BioOne COMPLETE

Ultrastructural Observations of Anthers, Staminodes, and Pollen Grains of Mango (Mangifera indica L. var. Beneshan; Anacardiaceae)

Authors: Muniraja, M., Vijayalakshmi, G., Naik, M. Lakshmipathi, Terry, Rg., and Valli Khan, P. S. Sha

Source: Palynology, 44(4) : 565-574

Published By: AASP: The Palynological Society

URL: https://doi.org/10.1080/01916122.2019.1633437

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Palynology on 09 Jan 2025
Terms of Use: https://bioone.org/terms-of-use

PALYNOLOGY
2020, VOL. 44, NO. 4, 565–574
https://doi.org/10.1080/01916122.2019.1633437

Taylor & Francis
Taylor & Francis Group

Check for updates

Ultrastructural observations of anthers, staminodes, and pollen grains of mango (*Mangifera indica* L. var. Beneshan; Anacardiaceae)

M. Muniraja[a], G. Vijayalakshmi[a], M. Lakshmipathi Naik[a], Rg. Terry[b] and P. S. Sha Valli Khan[a]

[a]Department of Botany, Yogi Vemana University, Kadapa, Andhra Pradesh, India; [b]Biology Department, Lamar University, Beaumont, TX, USA

ABSTRACT

The ultrastructure of anthers, staminodes, and pollen of *Mangifera indica* L. was studied using scanning electron microscopy (SEM), and pollen viability assessed using light (LM) and fluorescence microscopy (FM). Ultrastructural observation revealed anther surfaces with polygonal cells and hollow centres arranged in a reticulate manner, with swollen cells on the edges of the anther surfaces. Anther dehiscence is longitudinal, with pollen released through a long slit in both thecae. The average length and width of staminodes of male and hermaphroditic flowers measured 0.7 mm × 0.35 mm and 0.65 mm × 0.3 mm, respectively. Distinct ridge and hook-like outgrowths on the adaxial surface of staminodes are described, as are staminode surfaces comprised of long, irregular cells with stomata exuding nectar. Staminodes produced no pollen. Anthers of male flowers produced more pollen grains (536–537) than did anthers of hermaphrodite flowers (510–511). Pollen grains are tricolporate, have reticulate perforate exine ornamentation, and are bi-cellular at dispersal. Anther and staminode size and pollen production was greater but not significantly different in male versus hermaphrodite flowers. In contrast, the fluorochromatic reaction (FCR) test and FM observations found significantly higher pollen viabilities in hermaphroditic (50.1%) versus male (40.4%) flowers. This research provides new ultrastructural characters potentially useful in future taxonomic studies of mango and other Anacardiaceae. Results presented here may also be useful in pollination studies, and in the improvement of mango breeding programmes and commercial fruit production.

KEYWORDS

Anacardiaceae; *Mangifera*; fluorochromatic reaction; pollination

1. Introduction

Anacardiaceae Lindl. (83 genera, 850 species) is primarily a pantropical family well known for its edible fruits and seeds (e.g. *Anacardium occidentale* L., cashew; *Pistacia vera* L., pistachio; *Mangifera indica* L., mango; *Sclerocarya birrea* (A. Rich.) Hochst., marula; *Schinus terebinthifolia* L., peppercorns; fruits from *Spondias* L. and *Tapirira* Aubl.) (Pell 2004; Christenhusz and Byang 2016). Extreme variability in floral and fruit characteristics has confounded classification of the group, with the affinity of Anacardiaceae to other families, the placement of certain genera within the family, and intrafamilial classification and species delimitations all being contentious (Pell et al. 2010; Ramirez and Davenport 2016; Muniraja et al. 2018; Tolke et al. 2018). Although Engler's revisions (1881, 1883, 1892) historically have been the most detailed and widely accepted treatments, subsequent revisions based on morphological (Ramirez and Davenport 2016; Muniraja et al. 2018; Tolke et al. 2018) and molecular (Pell 2004) evidence suggest a sustained effort will be needed to better understand relationships within the family.

Common mango (*Mangifera indica* L.) is among the world's most important fruit crops and the most economically important member of Anacardiaceae (http://faostat.fao.org/; Mukherjee and Litz 2009). Mango is an andromonoecious tree producing male (staminate) flowers proximally and hermaphroditic flowers distally in terminal panicles (Mukherjee and Litz 2009; Pell et al. 2010; Ramirez and Davenport 2016; Tolke et al. 2018). Both male and hermaphrodite flowers produce one or two large stamens surrounded by three or more staminodes, with staminodes more variable in colour, size, and shape than stamens (Ramirez and Davenport 2016). In both types of flowers, stamens and staminodes are surrounded by flesh disks or nectaries (Singh and Shono 2005; Ramirez and Davenport 2016). Although the ratio of hermaphrodite to male flowers varies by cultivar and geographic location, sex ratios are generally less than 50% (Asif et al. 2002). To date, only general information is available regarding the morphology of anther, staminode, and pollen morphology (Li 1992). Similarly, knowledge on *M. indica* pollination is limited (Ramirez and Davenport 2016), although flowering and floral morphology recently have been described (Muniraja et al. (2018).

The most definitive taxonomy of *Mangifera* is that of Kostermans and Bompard (1993) who recognised 69 species, 47 and 11 of which were placed in the subgenera *Mangifera* and *Limus*, respectively, leaving 11 of uncertain placement. Shape of the floral disc, number of fertile stamens (and staminodes),

CONTACT P. S. Sha Valli Khan pssvkhan@yogivemanauniversity.ac.in Department of Botany, Yogi Vemana University, Vemanapuram – 516003, Kadapa, Andhra Pradesh, India.

© 2019 AASP – The Palynological Society

Downloaded From: https://bioone.org/journals/Palynology on 09 Jan 2025
Terms of Use: https://bioone.org/terms-of-use

inflorescence architecture, petal morphology, and the shape and size of petals and leaves were all important characters in distinguishing subgenera, sections, and species of *Mangifera* (Kostermans and Bompard 1993). The common mango (*M. indica*) was included with 14 other species in subg. *Mangifera* sect. *Mangifera* based on the number of fertile stamens and staminodes, staminode presence and size, and the number of flowering parts (5-merous; Kostermans and Bompard 1993). More recently, molecular data (Yonemori et al. 2002; Dinesh et al. 2015) or molecular combined with morphological, cytological, and palynological data (Sankaran et al. 2018) have been used to test traditional taxonomies of the genus.

Pollen structure and morphology have been described in several Anacardiaceae (Erdtman 1971; Wunnachit et al. 1992; Belhadj et al. 2007; Pradeep 2014; Bahramabadi et al. 2018), and pollen data hold great promise for addressing long-standing taxonomic issues in the family. For example, distinctive pollen has been described for genera (e.g. *Dobinia*, *Campylopetalum*, *Pistachia*) whose placement in Anacardiaceae has been problematic (Bentham and Hooker 1862; Forman 1954; Melchior 1964; Erdtman 1971; Cronquist 1981; Mitchell and Mori 1987; Mabberley 1997; Takhtajan 1997). Conversely, pollen more typical of Anacardiaceae characterises genera (e.g. *Amphipterygium* and *Orthopterygium*) placed in different families based on other morphological characters and subsequently transferred to Anacardiaceae using molecular evidence (Erdtman 1971; Pell 2004). Lastly, pollen data have been used to assess relationships at various taxonomic levels in Anacardiaceae, including among genera (Erdtman 1971), as well as between species within *Mangifera* (Sankaran et al. 2018).

