most widespread one is *T. stans* var. *stans*. This variety has many synonyms as *T. stans* var. *apiifolia* DC (1845), *Stenolobium stans* var. *pinnatum* Seem (1863), *S. stans* var. *multijugum* R.E.Fr (1903), *S. quinquejugum* Loes (1913), and *T. incisa* (Rose & Standl.) I.M. Johnst (1940).

Tecoma stans var. *stans* is commonly found in both Cairo and Alexandria streets and reach up to eight meters. The trees are flourished and attain their flowering time starting from March and last till October. The flowers have four epipetalous, didynamous stamens, each with two long versatile lobes producing considerable amount of pollen grains. The aims of this work are 1- to investigate the pollen morphological characters and densities in the different developmental stages, 2- to explore how much pollen characters can be used as a taxonomical tool and 3- to examine which flower stage can be used in pollen morphological study.

2. Material and methods

Flowers and flower buds in nine developmental stages were collected from four to six trees from Maadi district, Cairo University campus, Giza and Alexandria University, Faculty of Science campus during May and June 2023 (Figures 1,2 & 3). The flowers were opened carefully using two needles under stereo-microscope and the four anthers from each flower stage opened to obtain the pollen grains. Non-acetolysed pollen grains examined, measured and photographed using Motic (B-150D) light microscope fitted with USB digital-Video Camera and Computer Software with 10X40 lenses. The polar, equatorial axis or radius as well as the exine thickness and aperture measures using oculometer, were recorded in at least 30 pollen grains to obtain the minimum and maximum measures to calculate the mean and standard deviation of each item.

For Scanning electron microscope investigation, non-acetolysed pollen grains have been sputtered onto cleaned, Aluminum labeled stubs, coated with 20 nm Gold in a Polaron JFC-1100 coating unit, examined and photographed using JEOL-JSM.I T200 Series Scanning Electron Microscope allocated in the electron microscope unit, Faculty of Science, Alexandria University, Egypt. The terminology used for describing is that of [6].

3. Results

The results obtained didn't show any variations between the flowers stages collected from Cairo or Alexandria. The overall results are summarized in Table 1 and illustrated in figures 4 – 32. All the pollen grains under the light microscope have Ubisch bodies over their surfaces which seem like transparent droplets or unfocused areas (Figures 5, 7, 12, 14 & 17). SEM examinations, the Ubisch bodies appeared as rounded exinous protrusions (Figures 20, 28 & 29). In the first two stages, these Ubisch bodies were dense and decreased considerably in the rest of the stages.

3.1. Pollen density

The density of the pollen grains in first stage as well as the last stage (stage nine) was noticeably less than the rest of the examined stages. The pollen grains were dense in the stages from three to six then decrease gradually in stages seven and eight (Table 1).

3.2. Pollen morphology

Generally the pollen grains are apolar in the spherical forms (stage 1), isopolar, and bilaterally symmetric with small to medium polar axis lengths. In the first stage the radius varied from 14.8 µm to 25.0µm, while the polar axis length increased in all the other stages with the maximum length recorded in stages four and eight as it reaches 32.2 µm. The equatorial axis varied, as well, between the different flower stages with the maximum records were in the third and ninth stages, the first stage is spherical with radius reach (25.0 µm) and the lowest record was in the sixth and seventh stages (14.5 µm). Accordingly the general shape as indicated by P/E varied from the spherical (the 1st. stage, figure 20) to the sub-prolate (the 9th. stage, figures 9, 22 & 25) and mostly the prolate (the rest of the studied stages, figures 23, 24 & 25). The shape of the pollen grains is generally ovate with rounded poles, while elongated pollens with tapering poles can be found in the nearly matured flowers (7th., 8th. & 9th. stages, figures 10, 11, 12, 14 & 19). Generally the length of the polar and equatorial axis differs between the different flower stages as shown in graph 1.

3.3. Amb shape

The amb outline in the pollen grains of the studied flower stages is circular to straight triangular in the 2nd, 8th and 9th (Figures 5, 6 & 8) stages while it is convex triangular in the rest of the stages (Figures 16, 18, 21 & 28).

