



International Journal of Biological and Biomedical Research

Morphological Characteristics of *Solanaceae* Pollen Using Scanning Electron Microscopy (SEM)

Ajeng Putri Widowati¹, Aliffian Nisa' Cholida^{2*}, Mirza Nuryady³

¹⁻³ Master of Biology Education, Postgraduate Program, University of Muhammadiyah Malang, Indonesia

* Corresponding Author: Aliffian Nisa' Cholida

Article Info

E-ISSN: 3107-7137

Volume: 01

Issue: 01

January-February 2025

Received: 19-01-2025

Accepted: 13-02-2025

Page No: 23-28

Abstract

Pollen grains are the male reproductive structures produced by Spermatophyta plants. The family *Solanaceae* is one of the most important plant families that contributes significantly to human needs, including crops such as eggplant, tomato, chili pepper, and others. This study aimed to observe the morphological structure of pollen grains from the *Solanaceae* family. A qualitative descriptive method was employed, with observations conducted using a Scanning Electron Microscope (SEM). The results showed that the average equatorial diameter of pollen grains varied among species, with values of 43.12 μm in *Datura innoxia*, 35.98 μm in *Capsicum annum*, 29.28 μm in *Solanum melongena*, and 32.22 μm in *Solanum lycopersicum*. Pollen grains of the *Solanaceae* family were characterized as tricolporate, and three types of exine ornamentation were identified, namely scabrate, striate, and reticulate.

DOI: <https://doi.org/10.54660/IJBRR.2025.1.1.23-28>

Keywords: *Solanaceae*; pollen morphology; pollen grains; Scanning Electron Microscopy (SEM)

1. Introduction

Indonesia is one of the countries with the highest levels of megabiodiversity in the world, characterized by abundant biological natural resources and an exceptionally rich diversity of plant species that are highly beneficial to human life. Indonesia is home to approximately 25,000–30,000 species of flowering plants, with a significant proportion found on the island of Java. Java alone harbors around 4,600 species of flowering plants (Elfahmi *et al.*, 2014). Many of these plant species are utilized as traditional medicinal plants and as staple food sources, including members of the *Solanaceae* family. *Solanaceae* is a family of flowering plants belonging to the order Solanales (Eriawati, 2015). This family plays a crucial role in meeting human needs and is not limited to vegetables and fruits alone (Krisnawati & Febrianti, 2019). Several members of the *Solanaceae* family, such as potato, chili pepper, tomato, eggplant, and black nightshade (*Solanum nigrum*), are major components of human food production in many parts of the world (Hasanudin, 2018). In addition to their use as food sources, plants from the *Solanaceae* family are also utilized as ornamental plants, such as petunia, as well as medicinal plants, narcotics, anesthetic agents, and poisons (Setshogo, 2015). The diverse functions of plants as medicinal resources and staple foods may influence variations in pollination. Pollination variability can be affected by pollinators, environmental conditions, and the techniques or types of pollination involved (Azka, 2022; Budianto & Sukendah, 2023; Sari & Astija, 2020). Flower corollas play an important role in attracting insects such as butterflies, beetles, and bees to obtain nectar, thereby facilitating the pollination process in various plant species (Ramdhini *et al.*, 2018). Environmental factors that influence pollination variability include temperature, air humidity, and the duration of sunlight exposure (Sobari *et al.*, 2019). Different types of pollination that may affect pollination variability include self-pollination (autogamy), pollination between neighboring flowers (geitonogamy), and cross-pollination (allogamy) (Sari & Astija, 2020). Each plant produces secondary metabolites, which perform diverse functions depending on the species. In plants, secondary metabolites act as attractants for other organisms, plant growth regulators, defense mechanisms against pathogens, protection against ultraviolet radiation, and agents for protection and adaptation to environmental stress, as well as for competition with other plants through allelopathy (Dalimunthe & Rachmawan, 2017). One of the key roles of secondary metabolites is their function as attractants that draw pollinating organisms, such as insects, thereby facilitating the pollination

morphology, and seed quality, which ultimately affect seed viability for subsequent plant generations (Maintang & Nurdin, 2013; Sobari *et al.*, 2019).