In mango, the number of pollen grains per anther, pollen shape, and grain size differ among cultivars and between flower types (male or hermaphrodite; Ramirez and Davenport 2010; Gehrke-Velez et al. 2011). Depending on the extent of hydration, the shape of mango pollen varies from spherical to oblong, typically ranges in length from 20 to 45 μm (Mukherjee 1950, Ramirez and Davenport 2016), and is tricolpate (Singh 1961), with each sulcus having a pore in its centre (Mukherjee 1950). Although exine morphologies of male and hermaphrodite flowers are similar (Li 1992), hermaphrodite pollen grains are rounder and larger than those of male flowers (Li 1992).

Environmental conditions at anthesis are critical determinants of pollen viability in mango (Ramirez and Davenport 2016). Particularly important is temperature, with numerous studies documenting decreased viability at lower temperatures (Issarakraisilia and Considine 1994; de Wet and Robbertse 1986; de Wet et al. 1989; Dag et al. 2000; Huang et al. 2010; Geetha et al. 2016). A recent study examining cell wall composition and its influence on somatic–germline cell communications has shed light on the effect of decreased temperature on pollen development and viability in mango (Lora and Hormaza 2018). Muniraja et al. (2018) examined anther wall anatomy and pollen development in fertile anthers of male and hermaphroditic flowers of *M. indica*, as well as the anatomical features of staminodes in male flowers. Despite these advances, little attention has been paid to anther, staminode, and pollen grain ultrastructure in mango.

The current study examines the ultrastructure of anthers, staminodes, and pollen grains in *M. indica* using scanning electron microscopy (SEM). We also assessed pollen viability using fluorescein diacetate (FDA) and acetocarmine staining combined with light (LM) and fluorescence microscopy (FM). Our goal is to describe ultrastructural characters of possible taxonomic and reproductive significance in *M. indica*.

2. Materials and methods

2.1. Plant material

In summer 2016, fresh anthers, staminodes, and pollen were collected from newly opened floral buds of male and hermaphroditic flowers of a *Mangifera indica* L. var. Beneshan tree growing in the Yogi Vemana University Botanical Garden, YSR district, Andhra Pradesh, India (14°28′24″N, 78°42′44″E). A voucher of the collected specimen is deposited in the Yogi Vemana University herbarium (PSVK & MMR 4855). Mango (*Mangifera indica* L.) is a long-lived, andromonoecious tree, erect and 10–30 m in height, with a broad, rounded crown that may become oval to slender as the tree ages (Morton 1987). Leaves are alternate and evergreen or nearly so, and are borne mainly in rosettes at the tips of branches (Morton 1987). Hundreds or thousands of small yellowish to reddish flowers are borne in pyramid-shaped panicles, 25–98% of which are staminate, the remainder hermaphroditic, depending on cultivar and geographic locality (Morton 1987). *Mangifera indica* var. Beneshan flowers are yellow, densely puberulent and synchronous, with stamens equal and parallel to the pistil, staminodes well developed, and a large, obliquely oblong to oval fruit (Naik and Gangolly 1950).

2.2. Methods

2.2.1. Morphology of staminode, anther, and pollen

Sampled tissues (staminode, anther, and pollen) were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), rinsed in 0.05 M phosphate buffer (pH 7.4), dehydrated in a graded (0–100%) acetone series, and dried on a Bal-Tec 030 critical point dryer. Fixed samples were taped to aluminium stubs with two-sided carbon glue tape, and coated with gold–palladium using an Emitech K550 sputter coater. Observations were made and electron micrographs taken at the Sophisticated Analytical Instrument Facility, All India Institute of Medical Sciences, New Delhi, India, using a Zeiss SEM S-4700 scanning electron microscope (Carl Zeiss, Oberkochen, Germany) operating at 20 kV. Differences in mean anther length and width, staminode length and width, pollen diameter, number of pollen grains per anther, and pollen viability were compared for male and hermaphroditic flowers using *t* tests.

2.2.2. Anther, staminode, and pollen grain measurements

Lengths and widths of anthers and staminodes from five male and five hermaphroditic flowers at anthesis were

Downloaded From: https://bioone.org/journals/Palynology on 09 Jan 2025
Terms of Use: https://bioone.org/terms-of-use

measured to the nearest millimetre using an ocular micrometer on a Zeiss light microscope (Carl Zeiss, Oberkochen, Germany) at 100× magnification. Pollen grains were fixed in Carnoy's solution (3:1 absolute ethanol:acetic acid) for 24 h, and stored in 70% (v/v) ethanol at 4 °C until use. Pollen was stained with 2% aceto-carmine and examined under LM (Carl Zeiss, Germany) at 100× magnification, with pollen diameter measured to the nearest micrometre using the integrated software of the microscope. Photomicrographs were captured with a charged-coupled device (CCD) digital camera system (Prog.Res C3 Jenoptik, Jena, Germany). For anther, staminode, and pollen diameter measurements, mean percentages and standard errors were calculated from three replicates, with 12 samples per replicate.

2.2.3. Pollen grain number

Anthers from male and hermaphroditic flowers were dissected and pollen grains transferred to a 5% (v/v) tween-20 solution. After thorough mixing, a haemocytometer chamber was filled with 100 µL of homogeneous pollen grain suspension and grains counted using a light microscope (Carl Zeiss, Germany) at 40×. The number of grains per anther (A) was calculated using the following formula:

$$A = n \times B/N$$

where n = pollen grain count per chamber, B = fraction of suspension retained by each chamber in relation to the total suspension volume, and N = number of anthers per total suspension volume (Godini 1981). Three samples of 100 anthers each were prepared and grain counts repeated 3 times per vial, with the number of pollen grains per anther reported as the mean overall count.

2.2.4. Pollen viability

Pollen grains were harvested from dissected anthers and stained directly on a microscope slide in 2.5 mg/mL FDA solution for two minutes (Heslop-Harrison and Heslop-Harrison 1970). The fluorochromatic reaction (FCR) was excited using epifluorescence under blue-light excitation (510 nm dichroic mirror, 525 nm barrier filter, Carl Zeiss, Germany), with viable grains fluorescing yellow-green, and non-viable grains emitting ghost fluorescence. Percentage viability was calculated by dividing the number of yellow-fluorescent microspores by the total number of microspores per field of view multiplied by 100 (Subbarayudu et al. 2014). Mean percentage microspore viability was calculated by averaging percentages over all fields counted from five glass slides.

3. Results

Electron micrographs of ultrastructural features from anthers, staminodes, and pollen are provided in Plates 1–3, respectively. Plate 4 presents photomicrographs of pollen morphology and development (Plate 4, figures 1–2), grain diameter (Plate 4, figures 1–2), and viability (Plate 4, figures 3–4). Morphometric data and statistical results are provided in Table 1.

Table 1. Anther, staminode, and pollen grain characteristics in male and hermaphrodite flowers.