3.4. Aperture type

The aperture in all the stages is trizonocolpate, except the first stage it is aporate; as no apertures can be seen (Figure 20). Colpi are elliptic long, nearly reach the poles (Figures 22, 23, 24 & 25). It varied from 20 µm to 28 µm within the different stages as illustrated in graph 1. Colpi width is considered narrow; it ranges from 1.2 µm to 1.6 µm with slight variations between the flower stages (Graph 1). The distance between the two colpi; mesocolpi; differs according to the equatorial axis length. It varies from 10.2 µm to 12.8 µm. In the syncolpate apertures, the colpi meet before the poles leaving triangular area at the poles which measured as areas of this triangle. The apocolpi area ranged from 2. 8 µm to 4.8 µm (Figures 21 & 27). Syncolpate pollen grains are recorded in stages 2, 4 and 5, where each two colpi met near the poles (Figures 24, 26 & 28).

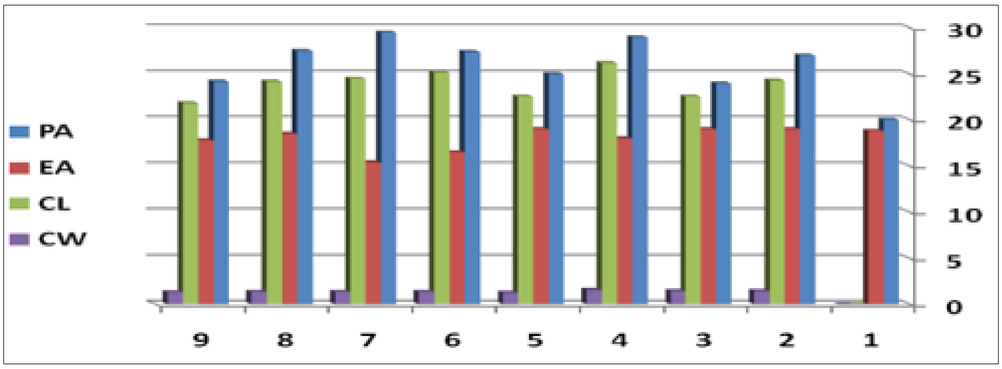
3.5. Exine description

The exine is considered thin and the pollen grains are subjected to deformations according to the process of vacuum for the SEM investigation. The exine thickness ranges from 1.9 µm to 2.7 µm with faintly reticulate ornamentation in the first flower bud stage (Figure 19) and reticulate to micro-reticulate in the rest of the flower stages (Figures 30, 31 & 32). The exine ornamentation was of the same type in both the poles and equators (Figures 21 & 27).

Table 1 The studied pollen morphological characters in the nine *Tecoma stans* flower stages