Pollen grains are essential biological materials used in the observation of pollen morphology. Pollen, or pollen grains, are the male reproductive structures produced by Spermatophyta, originating from both Gymnosperms and Angiosperms (Azizah *et al.*, 2016). Pollen consists of coarse powder-like particles that contain the male microgametophytes responsible for producing male gametes (sperm cells) (Sudarmono & Sahromi, 2012). Pollen grains are extremely small, granular structures produced in large quantities by flowering plants, functioning as the male reproductive units in plant reproduction (Umami *et al.*, 2021). Several morphological characteristics of pollen can be used for identification purposes, including size, shape, wall structure, stratification, exine ornamentation (sculpturing), surface folds, and aperture type (Gusmalawati, 2021). Therefore, pollen morphology serves as a reliable reference for plant identification.

Based on the study conducted by Perveen (2007) which examined the pollen morphology of 20 species from the *Solanaceae* family in Pakistan using a Scanning Electron Microscope (SEM), various exine characteristics were identified. The pollen grains were described as radially symmetrical, isopolar, and ranging in shape from prolate-spheroidal to oblate-spheroidal, including sub-oblate to sub-prolate forms. The pollen type was predominantly tricolporate, with colpi generally exhibiting costae. The colpal membranes ranged from psilate to sparsely or densely granulate, while the ora were lalongate. The sexine layer was reported to be equal in thickness to the nexine, or slightly thicker or thinner. Furthermore, Adedeji (2015) investigated pollen morphology for systematic purposes in several *Solanaceae* species at Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The study identified various pollen types, including acolpate, monocolpate, bicolpate, and tricolporate pollen. The pollen grain sizes were classified into the minuta group (10–25 µm in diameter) and the media group (25–50 µm in diameter). Based on these findings, research on pollen morphological identification is essential due to its potential application in plant taxonomy. This consideration forms the basis of the present study, which focuses on the pollen morphology of selected *Solanaceae* species, namely *Datura* (kecubung), tomato, chili pepper, and eggplant, commonly used as medicinal plants and staple food crops. The morphological structures of pollen grains were observed using Scanning Electron Microscopy (SEM). Therefore, the objective of this study was to determine the pollen morphological characteristics of several species within the *Solanaceae* family using Scanning Electron Microscopy (SEM).

2. Methodology

This study employed a qualitative descriptive research design. The research was conducted at the Biology Laboratory, Department of Biology, Universitas Muhammadiyah Malang, on April 5, 2024. The instruments used in this study included a Scanning Electron Microscope (SEM), a computer unit, vacuum pump, forceps, scissors, specimen holder, carbon tape, holder height gauge, and a blower. The materials used were pollen grains collected from the flowers of *Datura innoxia*, *Capsicum annum*, *Solanum melongena*, and *Solanum lycopersicum*, the pollen samples

were collected from Malang City and its surrounding areas. Sampling was conducted using a random sampling technique, as no specific criteria were applied for sample selection. Flower samples were collected and transported to the biology laboratory for further analysis.

Pollen observation was carried out using Scanning Electron Microscopy (SEM). Prior to observation, the samples were prepared by attaching pollen grains (*Datura innoxia*, *Capsicum annum*, *Solanum melongena*, and *Solanum lycopersicum*) onto carbon tape mounted on a specimen holder. The height of the specimen holder was measured using a holder height gauge, and the pollen grains were ensured to be firmly attached to the carbon tape to prevent detachment during the vacuum process inside the SEM chamber. The EVAC/AIR button was activated and allowed to run until the green LED indicator stopped blinking. Subsequently, the specimen holder containing the samples was placed into the SEM chamber (Hoten, 2020). The observation process was initiated by clicking the “start” button on the SEM software and terminated by clicking the “stop” button upon completion. Pollen morphological characteristics were observed at magnifications of 300×, 500×, 600×, 800×, 2,500×, 3,000×, and 4,000×. The observed morphological parameters included pollen size, exine ornamentation type, pollen type, and pollen shape, examined in both polar and equatorial views. The observed morphological features were then compared with relevant literature and pollen identification references.