	Flower			
Characteristic	Male	Hermaphrodite	*t* value	*p* value
Anther length (mm) (mean ± SE)	0.70 ± 0.03	0.65 ± 0.02	1.72	0.081
Anther width (mm) (mean ± SE)	0.35 ± 0.03	0.30 ± 0.02	−0.41	0.353
Staminode head length (mm) (mean ± SE)	0.28 ± 0.03	0.28 ± 0.0.02	−1.94	0.062
Staminode head width (mm) (mean ± SE)	0.20 ± 0.01	0.19 ± 0.02	−0.59	0.291
Pollen diameter (µm) (mean ± SE)	26.52 ± 0.48	29.38 ± 0.80	−0.73	0.251
Pollen grains (number per anther)	536.25 ± 22.30	510.41 ± 22.86	1.44	0.110
Pollen viability (mean ± SE)	40.41 ± 1.72	50.19 ± 1.5	−5.49	0.002*

SE: standard error. *: significant.
The mean percentages of anthers, staminodes, and pollen grains were compared using the *t*-test.

3.1. Morphology of anther and staminodes

Staminode shape and colour varied considerably within a flower compared to stamens; however, staminode and stamen shape, size, and colour did not differ significantly between male and hermaphroditic flowers (Table 1). Although anther dimensions were not significantly different between flower types, anthers of male flowers were longer and wider (0.7 mm × 0.35 mm) than those of hermaphroditic flowers (0.65 mm × 0.30 mm) (Table 1). The mean length and width of anthers from both flower types was greater than that of staminode heads (Table 1). In both flower types, anthers were two-lobed and dorsally elliptical in shape (Plate 1, figure 1); they had similar surface morphologies, consisting of reticulately arranged polygonal cells with hollow centres and swollen margins (Plate 1, figure 2). Irrespective of flower type, anther dehiscence was longitudinal and pollen was released from both thecae through a long slit (Plate 1, figures 3 and 4).

Staminodes had a short filament with the upper part resembling a head, usually swollen and capitate in shape (Plate 2, figure 1). The average length and width of staminode heads from male and hermaphroditic flowers was 0.28 mm × 0.2 mm and 0.28 mm × 0.19 mm, respectively, without any significant differences in their dimensions (Table 1). A distinct ridge was visible on the adaxial surface of staminodes (Plate 2, figure 1), and staminode surfaces had irregular and longer cells compared to those of anthers (Plate 2, figure 3), irrespective of flower type. A hook-like outgrowth was observed on staminode heads (Plate 2, figures 1 and 3), and open stomata (Plate 2, figures 4 and 5), some of which secreted nectar (Plate 2, figures 5 and 6), were observed on the adaxial surface of staminode heads. Neither dehiscence nor pollen release was observed in staminodes.

3.2. Pollen counts and morphology

Male and hermaphroditic flowers produced means of 536 and 510 pollen grains per anther, respectively (Table 1). Mean number of pollen grains per anther was not significantly different between male and hermaphrodite flowers (Table 1).

Downloaded From: https://bioone.org/journals/Palynology on 09 Jan 2025
Terms of Use: https://bioone.org/terms-of-use

Pollen grains were tricolporate (Plate 3, figures 1 and 2), and ranged in shape from oval to circular in polar view (Plate 3, figure 1), and from triangular to elliptical in equatorial view (Plate 3, figure 3), with grains of hermaphroditic flowers (Plate 3, figure 1) being rounder than those of male flowers (Plate 3, figure 3). Pollen grain exine had reticulate perforated ornamentation (Plate 3, figure 4), with the grain surfaces of male and hermaphroditic flowers having similar morphologies. Average grain diameters for hermaphrodite (Plate 4, figure 1) and male (Plate 4, figure 2) flowers were 29.3 μm and 26.5 μm, respectively (Table 1). The average grain diameter of pollen was not significantly different between male and hermaphrodite flowers (Table 1). Pollen grains were bi-cellular at the dispersal stage (Plate 4, figure 2).

3.3. Pollen viability

Pollen grain viability was assessed by FDA staining and epifluorescent excitation, with viable grains fluorescing yellow (Plate 4, figure 3), and non-viable ones appearing dull brown (Plate 4, figure 4). The viability of pollen from male and hermaphroditic flowers was 40.4% and 50.1%, respectively (Table 1). The pollen from hermaphrodite flowers had significantly higher viability compared to pollen from male flowers ($P < 0.002$; Table 1).

4. Discussion

We report ultrastructural features of anthers, staminodes, and pollen, as well as pollen counts and viabilities, in mango (*M. indica*). Several features of potential taxonomic utility are either described here for the first time, or were previously underreported in Anacardiaceae and very poorly known (Muniraja et al. 2018). These include size differences between anthers and staminodes within and between flower types, differences in anther size between flower types, and numerous staminode characteristics that varied between and sometimes within flower types, including the presence of distinct ridges (Plate 2, figure 1), the presence of hook-like outgrowths (Plate 2, figure 2), cell shape and size variability (Plate 2, figure 3), and the occurrence of nectar-secreting stomata on staminode heads (Plate 2, figures 4–6).

Mango anther and staminode heads differed in shape, size, and colour. This dimorphism apparently is a consequence of natural selection for pollen production, or for other functions generally related to pollination and reproductive success (Rodriguez-Riano et al. 2015). We observed differences in anther size between male and hermaphroditic flowers that could result from differing patterns of resource allocation between male and female reproductive functions (Diggle 1993). Alternatively, natural selection may have favoured larger anthers in male flowers, as large anthers are preferred by visiting insect pollinators due to their size and prominence (Wunnachit et al. 1992), or for their pollen-producing capacity in pollen-rewarding species (Johnson and Schiestl 2016). Although the anther lobes of male and hermaphroditic flowers had similar surface morphologies, staminode surfaces were highly variable both within and between flower types compared to anthers (see Plates 1 and 2) (Scholefield 1982). The potential of staminode variation to illuminate phylogenetic relationships in flowering plants has been appreciated by previous authors (Endress and Matthews 2006) and, given the variation documented here, may inform classifications at lower (inter- and intraspecific) taxonomic levels in *Mangifera* and other Anacardiaceae.

Staminodes may be either vestigial or functional (Decraene and Smets 2001). Vestigial staminodes are derived from functional stamens, and may be vascularised and morphologically similar to or indistinguishable from pollen-producing stamens, depending on the extent of reduction (Decraene and Smets 2001). In contrast, functional staminodes have particular floral functions (e.g. production of food rewards such as sterile pollen or nectar), and may be derived from sterile stamens by a comparatively simple transition involving the origin of a vascular connection (Decraene and Smets 2001).

