

Pollen morphology of *Fabaceae* from the lower Casamance forest, Senegal

DIATTA Kady^{*1,2}, DIATTA William¹, MBAYE Amadou Ibrahima¹, SARR Abdou¹,

DIENG Serigne Ibra Mbacké¹, KABOU Marie Léa¹ and FALL Alioune Dior¹

¹Laboratory of Pharmacognosy and Botanical, Cheikh Anta Diop University (UCAD), Senegal

²International Laboratory Research 3189 (ILR 3189) « Environment, Health, Society » CNRS-UCAD-CNRST-USTTB-UGB, Dakar, Senegal

Citation: DIATTA Kady, DIATTA William, MBAYE Amadou Ibrahima, SARR Abdou, DIENG Serigne Ibra Mbacké, KABOU Marie Léa and FALL Alioune Dior (2025). Pollen morphology of *Fabaceae* from the lower Casamance forest, Senegal. *Plant Science Archives*.

DOI: <https://doi.org/10.51470/PSA.2025.10.4.28>

Corresponding Author: **DIATTA Kady** | E-Mail: (khadydiose@yahoo.fr)

Received 25 July 2025 | Revised 22 August 2025 | Accepted 23 September 2025 | Available Online 19 October 2025

ABSTRACT

Biologists have long been fascinated by the study of pollen grains. They have used their great intra- and interspecific diversity as an indicator to reconstitute and interpret ancient and modern plants and environmental conditions. Objective: This study aimed to investigate Palynological research of thirteen (13) *Fabaceae* species (*Afzelia africana* Sm. & Pers, *Caesalpinia pulcherrima* (L.) Sw, *Cassia sieberiana* DC, *Daniellia oliveri* (Rolfe) Hutch. & Dalziel, *Detarium senegalensis* J. Gmelin, *Dialium guineensis* Willd, *Faidherbia albida* Del.Chev, *Parkia biglobosa* Jacq.Benth, *Pterocarpus erinaceus* Poir, *Senna alata* L, *Senna occidentalis* (L.) Link, *Senna siamea* (Lam) and *Tamarindus indica* L) which occur in the lower Casamance forest (Senegal). Pollen grains were acetolyzed and observed both qualitatively and quantitatively through light microscopy. The results showed that *Fabaceae* are eurypalynous, dispersed in monads, subcircular polyads, subspherical and subtriangular forms, reticulate, tricolporate apertures. The polar axis, equatorial diameter of the pollen grains, colpus length and width were measured. The smallest pollen grain size was found in *Dialium guineense* (16.00 x 18.00 μ m), while the largest pollen size was found in *Parkia biglobosa* (94.00 x 94.00 μ m). The ornamentation of the exine is thick reticulate, fine, scabrous, pseudoreticulate, cracked ectexine, reticulate with granular cytoplasm, striato reticulate, reticulate with large apertures, and fine exine with thick striations. Although pollen characteristics within the *Fabaceae* family are generally similar, variations in the apertures and surface ornamentation of pollen grains provide useful criteria for distinguishing between species.

Keywords: *Fabaceae* species, Pollen morphology, ornamentation, Casamance, Senegal.

INTRODUCTION

The *Fabaceae* family comprises herbaceous, shrubby, arboreal, and climbing plants with a cosmopolitan distribution. *Fabaceae* is the third-largest family of flowering plants. *Fabaceae* includes three subfamilies: Mimosoideae, Caesalpinioideae, and Faboideae. It includes approximately 751 genera and 19,500 species, making it the third-largest family of angiosperms. This family holds significant ecological and economic importance [1]. The *Fabaceae* family ranks among the most economically significant plant groups worldwide. Its importance stems from the wide range of uses humans have derived from it over time, including carbon sequestration, mitigation of greenhouse gas emissions, biodiversity conservation, diversification of livestock feed, provision of flowers for pollinators, and shade for grazing animals [2]. In Senegal, the flora comprises approximately 3,645 plant species distributed among 1,277 genera, including 2,504 flowering plants belonging to 165 families [3]. Among these families, legumes occupy a particularly important place, with about 330 species divided into 94 genera, representing nearly 16% of the country's total flora [3].