3. Results

The study of pollen morphology is commonly referred to as palynology. Palynology is the scientific discipline that studies pollen and spores, both in living conditions and as fossils (Kumaladita, 2014). The palynological approach applied in this study is actuopalynology, which utilizes pollen obtained from fresh flowers as the observation material (Nugroho & Abdullah, 2021). Pollen, or pollen grains, are floral structures in the form of sacs that contain the male gametophyte in flowering plants, including both angiosperms and gymnosperms (Bria, 2017). Pollen and spores originate from plants growing in specific habitats and environments and are capable of forming particular vegetation types; therefore, they can be used to reconstruct surrounding vegetation. In addition, palynological evidence represents plant assemblages that reflect vegetation patterns and their associated environmental conditions (Widodo *et al.*, 2012). Pollen and spores are useful for plant identification because they possess an exine layer with distinctive structures and ornamentation. This layer is well preserved due to the presence of sporopollenin, a compound that is resistant to extreme environmental conditions (Azizah *et al.*, 2016). Pollen grains exhibit diverse sizes, shapes, and patterns of surface ornamentation, allowing variations in pollen size and form even among flowers of the same species (Sobari *et al.*, 2019). In general, pollen observations focus on characteristics such as pollen size and shape, aperture type or pollen type, polarity and symmetry, and exine ornamentation. These pollen characteristics can be used as indicators of phylogenetic relationships among taxa and are frequently applied as references in plant classification and identification (Qodriyyah & Suedy, 2016). Palynology can also be used as a reference for understanding past climatic conditions and serves as a basis for reconstructing environmental vegetation,

climate, floral history, conservation efforts, and natural disaster mitigation (Sarah *et al.*, 2017).

Table 1: Results of Solanaceae pollen observations

No.	Species Name	Equatorial view (μm)	Tipe Ornamentasi Eksin	Pollen Type	Spore and Pollen Shape	
					Polar view	Equatorial view
1.	<i>Datura innoxia</i>	1. 42,1 2. 46,1 3. 42,6 4. 42,0 5. 42,8	Striate dan reticulate	Tricolporate	Circular	Circular oval
2.	<i>Capsicum annuum</i>	1. 35,6 2. 36,1 3. 37,5 4. 35,3 5. 35,4	Scabrate	Tricolporate	Circular	Circular
3.	<i>Solanum melongena</i>	1. 28,7 2. 31,6 3. 35,0 4. 26,2 5. 24,9	Scabrate	Tricolporate	Circular	Circular
4.	<i>Solanum lycopersicum</i>	1. 28,6 2. 39,3 3. 28,7 4. 32,4 5. 32,1	Scabrate	Tricolporate	Circular	Circular oval

Based on the observation results, *Datura innoxia* pollen observed from the equatorial view had an average pollen size of 43.12 μm . This average value was obtained from measurements of five *Datura innoxia* pollen grains based on the equatorial view, with sizes of 42.1 μm , 46.1 μm , 42.6 μm , 42.0 μm , and 42.8 μm . This result is consistent with the study conducted by Gavrilova *et al.*, (2018), which reported the average pollen size of *Datura innoxia*.

Based on the equatorial view, the pollen size ranged from 35.6 to 45.9 μm . The observation results showed that *Capsicum annuum* pollen observed from the equatorial view had an average pollen size of 35.98 μm . This average value was obtained from measurements of five *Capsicum annuum* pollen grains with sizes of 35.6 μm , 36.1 μm , 37.5 μm , 35.3 μm , and 35.4 μm . However, the average pollen size of *Capsicum annuum* based on the equatorial view reported in previous studies ranged from 30 to 32.50 μm . This difference may occur due to variations in pollen size, which can be influenced by external factors such as temperature, mineral availability, and soil water supply where the plant grows, as well as internal factors including chromosome number, floral characteristics, and water conditions within the plant (Zahrina *et al.*, 2017). In addition, another possible factor contributing to pollen size variation is the growing location, because Ashfaq *et al.*, (2020) conducted their study on *Capsicum annuum* pollen in Pakistan, whereas the present observation used pollen samples collected from the Malang region. According to Lelono, (2007) pollen zonation in a particular area can differ from that of other regions.

Based on the observation results, *Solanum melongena* pollen observed from the equatorial view had an average pollen size of 29.28 μm . This average value was obtained from measurements of five *Solanum melongena* pollen grains based on the equatorial view, with sizes of 28.7 μm , 31.6 μm , 35.0 μm , 26.2 μm , and 24.9 μm . This result is consistent with the study conducted by Chatri & Yesti, (2012) which reported that the average pollen size of *Solanum melongena* based on the equatorial view ranges from 27.5 to 30 μm .