Stages→ ↓Characters	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Density	+	++	++++	+++	+++	++	++	++	+
Polar axis	14.8-25.0 20.0±5.0	25.2-31.5 27.0±4.5	22.4-27.5 24.0±2.5	24.0-32.0 29.0±3.0	24.0-28.4 25.0±3.0	25.2-31.5 27.4±4.0	25.6-31.2 29.5±1.8)	26.8-32.2 27.5±4.7	22.8-26.4 24.2±2.2
Equatorial axis	14.5-25.0 18.8±7.7	16.5-20.0 19.0±1.0	16.0-21.0 19.0±1.0	15.0-20.0 18.0±2.0	17.0-20.0 19.0±1.0	14.5-18.2 16.5±1.8	14.5-18.6 15.4±3.2	17.2-19.6 18.5±1.1	16.5-21.2 17.8±3.4
P/E	0.98-1.04	1.52-1.55	1.31-1.4	1.6	1.42-1.41	1.73-1.74	1.68-1.77	1.56-1.64	1.25-1.38
Shape	Spherical	Prolate	Prolate	Prolate	Prolate	Prolate	Prolate	Prolate	Subprolate
Amb shape	Circular	Triangular	Convex	Convex	Convex	Convex	Convex	Triangular	Triangular
Aperture type	Aporate	Trizono-colpate	Trizono-colpate	Trizono-colpate	Trizono-colpate	Trizono-colpate	Trizono-colpate	Trizono-colpate	Trizono-colpate
Aperture position	Absent	Zono-colpate	Para-syn-colpate	Zono-colpate	Para-syn-colpate	Para-syn-colpate	Zono-colpate	Zono-colpate	Zono-colpate
Colpus length	0.0	21.8-27.2 24.3±2.9	22.2-24.8 22.5±2.3	21.2-27.5 26.2±1.3	21.8-25.5 22.5±3.0	23.0-27.2 25.2±2.0	23.0-28.2 24.5±3.3	23.5-28.2 24.2±4.0	20.2-23.5 21.8±1.3
Colpus width	0.0	1.4-1.6 1.45±0.15	1.4-1.6 1.45±0.15	1.5-1.6 1.55±0.05	1.2-1.4 1.25±0.15	1.2-1.4 1.35±0.05	1.2-1.4 1.35±0.05	1.2-1.4 1.35±0.05	1.2-1.4 1.30±0.10
Colpus state	Absent	Apo-colpate	Syn-colpate	Apo-colpate	Syn-colpate	Syn-colpate	Apo-colpate	Apo-colpate	Apo-colpate
Apocolpi	Absent	Present	Absent	Present	Absent	Present	Present	Present	Present
Apocolpi area	Absent	2.8-4.4 4.2±0.2	Absent	3.2-4.6 4.2±0.4	Absent	2.8-4.4 4.2±0.2	3.2-4.8 4.4±0.4	2.8-4.8 4.4±0.4	2.8-4.8 4.4±0.4
Meso-colpi Width	Absent	10.2-12.5 11.8±0.7	10.5-11.8 11.2±0.6	10.2-12.5 11.8±0.7	11.2-12.2 11.8±0.4	10.5-12.0 11.2±0.8	10.4-11.0 10.8±0.2	11.0-12.8 12.2±0.6	10.8-12.5 11.8±0.7
Exine thickness	1.9±0.4	2.6 ±0.5	2.5 ±0.5	2.6 ±0.5	2.6 ±0.5	2.6 ±0.5	2.5 ±0.5	2.7 ±0.4	2.7 ±0.5

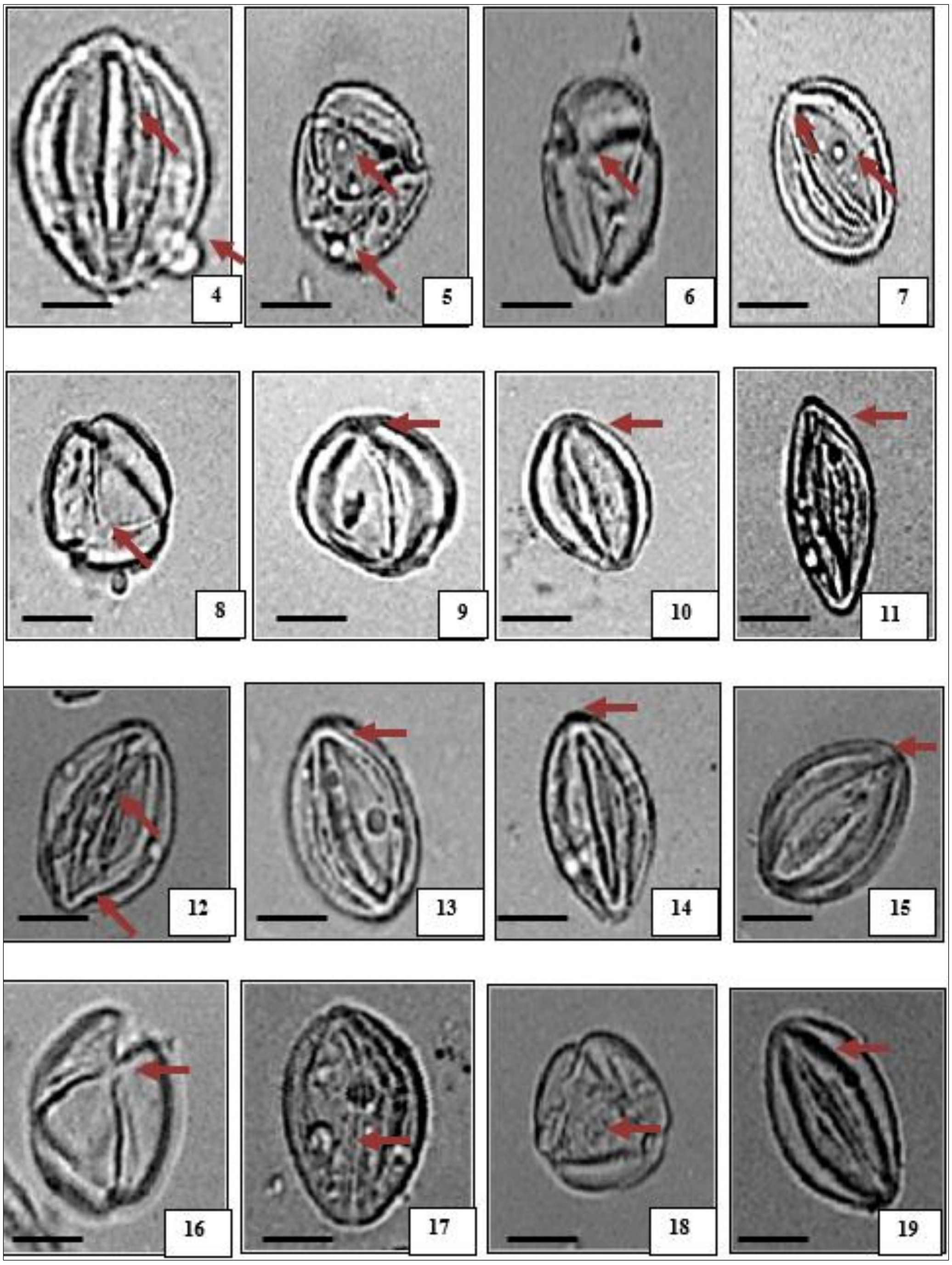
Exine ornamentation	Faint reticulate	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate	Micro Reticulate	Micro Reticulate