This family therefore represents around 12 to 13% of the country's vascular flora, demonstrating its ecological and floristic importance in the country's various ecosystems. Floristic inventories carried out at the local level confirm this predominance. *Fabaceae* constitute approximately 23% of the woody species in Hann Park in Dakar [4] and nearly 19% of those recorded at the Michel Adanson Botanical Conservatory in

Mbour [5]. *Fabaceae* pollen grains can exhibit a wide range of features, including monads, tetrads, ditetrads, or polyads, as well as various aperture patterns and exine configurations such as psilate, granulate, foveolate, rugulate, and many others [6, 7, 8]. Pollen analysis, or palynology, offers valuable insights that support both food security and sustainable development. The morphological features of plant pollen grains exhibit distinct variations that are stably inherited across generations [9]. Since these morphological traits are genetically determined and minimally influenced by external environmental factors, pollen morphology plays a crucial role in plant taxonomy, evolutionary studies, and species identification.

Pollen morphology represents the basis of applied palynological studies, providing essential tools and knowledge for meaningful research and valuable information for various scientific fields [10]. The identification and classification of pollen grains according to their morphological characteristics remain essential skills for any practical application of palynology [11,12,13]. Pollen grains are distinguished by notable morphological traits found in their exine, which serve as key criteria for comparative analyses among flowering plants [14]. Exine ornamentation, in particular, represents an important taxonomic characteristic for species identification. This feature is typically highly distinctive and consistent within taxa [15], thereby aiding in the differentiation of species [16]. In Senegal, pioneer Kady Diatta, who cataloged the pollen flora of Casamance, made a significant contribution.

This research aimed to identify the morphological characteristics of pollen from 13 *Fabaceae* specimens collected in the lower Casamance forest. The results will contribute to their taxonomic classification and to knowledge of the local pollen flora. In fine, this study demonstrated that new observations continue to refine and deepen our understanding, and highlights the need for broader sampling to capture the full extent of pollen diversity in the genus [17].

MATERIAL AND METHODS

We studied the pollen grains of 13 species, belonging to the *Fabaceae* family in the Casamance forest, Senegal. The botanical material was collected from the flowers and placed in paper envelopes for proper preservation. The plant specimens were identified using Berhaut flora as well as online resources such as the Royal Botanic Gardens.

The Wodehouse method (1935) [18] was used to prepare the reference pollen slides. Flowers of all species in bloom were collected around the apiaries in the study areas. Fresh pollen from these different species was placed in a drop of distilled water directly onto a microscope slide, labeled with the date of preparation, species name and degree of gelatine staining. The slide was then placed on a histological stage to evaporate the water. The grains were then degreased with ether to remove the

'pollen coat', formed of lipid substances, so that the characteristics of the envelope or sporoderm could be observed correctly. One or two drops of Kaiser's glycerol gelatin, coloured with Ziehl's fuchsin and previously liquefied in a water bath at approximately 40°C, are placed on the dry, degreased pollen grains. This inclusion liquid is immediately covered with a slide cover glass. These slides are dried, sealed with varnish and then examined under a microscope.

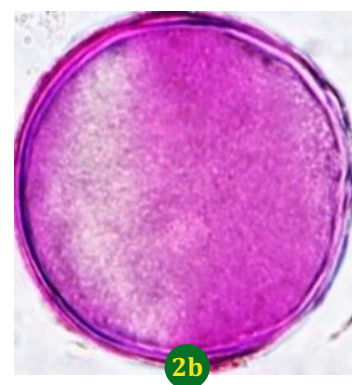
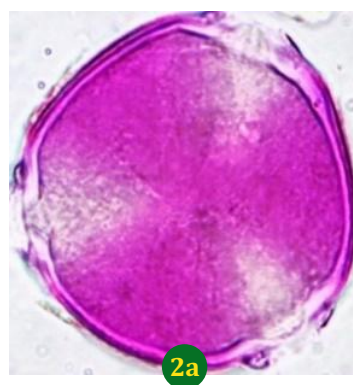
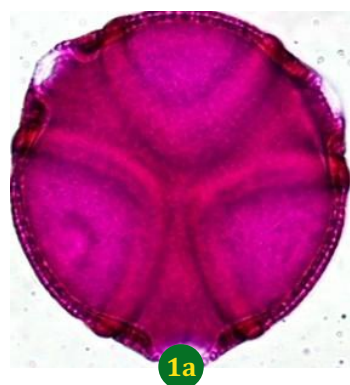
The preparations are observed using a Zeiss research microscope (Axioscope type) allowing for the adjustment of the bright field in transmitted light according to KÖHLER, using 20 to 40 objectives for species inventories, 50 for counts and 100 for studies of stratification and exine structure. The recognition and identification of pollen grains was carried out based on the palynological expertise acquired in the context of the AOC-AOP certification of Corsican honeys from Battesti. Approximately 20–25 pollen grains were observed on each slide to measure all quantitative characteristics of the pollen grains.