Based on the observation results, *Solanum lycopersicum* pollen observed from the equatorial view had an average pollen size of 32.22 μm . This average value was obtained from measurements of five *Solanum lycopersicum* pollen grains based on the equatorial view, with sizes of 28.6 μm , 39.3 μm , 28.7 μm , 32.4 μm , and 32.1 μm . In contrast, a study conducted by Muhammed (2022) reported that the average pollen size of *Solanum lycopersicum* based on the equatorial view ranged from 18.2 to 23.9 μm . This difference may occur because variations in pollen size can be influenced by differences in pollen maturity levels (Sanjaya *et al.*, 2020). In addition, another possible factor contributing to pollen size variation is the growing location, because Muhammed, Fadil., (2022) conducted their study on *Solanum lycopersicum* pollen in Iraq, whereas the present observation used pollen collected from the Malang region. According to (Anis *et al.*, 2021) pollen variation is associated with the geographical region in which the pollen is found.

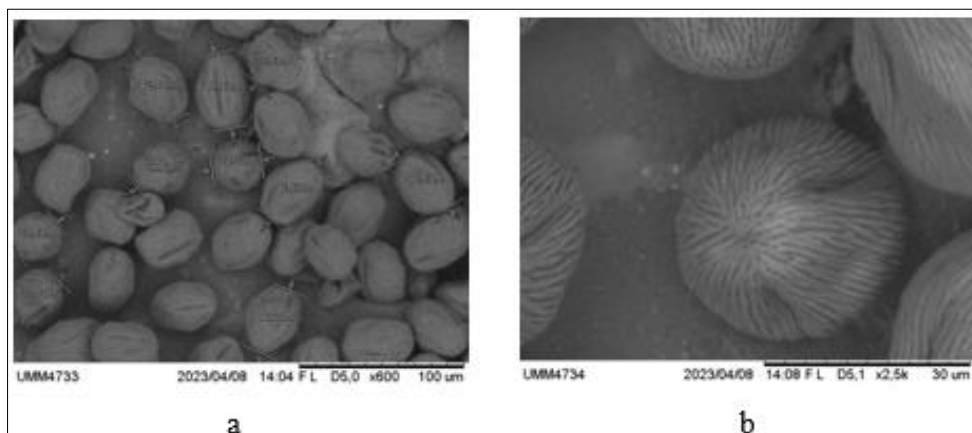


Fig 1: *Datura innoxia* (a) polar view and equatorial view (600×), (b) exine ornamentation type and pollen type (2.500×)

The exine ornamentation types of *Datura innoxia* pollen were striate and reticulate. This finding is consistent with the study conducted by Dhanya & Devipriya, (2016) which reported that the exine ornamentation of *Datura innoxia* pollen is striate and reticulate. In addition, the pollen type of *Datura innoxia* was identified as tricolporate. The pollen shape

observed in the polar view was circular, while in the equatorial view it was circular-oval. These results are in agreement with the findings of Song, Y & Liu, (2018), which stated that *Datura innoxia* pollen is tricolporate and exhibits a circular shape in both polar and equatorial views.

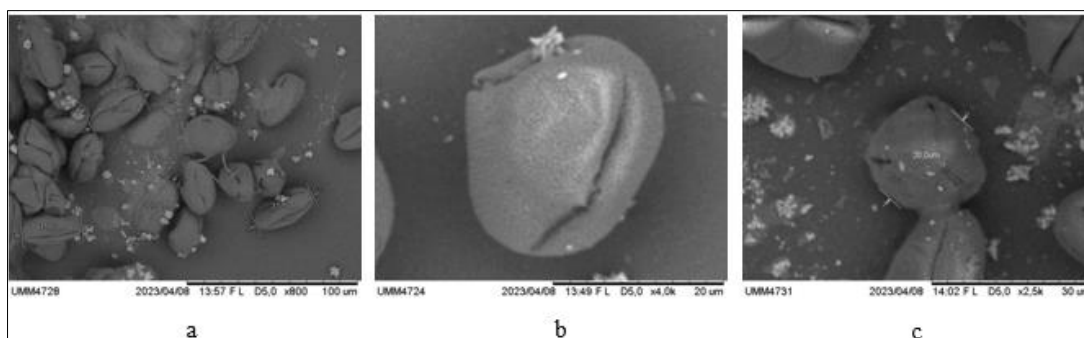


Fig 2: *Capsicum annuum* (a) equatorial view pollen shape (800×), (b) exine ornamentation type (4,000×), (c) pollen type (2.500×).

The exine ornamentation type of *Capsicum annuum* pollen was scabrate, with a tricolporate pollen type. The pollen grains exhibited a circular shape when observed in both polar and equatorial views. These findings are consistent with the

study conducted by Ashfaq *et al.*, (2020), which reported that *Capsicum annuum* pollen has scabrate exine ornamentation, a tricolporate pollen type, and a circular shape in both polar and equatorial views.