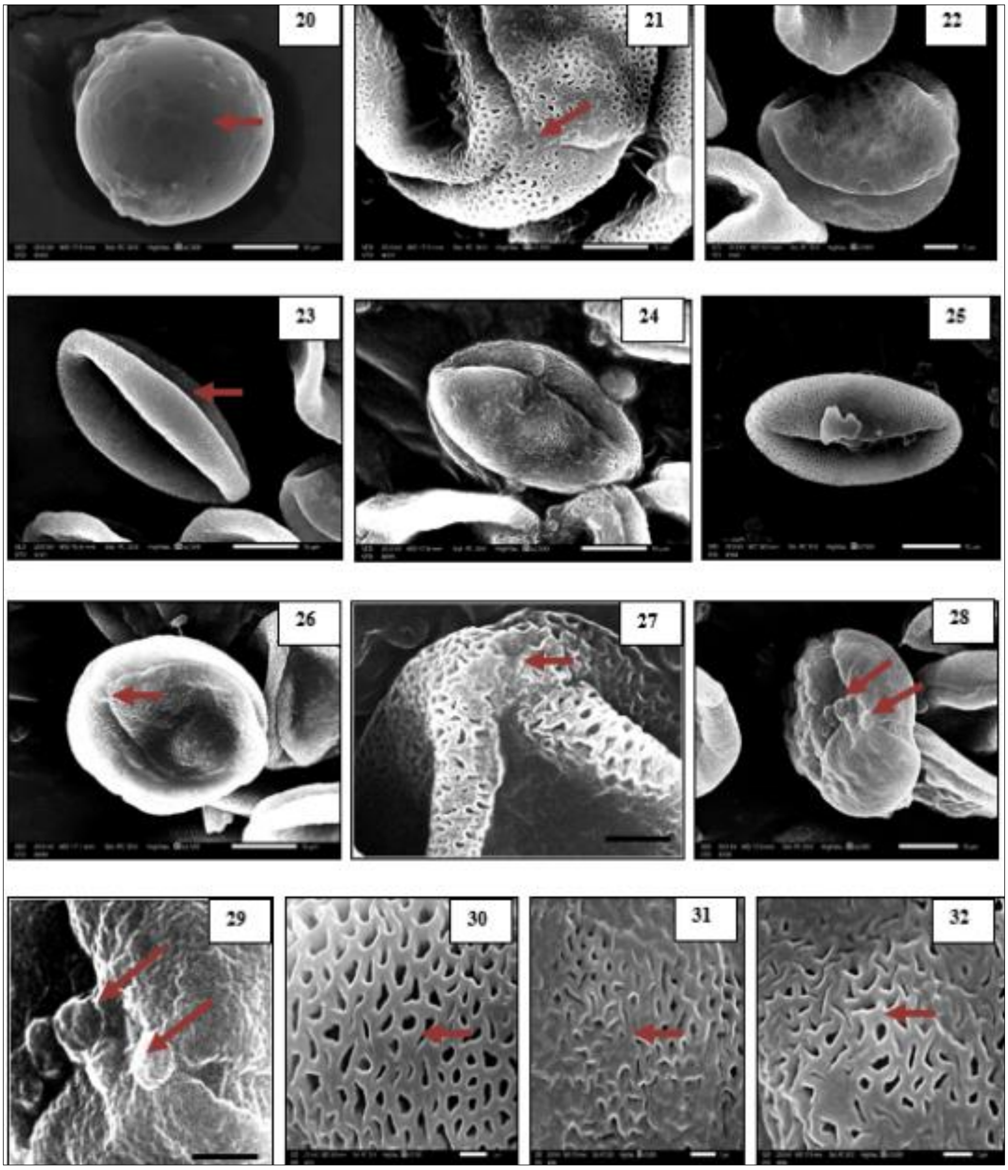
Graph 1 Relation between Polar axis (PA), Equatorial axis (EA) lengths, and Colpi length (CL) and width (CW) within the nine flower stages