In the current study, we described nectary pores and nectar drops on the surface of staminode heads in mango. Several insect-pollinated genera of flowering plants have nectaries which develop on modified stamens and staminodes, which are considered a primitive manifestation of *nectaria cauda* (Smets 1986; Weryszko-Chmielewska et al. 2003; Weryszko-Chmielewska and Sulborska 2011). In mango staminodes, nectariferous tissue is usually fed by a single vascular bundle surrounded by multi-layered parenchyma and a single-layered epidermis at the centre of the nectar (Muniraja et al. 2018). Muniraja et al. (2018) provide additional anatomical details of the vasculature bundle of staminodes in mango. Although the presence of vascularisation alone does not support *M. indica* staminodes as functional, pronounced morphological differentiation (compare Plates 1 and 2), the production of nectar and its secretion from pores or stomata (Plate 2, figures 5 and 6), and the presence of vascularisation in staminodes and staminodal nectaries (Muniraja et al. 2018) all support a biological function for staminodes in mango (Decraene and Smets 2001). Studies of pollination/pollinator reward and comparative studies of stamen and staminode vascular anatomy may provide additional insight into the origin and function of staminodes in mango.

Anthers of male and hermaphroditic flowers produced an average of 536 and 510 pollen grains, respectively (Table 1). Previous studies in mango report an average of 400 to 800 pollen grains per anther, and decreased pollen production in male compared to hermaphroditic flowers, depending on the cultivar (Gehrke-Velez et al. 2011). Pollen production is a function of male reproductive fitness and is a key parameter in plant–pollinator interactions (Costa and Yang 2009). Although pollen production from staminodes has not been observed in *M. indica*, differences in pollen production between anthers of male and hermaphroditic flowers may be useful in assessing male fertility in other Anacardiaceae (Wunnachit et al. 1992; Mert and Soylu 2006; Ramirez and Davenport 2016), where male pollen appears to be specialised for fertilisation and fruit set, and hemaphrodite pollen may serve as an attractant (Wunnachit et al. 1992).

Pollen apertures are the reduced or altered part of the pollen wall where pollen tubes emerge upon grain

Downloaded From: https://bioone.org/journals/Palynology on 09 Jan 2025
Terms of Use: https://bioone.org/terms-of-use

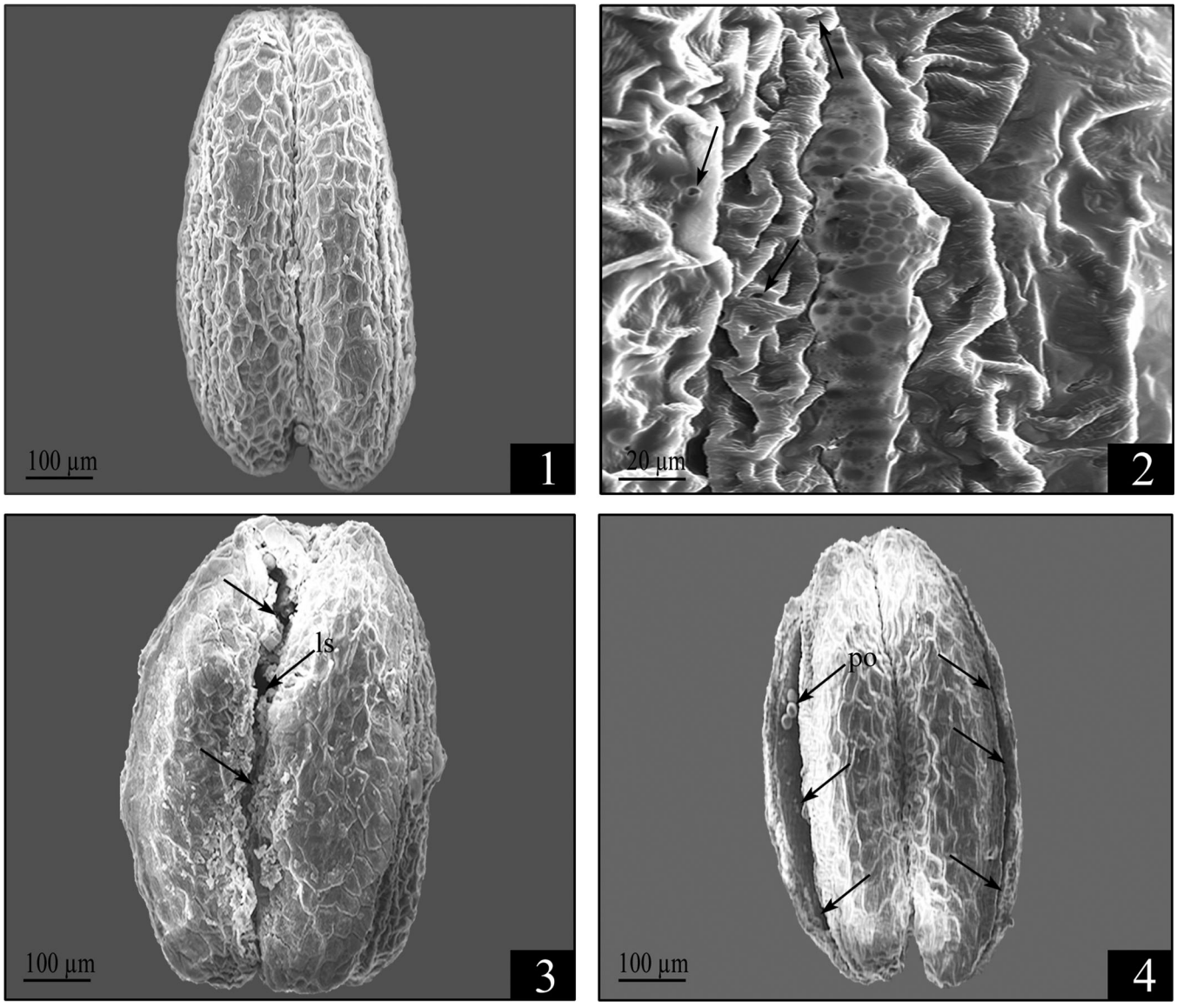

Plate 1. Scanning electron micrograph of mature anthers: 1. Abaxial side of a pre-dehiscent anther. Scale bar: 100 µm. **2.** Irregular-shaped groove and stomata (arrows) on the surface of the anther. Scale bar: 20 µm. **3.** Development of longitudinal slit (ls) (arrows) on the anther. Scale bar: 100 µm. **4.** Side view of a dehisced anther (arrows) with residual pollen grains (po). Scale bar: 100 µm.

germination. Apertures vary in shape, number, orientation, and location between angiosperm taxa (Furness et al. 2004), with eudicots usually producing tricolpate (three apertures) pollen, monocots monocolpate (one aperture) pollen, and basal angiosperms pollen with two or more apertures (discussed in APG II 2003; Furness et al. 2004; Pell et al. 2010). Lora and Hormaza (2018) described mango pollen as tricolpate at anther dehiscence. Consistent with the findings of Ramirez and Davenport (2016), the *M. indica* pollen described herein was tricolporate, spherical in shape with pitted walls, and two-celled at dispersal (see Plate 3). In contrast to other Anacardiaceae (Pradeep 2014), exine ornamentation was reticulate perforate (Plate 3, figures 2–4).

Pollen grains of male and hermaphroditic flowers reported here averaged 26.5 µm and 29.3 µm in diameter, respectively, values which fall within the size range reported for angiosperms (Erdtman 1952), Anacardiaceae, and *Mangifera indica* (Jayarajan et al. 2016). Furthermore, and in contrast to other mango varieties, the pollen of hermaphroditic flowers in *Mangifera indica* L. var. Beneshan was slightly smaller than that of male flowers (Table 1; Li 1992), although the somewhat rounder shape reported here appears typical for hermaphrodite pollen in *Mangifera indica* (Ramirez and Davenport 2016).