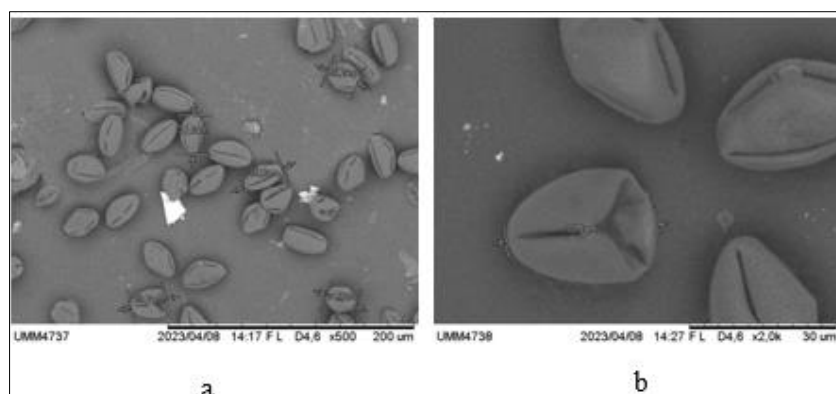


Fig 3: *Solanum melongena* (a) equatorial view (500×), (b) exine ornamentation type and pollen type (2.000×)

The exine ornamentation type of *Solanum melongena* pollen was scabrate. This finding is consistent with the study conducted by Pariat *et al.*, (2010) which reported that the exine ornamentation of *Solanum melongena* pollen is scabrate. In addition, the pollen type of *Solanum melongena* was identified as tricolporate, and the pollen shape observed

in both polar and equatorial views was circular. These results are in accordance with the findings of mahdy *et al.*, (2019) which stated that *Solanum melongena* pollen is tricolporate and exhibits a circular shape in both polar and equatorial views.

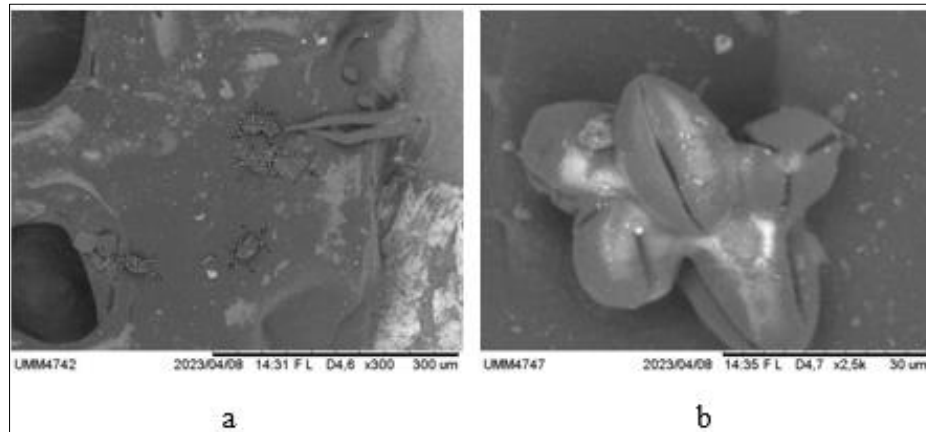


Fig 4: *Solanum lycopersicum* (a) equatorial view (500 \times), (b) exine ornamentation type and pollen type (2.000 \times)

The exine ornamentation type of *Solanum lycopersicum* pollen was scabrate, with a tricolporate pollen type. The pollen grains exhibited a circular shape when observed from both polar and equatorial views. These findings are consistent with the study reported by Roubik (2018), which described the pollen of *Capsicum annuum* as having scabrate exine ornamentation, a tricolporate pollen type, and a circular shape in both polar and equatorial views.

4. Conclusion

Pollen structural observations were conducted using Scanning Electron Microscopy (SEM). Based on the results, the pollen size range of the *Solanaceae* family varied from approximately $\pm 24.9 \mu\text{m}$ to $\pm 46.1 \mu\text{m}$. The observed exine ornamentation types included striate, reticulate, and scabrate patterns. The pollen grains were classified as tricolporate. In equatorial view, the pollen shape was predominantly circular, while in polar view, the pollen grains exhibited circular and circular-oval shapes.

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