RESULTS

In present work, pollen morphology of 13 species, representing 11 genera and 1 family, was studied. These species are listed in Table 1. The latter contains scientific names, size range, symmetry and form, apertures and exine ornamentation.

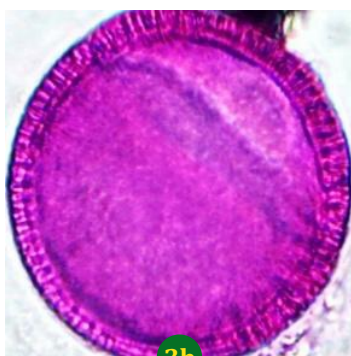
Table 1. Morphological characteristics of pollen grains in selected *Fabaceae* species

Scientific names (Families)	Size range (µm)	Symmetry and Form	Apertures	Exine ornamentation
<i>Caesalpinia pulcherrima</i> (Fabaceae)	E=P= 70 µm	circular isopole	syncolped triaperture	thick, reticulated
<i>Daniellia oliveri</i> (R.) Hutch. et Dalz. (Fabaceae)	E=62µm, P=38µm	Subcircular isopole	tricolporate	fine, scabrous
<i>Detarium senegalensis</i> J. Gmelin. (Fabaceae)	E=P= 38µm	subcircular isopole	tricolporate	pseudoreticulum
<i>Parkia biglobosa</i> Jacq. Benth (Fabaceae)	E=P= 94 µm	Subcircular polyad	32 monads	Cracked ectexine
<i>Senna siamea</i> (Lam.) H.S. Irwin & Barneby (Fabaceae)	E=P= 50µm	subtriangular isopole	tricolporate	thick reticulated
<i>Senna alata</i> L. (Fabaceae)	E=P= 32µm	subtriangular isopole	tricolporate	fine, scabrous
<i>Senna occidentalis</i> (Fabaceae)	E=P= 36µm	subtriangular isopole	tricolporate	Thick, scabrous
<i>Cassia sieberiana</i> DC. (Fabaceae)	E=P= 28µm	subtriangular isopole	tricolporate	reticulated granular cytoplasm
<i>Dialium guineensis</i> Willd. (Fabaceae)	E=16µm P= 18µm	subtriangular isopole	tricolporate	reticulate striato
<i>Tamarindus indica</i> L. (Fabaceae)	E=P= 36µm	subtriangular isopole	tricolporate	reticulate striato
<i>Afzelia africana</i> Sm. & Pers. (Fabaceae)	E=P= 56µm	isopolar, subtriangular, heteroapertured, 6 lobes grouped in 3 pairs	tricolporate	reticulated with large meshes
<i>Faidherbia albida</i> Del. Chev. (Fabaceae)	E=P= 92 µm	subcircular polyad	32 monads	fine
<i>Pterocarpus erinaceus</i> Poir. (Fabaceae)	E=P= 26µm	subtriangular isopole	tricolporate	thick striato





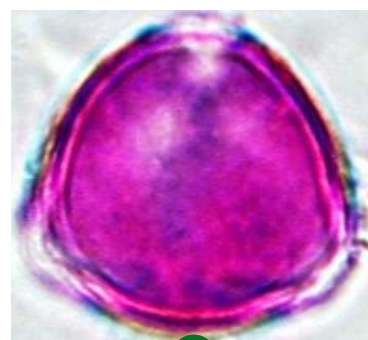
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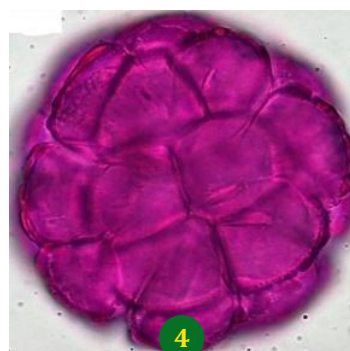
3b



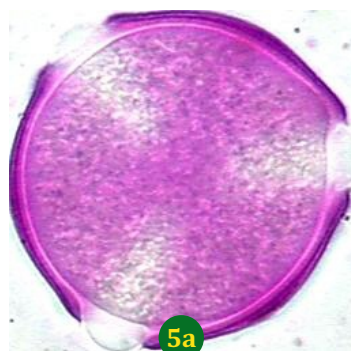
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9a