In this study, the mean pollen viabilities of hermaphroditic and male flowers were 50.1% and 40.4%, respectively. These findings are consistent with those of Gehrke-Velez et al. (2011), who reported higher viabilities in hermaphroditic flowers compared to male flowers in *Mangifera indica* L. cv. Ataulfo. However, the percentages of pollen viability documented in the current study are lower than those reported for other mango varieties such as 'Kent' (23–96%; Dag et al. 2000) and 'Sensation', 'Tommy Atkins', and 'Janardhan Pasand' (83.4–88.2%; Dutta et al. 2013). A number of variables not rigorously controlled for in this study are known to influence pollen viability in *Mangifera* and may account for the relatively low viabilities reported here. These include decreased temperature at anthesis (Ramirez and Davenport 2016) and

Downloaded From: https://bioone.org/journals/Palynology on 09 Jan 2025
Terms of Use: https://bioone.org/terms-of-use

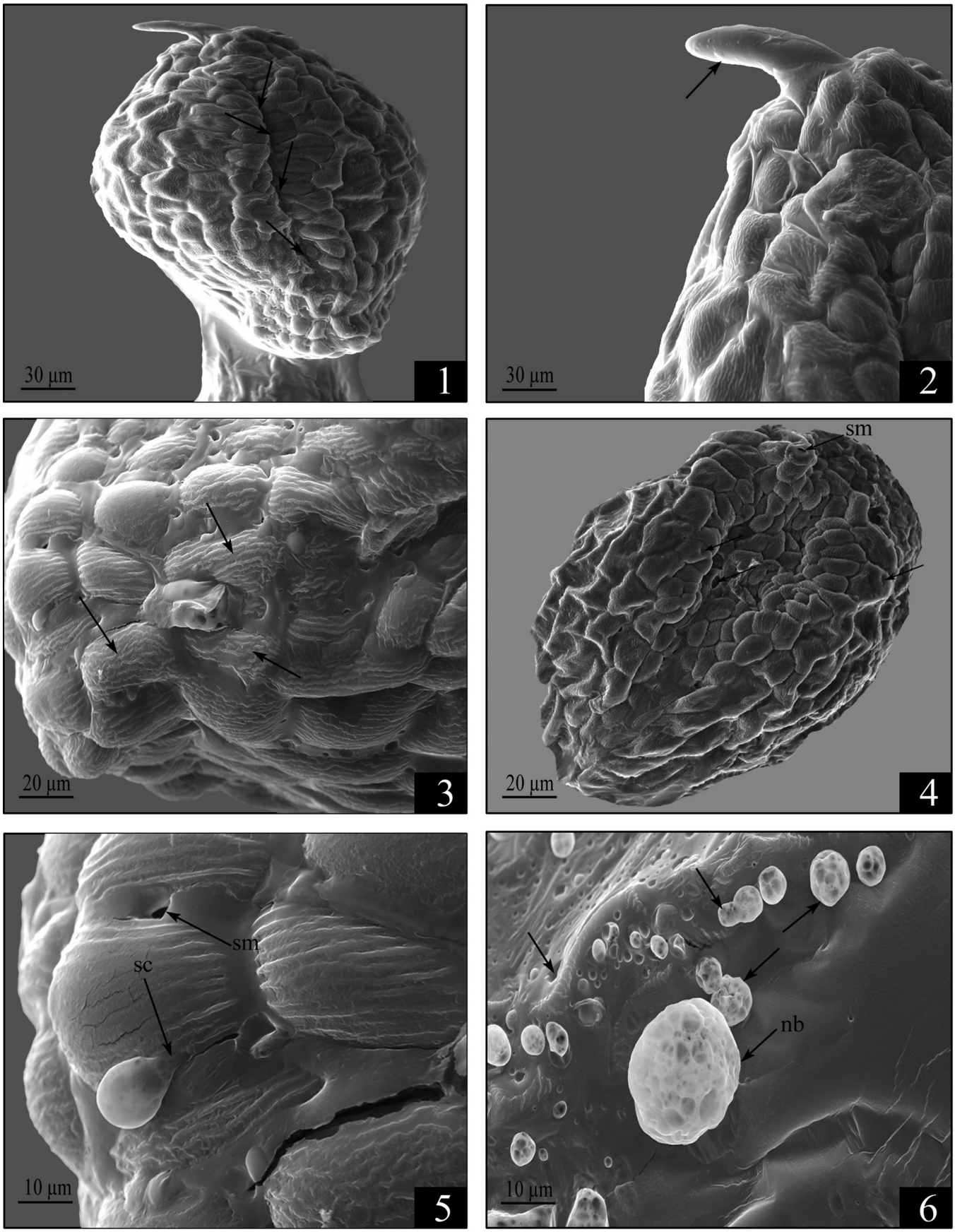

Plate 2. Scanning electron micrograph of staminodes of *Mangifera indica* L.: 1. Capitate staminode with a filament from a male flower (arrows). Scale bar: 30 µm. **2.** Staminode with a terminal hook (arrow). Scale bar: 30 µm. **3.** A staminode with irregular cells on the surface and parallel ornamentation (arrows). Scale bar: 10 µm. **4.** Stomata (sm) (arrows) on the surface of the staminode. Scale bar: 20 µm. **5.** The presence of stomata (arrows) and the secretion (sc) (arrow) of nectar from the stomata (arrows). Scale bar: 10 µm. **6.** Expanded view of nectar drops (nb) (arrows). Scale bar: 10 µm.

Downloaded From: https://bioone.org/journals/Palynology on 09 Jan 2025
Terms of Use: https://bioone.org/terms-of-use