		
1	2	3

Figure 1 and 2 Morphology of the *T.s.* flower. **Figure 3** the different stages of the flowers



Figures 4-19 Pollen grains of the nine stages under light microscope. Bar = 5 µm. Figure 4, Equatorial view in (stage 2), arrow indicates to the wide colpi. Figures 5, 6, 8, 16 & 18 Polar views, arrows indicate to Ubisch bodies. Figure 5 stage 2, arrow indicates to apocolpi in 6 & 16, arrows indicate to syncolpi with triangular apocolpi region in 8 & 18 (stages 6 & 7). Figures 7 & 12 Equatorial views, arrows indicate to Ubisch bodies and syncolpi (stages 3 & 4). Figures 9, 10, 11, 12, 13, 14, 15, 17 & 19 Equatorial views showing apocolpi and subprolate shape in (stage 9), Prolate pollen grains with tapering poles in 10, 11, 12, 14 & 19, Prolate pollen grains with rounded poles in 13 & 15, arrows indicate to the rounded poles



Figures 20-32 Pollen grains of the different character under SEM. Bar = 10 µm in 20, 23, 24, 25, 26 & 28. Bar = 5 µm in 20, 21, 26 & 28. Bar = 1 µm in 30, 31 & 32

Figure 20 Spherical pollen grains with faint exine reticulation (first stage). Figures 21 & 27 Polar views showing syncolpate aperture with triangular apocolpi. Figures 22 & 26 Equatorial views showing subprolate pollen grains with long colpi. Figures 23, 24 & 25 Equatorial views for prolate pollen grains with long narrow colpi. Figures 28 & 29 Polar views showing Ubisch bodies. Figures 30, 31 & 32 Part of the exine showing reticulate (30) and microreticulate ornamentation (31 & 32).

4. Discussion

Palynological studies provide a reliable basis for classifying and identifying the origin and evolution of plants since the last century till now [7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19]. [20] pointed out to the importance of studying the variability of pollen characteristics occurred in the same species to understand of the biological diversity and solve problems related to taxonomy. Within the taxonomical studies, pollen morphology considered as an important tool in plant discrimination and classification to solve many taxonomic problems since [1, 21, 8]. Despite of the significant role of pollen studies in taxonomical works, there are many errors mentioned by [2] which taxonomists ignored in their conclusions and interpretation and led to wrong decisions. [22] found that pollen morphology is affected by environmental factors as they exposed to dispersing agents in their developmental state which is affected by their water content. [23] found that the size and shape of the pollen grain can be affected by their hydration state which correlated with the environmental conditions. Concerning the study of the pollen grains in taxonomical decisions, it must keep into consideration all the phenotypical characteristics in the different developmental stages. Many plant species produce pollen grains that are variable in shape; such variability can also be observed in pollens produced by a single flower. Taxonomists must thus be careful to select the proper developmental flower stage when examining pollen characteristics in connection to various tools of plant classification.