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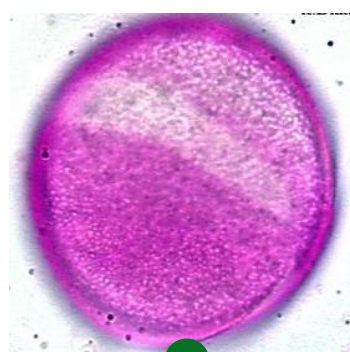
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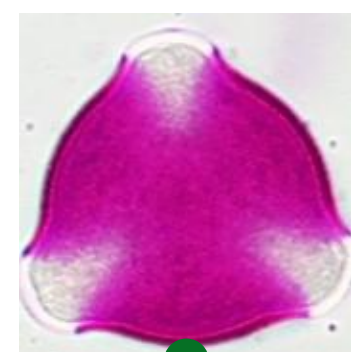
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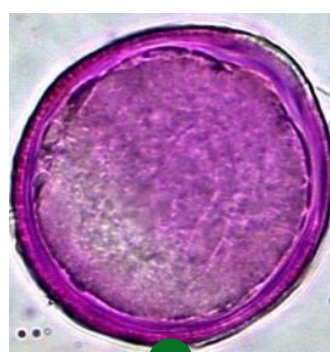
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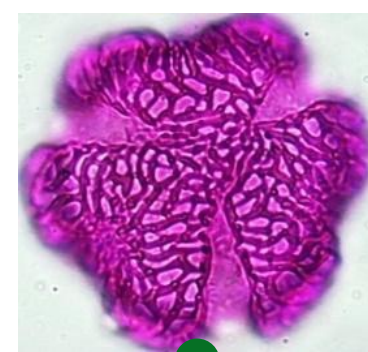
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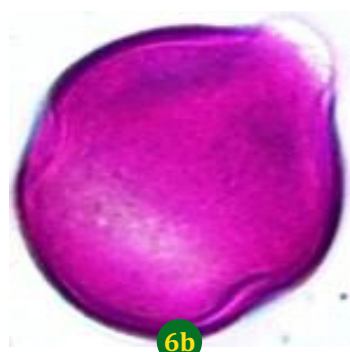
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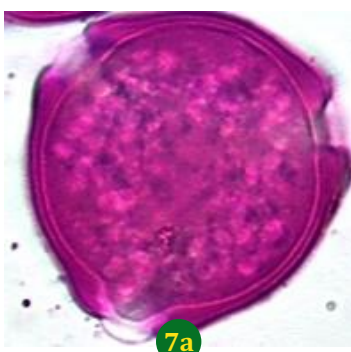
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11a



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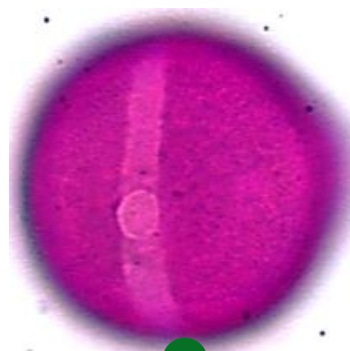
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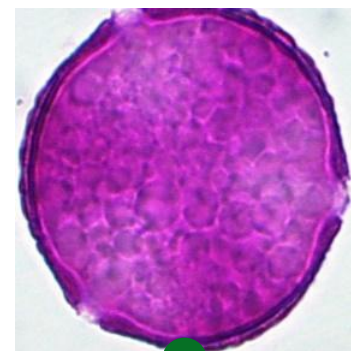
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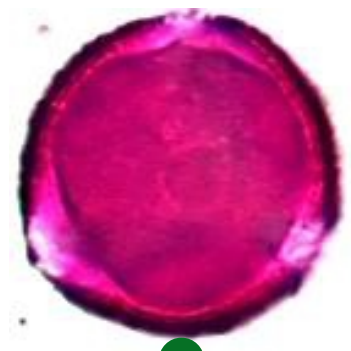
12