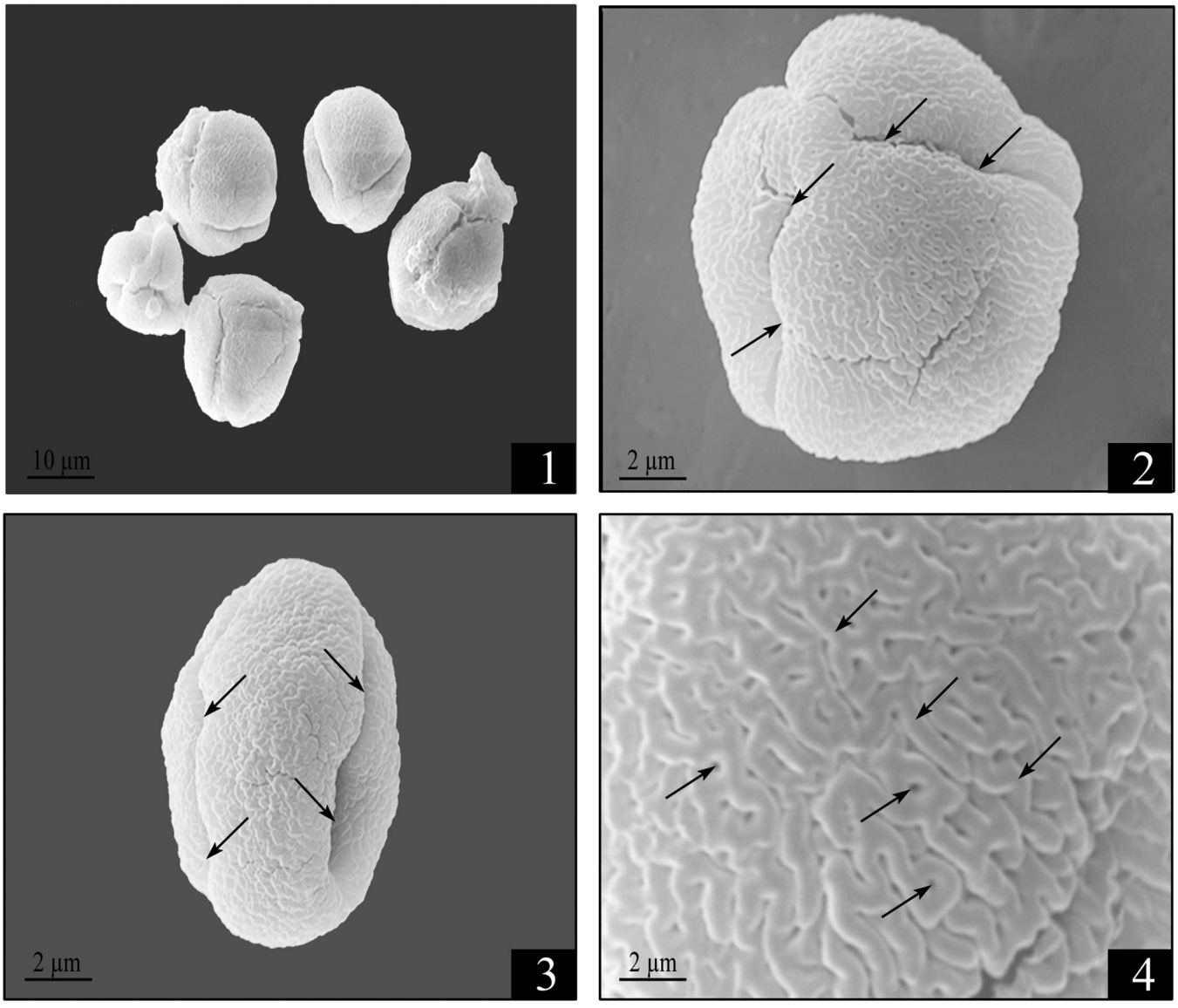

Plate 3. **Scanning electron micrographs of mature pollen grains with different proximal faces: 1.** A group of pollen grains from hermaphrodite flowers. Scale bar: 10 µm. **2.** Tricolpate hermaphrodite pollen grain in polar view (arrows). Scale bar: 2 µm. **3.** Tricolpate male pollen grain in equatorial view (arrows). Scale bar: 2 µm. **4.** Pollen exine with psilate perforate ornamentation (arrows). Scale bar: 2 µm.

increased time between pollen collection and analysis (de Wet et al. 1989), both of which negatively impact viability.

5. Conclusions

In this study, we described numerous ultrastructural features of potential taxonomic utility in mango (*M. indica* var. Beneshan). These include differences in anther and staminode size within and between hermaphroditic and male flowers, differences in anther size between flower types, and numerous staminode characteristics that varied between and sometimes within flower types, including the presence of distinct ridges (Plate 2, figure 1), variability in cell shape and size (Plate 2, figure 2), the presence of hook-like outgrowths (Plate 2, figure 3), and the occurrence of nectar-secreting stomata on staminode heads (Plate 2, figures 4–6). Pollen of *M. indica* var. Beneshan is morphologically similar to that of the other members of the subfamily Anacardioideae, including similarities in size, shape, and number of apertures. However, in mango exine ornamentation is reticulate perforate, whereas it is reticulate or reticulate striate in other members of Anacardioideae (Pradeep 2014). Pollen viability studies are of great importance in mango breeding programmes and commercial fruit production. We report moderate (40–50%) pollen viabilities in *M. indica* var. Beneshan compared to other varieties. However, mango pollen is highly sensitive to desiccation and there is rapid loss of moisture when pollen is exposed to higher temperatures (Dutta et al. 2013). Moreover, decreased temperatures at anthesis negatively impact mango pollen viability, as does the overall time between pollen collection and viability analysis (Ramirez and Davenport 2016). Heslop-Harrison et al. (1984) suggested correlation studies on pollen viability with FDA and *in vitro* germination of mature pollen to reduce erroneous viability estimates, and such studies are needed in *M. indica* var. Beneshan to corroborate the relatively low viabilities reported here. This research provides new ultrastructural characters potentially useful in future taxonomic studies of *Mangifera*. Results presented here may also be useful in pollination

Downloaded From: https://bioone.org/journals/Palynology on 09 Jan 2025
Terms of Use: https://bioone.org/terms-of-use