In the present study, the pollen grain morphology of nine developmental flower stages from *Tecoma stans* tree was carefully examined. The microscopic observations under the light microscope revealed that the density of the pollen grains in both the first and last flower stages is scarce. The first flower stage has different morphological characters than the rest of the studied stages. They have spherical, inaperturate pollen grains with faint undeveloped exine ornamentation. The inaperturate pollen grains and the presence of Ubisch bodies have been reported before within members of the Bignoniaceae by [24], but it is the first record within *T.s* tree. [25] and [26] described the pollen grains of *T.s.* by being spheroidal with tricolpate aperture and reticulate exine. Our data in partial agreement with them as the spheroidal shape recorded in the immature pollen grains only (1st. stage), while the shape of the eight bigger stages have prolate to subprolate pollen grains with tricolpate aperture and reticulate to micro-reticulate exine. [27] reported some pollen morphological features with more non-viable ones which were not observed in the natural plants, like wrinkled pollens, with abnormally shapes. This observation can explain the completely different pollen characters reported in the first flower stage, as it can be referred to the incomplete developmental process with high percentage of water contents which gave them the spherical shape. [28] explained the difference in pollen shapes by being dehydrated after dehiscence until rehydrated by the stigma exudates, especially in pollen grains with thin exine. [29] recorded five distinct pollen types in *Atriplex* individuals grown in different altitudes and they referred their data to different humidity. Our data agree with this explanation as the studied pollen grains showed different shapes after the dehiscence of the anthers.

The amb polar view shape, with a long tricolpate aperture is reported before by [30] but their measurements are not of the same as the data obtained in this investigation and this may refer to the different altitudes.

5. Conclusion

This investigation revealed that pollen morphological characters are not stable throughout their developmental stages. They differ according to the flowering stage, humidity beside other climatic factors. In taxonomical works, researchers must be precise in choosing the correct mature unexposed flowers to give the correct morphological descriptions. Aperture type is more stable than pollen shape and size.

Compliance with ethical standards

Acknowledgments

The authors are grateful to Mr. Moatamed Mohamed, Electron microscope unit technician, Faculty of science, Alexandria University.

Disclosure of conflict of interest

Authors have declared that no conflict of interests exists.

References

[1] Erdtman G. Pollen Morphology and Plant Taxonomy. Angiosperms. Stockholm: Almqvist & Wiksell; 1952.

[2] Halbritter H, Ulrich S, Grimsson F, Weber M, Zetter R, Hesse M, Buchner R, Svojtka M, Frosch-Radivo A. Misinterpretations in Palynology. Illustrated book terminology; 2018. Pp. 67-84.

[3] Halbritter H. and Hesse M. Sulcus morphology in some monocot families. Grana. 1993; 32 (2): 87-99.

[4] Wood JRI. A revision of *Tecoma* Juss. (Bignoniaceae) in Bolivia. The Linnean Society of London, Environmental Science Botanical Journal of the Linnean Society. 2008; 156: 143–172.

[5] Gentry AH. Bignoniaceae. Part II (Tribe Teco- meae). Flora Neotropica Monograph. 1992; 25: 1-370.

[6] Faegri K. and Iversen J. Textbook of pollen analysis. (4th edn by K. Faegri, P. E. Kaland, K. Krzywinski). Chichester: John Wiley and Sons; 1989. (328 pp).

[7] Wodehouse RP. The phylogenetic value of pollen grain characters. Annals of Botany. 1928; 42: 891–934.

[8] Wodehouse RP. Pollen grains. Their Structure, Identification, and Significance in Science and Medicine. New York: Hafner Publishing Company; 1965.

[9] Taia WK. On the pollen morphology of some Egyptian Caryophyllaceae. Journal of King Saud University. 1994; 6 (2): 149-165.

[10] Taia WK. Palynological studies on the Egyptian species of the Amaranthaceae. The Conference Proceeding of Assiut University. 1996; 13-22.