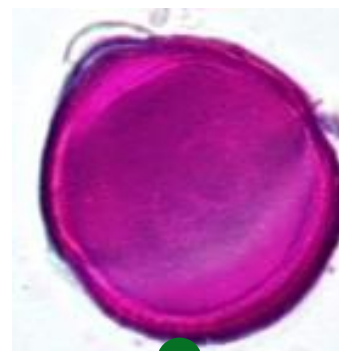
7b



8a



13a



13b

Figure 1. Microscopic pollen taken at 10 µm from different species of Fabaceae. a : Polar view and **b :** equatorial view. 1 : *Caesalpinia pulcherrima* ; 2 : *Daniellia oliveri* ; 3 : *Detarium senegalense* ; 4 : *Parkia biglobosa* ; 5 : *Senna alata* ; 6 : *Senna alata* ; 7 : *Senna occidentalis* ; 8 : *Cassia sieberiana* ; 9 : *Dialium guineense* ; 10 : *Tamarindus indica* ; 11 : *Afzelia africana* ; 12 : *Faidherbia albida* ; 13 : *Pterocarpus erinaceus*

Discussion

Several researchers have employed pollen characteristics to delimit or distinguish species and varieties across different taxonomic groups. For instance, [19] analyzed variations in pollen morphology and their taxonomic significance among *Strobilanthes* species in Sumatra. Similarly, [20] investigated the effects of elevated temperatures on pollen viability, photosynthetic and enzymatic activities, and their subsequent impact on the yield of cowpea cultivars. The morphology of pollen grains represents an important diagnostic tool for taxonomists and breeders to distinguish and identify the possible differences between cultivars within species [21]. While studying pollen morphology, four parameters, namely, pollen size, shape, aperture and exine ornamentation, were taken into consideration. The length (polar diameter) and width (equatorial diameter) exhibited significant differences between the accessions (Table 1) and ranged from 16 to 94 µm [21]. [22] reported the pollen grains of the *Fabaceae* family measured 13,26 to 40,85 µm in length and 15,3 to 43,3 µm in width. Our results were in accordance with these values. [23] reported in the *Arecaceae* family that the variation in length and width observed ranged from 55.06 -71.25 µm and 24.38 -36.27 µm amongst the accessions. The majority of pollen grains of our species have subcircular and isopolar shapes and symmetries, except for the species *C. pulcherrima*, which is isopolar and circular. Our results were in accordance with [22] values. Tricolporate apertures of the pollens noted in *Daniellia oliveri*, *Detarium senegalense*, *Senna siamea*, *Senna alata*, *Senna occidentalis*, *Cassia sieberiana*, *Dialium guineense*, *Tamarindus indica*, *Afzelia africana* and *Pterocarpus erinaceus*. However, *Caesalpinia pulcherrima* and *Faidherbia albida* have 32 monads. One of the most consistent features in *Fabaceae* is the presence of tricolporate pollen grains [24,25]. In Papilionoideae and Caesalpinioideae, the grains are predominantly isopolar tricolporate, subprolate to spheroid in shape, with a finely reticulate exine [24,26]. However, some variability is observed within the *Mimosoideae*, where pollen grains are often grouped into polyads and may have triporate or multiporate apertures [27]. These particular characteristics are considered to be adaptations to wind or insect pollination specific to this group. [28] confirmed in these studies that pollen grains species were tricolporate. The sizes of pollens are small, medium and large. The smallest pollen was found in *Dialium guineensis* and largest pollen observed in *Parkia biglobosa*. [29] confirmed these sizes in the *Fabaceae* family. In another study of *Fabaceae*, the authors found small and medium sizes [30]. The ornamentation of the exines was diverse and included reticulated, scabrous, fine and Cracked ectexine. [30] found same ornamentation of the pollen's exine. According to [31], the structure and ornamentation of the exine represent features of high diagnostic significance in pollen analysis. However, they also emphasized that other morphological attributes can be equally, or even more, important in the accurate identification and classification of pollen grains.

These pollen characters serve as important tools for the identification and classification of taxa. All of these distinctive pollen features are undoubtedly valuable and will contribute to future taxonomic studies. Furthermore, this research provides comprehensive knowledge of *Fabaceae* pollen morphology to botanists and other scientists with an interest in this field.

Conclusion

Thirteen species of the *Fabaceae* family were examined palynologically to identify their distinctive features at both the species and genus levels. The study primarily focused on the shapes and detailed surface ornamentation of pollen grains, intending to assess how morphological variations could reveal unique characteristics of *Fabaceae* taxa. Analyses were conducted using both light microscopy (LM) to ensure observation of pollen morphology. The primary aim of this study was to assess the taxonomic relationships among various *Fabaceae* using pollen morphological data. This approach aimed to illustrate the potential of pollen grains as reliable taxonomic indicators. By identifying and analyzing variations in pollen characteristics, the research contributes to the differentiation of closely related species within the family.

Disclaimer (artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of this manuscript.

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