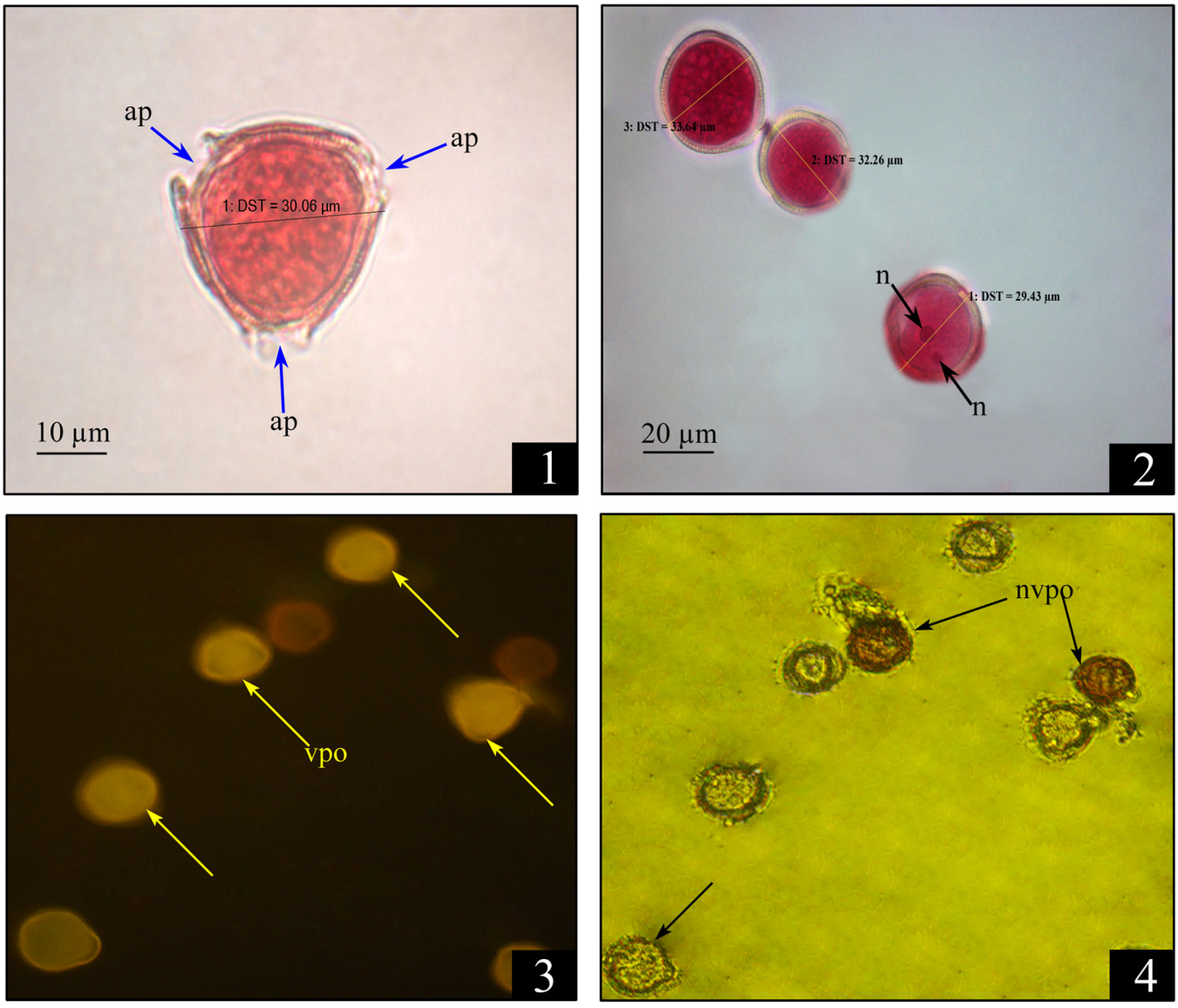

Plate 4. Photomicrographs related to pollen size, pollen development, and pollen viability: 1. Pollen grain of a hermaphrodite flower showing the three-aperture (ap) stage, stained with aceto-carmine (arrows). Scale bar: 10 µm. (in 100×). **2.** Just-released pollen grains with three apertures (ap) (arrows), (at 100×). Scale bar: 10 µm. **3.** Viable pollen grains (vpo) stained with FDA (arrows) (at 40×). **4.** Non-viable pollen grains (nvpo) Differential Interference Contrast (DIC) stained with fluorescein diacetate (FDA) (arrows) (at 40×).

studies, and in the improvement of mango breeding programmes and commercial fruit production.

Acknowledgements

We thank Prof. K.R. Shivanna, University of Delhi, New Delhi, Prof. M.A. Germana, University of Palermo, Palermo, Italy, and Dr Kjell Sergeant, Luxembourg Institute of Science and Technology, Luxembourg, for their valuable suggestions and critical comments. We also thank SAIF – All India Institute of Medical Sciences (AIIMS), New Delhi, for their help with SEM studies.

Disclosure statement

The authors declare that they have no conflicts of interest.

Notes on contributors

M. MUNIRAJA is undergraduate in biological sciences and chemistry from Sri Venkateswara University, Tirupathi (2009), a Master's degree in Botany obtained from Yogi Vemana University, Kadapa (2014) and a Ph.D in Botany by the same institution (2019). He has experience in plant biology with emphasis on plant reproduction and palynology.

G. VIJAYALAKSHMI is undergraduate in biological sciences and chemistry from Sri Venkateswara University, Tirupathi (2010), a Master's degree in Botany obtained from Yogi Vemana University, Kadapa (2012) and a Ph.D in Botany by the same institution (2018). She has research experience in the plant biology with emphasis on food chemistry, gynogenic haploids and palynology.

M. LAKSHMIPATHI NAIK is undergraduate in biological sciences and chemistry from Sri Krishnadevaraya University, Anantahpuram (2013) and a Master's degree in Botany obtained from Yogi Vemana University, Kadapa (2015). He is currently pursuing Ph.D in Botany by the same institution in plant stress physiology and plant reproduction with emphasis on pollen biology.

RG. TERRY received his Ph.D. in Botany from the University of Wyoming in 1996. His dissertation research examined the phylogenetic relationships in Bromeliaceae (pineapple family). This was followed by postdoctoral research with Drs. Robert Nowak and Robin Tausch at the University of Nevada Reno, where he studied hybridization in Juniperus

Downloaded From: https://bioone.org/journals/Palynology on 09 Jan 2025
Terms of Use: https://bioone.org/terms-of-use

(Cupressaceae). Dr. Terry joined the Biology Department at Lamar University in 2000 following three years as a Visiting Assistant Professor at the University of Montana, Research in his lab examines evolutionary processes and phylogenetic relationships in plants. He also has experience in plant physiology and plant reproduction with emphasis on pollen biology.

P. S. SHA VALLI KHAN is undergraduate in biological sciences and chemistry from Sri Venkateswara University, Tirupathi (1987), a Master's degree in Botany (1990) and a Ph.D in Botany by the same institution (1995). Post doctorate at Luxembourg Institute of Science and Technology, Luxembourg, Chiba University, Japan and Institute of Botany, Academic Sinica, Taiwan. Visiting researcher at University of Palermo, Italy to work on pollen biology and androgenic haploids of fruit trees in association with Prof. M.A. Germana. Since 2013, he is full Professor at the Department of Botany, Yogi Vemana University, Kadapa, teaching plant physiology and plant reproduction with emphasis on pollen biology and doubled haploids. He is supervisor of doctoral students and has several research grants from DST, SERB, DBT and UGC in India.

Funding

This study was financially supported by the University Grants Commission, New Delhi [F. No. 41-451/2012].

References

APG II 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnaean Society. 141:399–436.

Asif M, Usman M, Jaskani MJ, Khan MM. 2002. Comparative study of flower sex ratio in different cultivars of mango (*Mangifera indica* L.). International Journal of Agriculture and Biology. 8530(4–2):220–222.

Bahramabadi EZ, Jonoubi P, Rezanejad F. 2018. Some cytological and physiochemical features relating to non-storability of pistachio (*Pistacia vera* L.) pollen. Grana. 57:456–463.

Belhadj S, Derridj A, Civeyrel L, Gers C, Aigouy T, Otto T, Gauquelin T. 2007. Pollen morphology and fertility of wild Atlas pistachio (*Pistacia atlantica* Desf., Anacardiaceae). Grana. 46(3):148–156.

Bentham G, Hooker JD. 1862. Anacardiaceae. In Genera Plantarum. London, UK: Reeve and Company; p. 415–428.

Christenhusz MJM, Byang JW. 2016. The number of known plant species in the world and its annual increase. Auckland, New Zealand: Phytotaxa. Magnolia Press; p. 201–217.

Costa CM, Yang S. 2009. Counting pollen grains using readily available, free image processing and analysis software. Annals of Botany. 104(5): 1005–1010.

Cronquist A. 1981. An integrated system of classification of flowering plants. New York: Columbia University Press.

Dag A, Eisenstein D, Gazit S. 2000. Effect of temperature regime on pollen and the effective pollination of "Kent" mango in Israel. Scientia Horticulturae. 86(1):1–11.

de Wet E, Robbertse PJ. 1986. A preliminary study of the pollen of *Mangifera indica* L. cv. Haden in South Africa. South African Journal of Plant and Soil. 3(2):87–89.

de Wet E, Robbertse PJ, Groeneveld HT. 1989. The influence of temperature and boron on pollen germination in *Mangifera indica* L. South African Journal of Plant and Soil. 6(4):228–234.