[11] Taia WK. tribe Vicieae: Evidence from palynology, seed and leaf micro-morphology. Journal of Union of Arab Biologists Cairo 4 (B), Botany. 1997; 285-302.

[12] Taia WK. Palynological Study Within Tribe Trifolieae (Leguminosae). P.J.Bio.Sci. 2004; 7 (7):1303-1315.

[13] Rosenfeldt S. and Galati BG. Pollen morphology of *Oxalis* species from Buenos Aires province (Argentina). Biocell. 2007; 31:13–21.

[14] Fukuda T, Naiki A, Nagamasu H. Pollen morphology of the genus *Skimmia* (Rutaceae) and its taxonomic implications. Journal of Plant Research. 2008; 121:463–71.

[15] Erik BMS. Pollen morphology and its taxonomic significance of the genus *Arabis* (Brassicaceae) in Turkey. Plant Systematics and Evolution. 2012; 298:1931–1946.

[16] Baser B, Firat M, Aziret A. The pollen morphology of *Pelargonium endlicheri- anum* and *Pelargonium quercetorum* (Geraniaceae) in Turkey. PhytoKeys. 2016; 11:153–62.

[17] Taia WK, Ibrahim MM, Abdel-Satar M. Pollen Morphological Variations among some Cultivated *Citrus* species and its Related Genera in Egypt. Jordon Journal of Biological Sciences. 2020; 13(4): 499-508.

[18] Taia WK, Ibrahim MM, Hassan SA, Asker AM. Palynological Study of the genus *Fagonia* L. (Zygophyllaceae) in Libya. Libyan Journal of Science & Technology. 2021; 13 (1): 1-9.

[19] Taia WK, Mahdy RA, Bassiouni EM. Pollen morphological study in some *Bauhinia* L. species and their phylogenetic indications. International Journal of Botany Studies. 2022; 7 (2); 515-526.

[20] Polyakovaa TA. and Gataulina GN. Morphology and Variability of Pollen of the Genus *Spiraea* L. (Rosaceae) in Siberia and the Far East. Contemporary Problems of Ecology. 2008; 1 (4): 420-424.

[21] Nair PKK. A modification in the method of pollen preparation. Ibid. 19c. 1960; (1): 26-27.

[22] Pacini E, Guarnieri M, Nepi M. Pollen carbohydrates and water content during development, presentation, and dispersal: a short review. Protoplasma. 2006; 228: 73–77.

[23] Grant-Jacob JA, Praeger M, Eason RW, Mills B. Generating images of hydrated pollen grains using deep learning. IOP SciNotes 3. 2022; 024001.

[24] Bove CP. Pollen morphology of the Bignoniaceae from a south Brazilian Atlantic forest. Grana. 1993; 32(6): 330-337.

[25] Ragho KS. Role of pollen morphology in taxonomy and detection of adulterations in crud drugs. Journal of Plant Science and Phytopathology. 2020; 4: 024-027.

[26] Pratignya S, Ganneshwar S, Manjusha T, Saisravya T, Umarani K, Rajesh D. Pollen Diversity of Sri Ram Narayan Khedia Government Degree College Banswada, Kamareddy District, Telangana State, India. International Journal of Scientific Research in Science, Engineering and Technology. Online ISSN: 2394-4099, Print ISSN: 2395-1990. 2022; 9 (4): 104-111.

[27] Candan F. and Cali IO. Studies on the comparison of pollen morphology and viability of four naturally distributed and commercial varieties of *Anemone coronaria* L. Pakistan Journal of Botany. 2015; 47(2): 517-522.

[28] Bozic A. and Siber A. Mechanical design of apertures and the infolding of pollen grain. Proceedings of the National Academy of Sciences of the United States of America. 2020; 117 (43): 26600–26607.

[29] Fatmi H, Mâalem S, Harsa B, Dekak A, Chenchouni H. Pollen morphological variability correlates with a large-scale gradient of aridity. Web Ecology. 2020; 20: 19–32.

[30] Maw Kh, Than KN, Yee NN. Pollen Morphology of Some Ornamental Plants in Yadanabon University Campus, Mandala. 3rd Myanmar Korea Conference Research Journal. 2018; 3 (1): 203-210.