Decraene LPR, Smets EF. 2001. Staminodes: their morphological and evolutionary significance. The Botanical Review. 67:351–402.

Diggle PK. 1993. Developmental plasticity, genetic variation, and evolution of andromonoecy in *Solanum hirsutum* (Solanaceae). American Journal of Botany. 20:967–973.

Dinesh MR, Ravishankar KV, Nischita P, Sandya BS, Padmakar B, Ganeshan S, Chithiraichelvan R, S. Sharma TVR. 2015. Exploration, characterization and phylogenetic studies in wild *Mangifera indica* relatives. American Journal of Plant Sciences. 06(13):2151–2160.

Dutta SK, Srivastav M, Chaudhary R, Lal K, Patil P, Singh SK, Singh AK. 2013. Low temperature storage of mango (*Mangifera indica* L.) pollen. Scientia Horticulturae. 161:193–197.

Endress PK, Matthews ML. 2006. Elaborate petals and staminodes in eudicots: diversity, function, and evolution. Organisms, Diversity and Evolution. 6(4):257–293.

Engler A. 1881. Uber die morphologische verhaltnisse und die geographische varbreitung der gattung *Rhus*, wie der mitihrverwandten, lebenden und ausgestorbenen Anacardiaceae. Botanische Jahrbucher Für Systematik Pflangengeschichte und Pflanzeneographie. 1:365–426.

Engler A. 1883. Anacardiaceae. In: A. P, De Candolle AC, editors. Monographie Phanerogamarum. Paris, France: G. Masson; p. 171–546.

Engler A. 1892. Anacardiaceae. In: Engler A, Prantl K, editors. Die Naturlichen Pflanzenfamilien, Vol. III, Parts 4 & 5. Leipzig: Engelmann; p. 138–178.

Erdtman G. 1952. Pollen morphology and plant taxonomy Angiosperms. Stockholm: Almqvist and Wiksell; p. 539.

Erdtman G. 1971. Pollen morphology and plant taxonomy. New York (NY): Hafner Publishing Company.

Forman LL. 1954. A new genus from Thailand. Kew Bulletin. 4:555–564.

Furness CA, Rudall PJ, Sampson FB. 2004. Pollen aperture evolution-a crucial factor for eudicot success? Trends Plant Science. 9(3):154–158.

Geetha GA, Shivashankara KS, Reddy Y. 2016. Varietal variations in temperature response for hermaphrodite flower production and fruit set in mango (*Mangifera indica* L.). South African Journal of Botany. 106: 196–203.

Gehrke-Velez MR, Castillo-Vera A, Ruiz-Bello C, Moreno-Martinez JL. 2011. Viabilidad y germinacion delpolenen mango (*Mangifera indica* L.) cv. Ataulfo. Sistema de Informacion Cientifica. 36:378–385.

Godini A. 1981. Counting pollen grains of some almond cultivars by means of an haemocytometer. Option Mediterraneennes. 81:83–86.

Heslop-Harrison J, Heslop-Harrison Y, Shivanna KR. 1984. The evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test procedure. Theoretical and Applied Genetics. 67(4):367–375.

Heslop-Harrison J, Heslop-Harrison Y. 1970. Evaluation of pollen viability by enzymatically induced fluorescence: intracellular hydrolysis of fluorescein diacetate. Stain Technology. 45(3):115–120.

Huang JH, Ma WH, Liang GL, Zhang LY, Wang WX, Cai ZJ, Wen SX. 2010. Effects of low temperatures on sexual reproduction of "Tainong 1" mango (*Mangifera indica*). Scientia Horticulturae. 126(2):109–119.

Issarakraisilia M, Considine JA. 1994. Effects of temperature on microsporogenesis and pollen viability in mango cv. 'Kensington'. Annals of Botany. 73:231–234.

Jayarajan MR, Preethy TT, Aswathi KK, Asha VP. 2016. Reproductive biology and palynology in mango. International Journal of Recent Scientific Research. 7:9343–9346.

Johnson SD, Schiestl FP. 2016. Floral mimicry. Oxford, UK: Oxford University Press.

Kostermans A, Bompard JM. 1993. The mangoes: their botany, nomenclature, horticulture and utilization. Waltham: Academic Press.

Li K. 1992. Studies on flowering habit, pollen morphology and activity of Irwin mango (*Mangifera indica* L.). Taiwan: National Taiwan University. (In Chinese).

Lora J, Hormaza JI. 2018. Pollen wall development in mango (*Mangifera indica* L., Anacardiaceae). Plant Reproduction. 31(4):385–397.

Mabberley DJ. 1997. The plant book. Cambridge, UK: Cambridge University Press.

Melchior HH. 1964. Aengler's syllabus der pflanzenfamilien, Gebruder Borntraeger, Berline, Germany.

Mert C, Soylu A. 2006. Flower and stamen structures of male-fertile and male sterile chestnut (*Castanea sativa* Mill.) cultivars. Journal of the American Society for Horticultural Science. 131(6):752–759.

Mitchell SD, Mori SA. 1987. The cashew and its relatives (*Anacardium*: Anacardiaceae). Memoirs of the New York Botanical Garden. 42:1–72.

Morton J. 1987. Mango. In: Julia F. Morton, editor. Fruits of warm climates. Miami (FL): Florida Flair Books; p. 221–239.

Mukherjee SK, Litz RE. 2009. Introduction: botany and importance. In: Litz RE editor. The mango: botany, production and uses. Wallingford; CABI; p. 1–18.

Downloaded From: https://bioone.org/journals/Palynology on 09 Jan 2025
Terms of Use: https://bioone.org/terms-of-use

Mukherjee SK. 1950. Cytological investigation on the mango (*Mangifera indica* L.) and the allied Indian species. Proceedings of the National Institute of Sciences of India. 16:287–303.

Muniraja M, Vijayalakshmi G, Lakshmipathi Naik M, Vijaya Bhaskar V, Sha Valli Khan PS. 2018. A developmental study on anther wall and pollen in *Mangifera indica* L. var. Beneshan (Anacardiaceae). South African Journal of Botany. 119:142–153.

Naik KC, Gangolly SR. 1950. A monograph on classification and nomenclature of South Indian mangoes. Madras: The Superintendent Government Press.

Pell SK, Mitchel JD, Miller AJ, Lobova TA. 2010. Anacardiaceae. In: Kubitzki K, editor. Flowering Plants. Eudicots. The families and genera of vascular plants. Berlin, Heidelberg: Springer.

Pell SK. 2004. Molecular systematics of the cashew families (Anacardiaceae) [dissertation]. Baton Rouge (LA): Dept. of Biological Science, Louisiana State University.

Pradeep AS. 2014. LM and SEM Studies on the pollen morphology of family Anacadiaceae from Chandrapur and Gadchiroli Districts of Maharastra state. International Journal of Life Sciences. A2:11–12.

Ramirez F, Davenport TL. 2010. Mango (*Mangifera indica* L.) flowering physiology. Scientia Horticulturae. 126:65–72.

Ramirez F, Davenport TL. 2016. Mango (*Mangifera* indica L.) pollination: a review. Scientia Horticulturae. 203:158–168.

Rodriguez-Riano T, Valtuena FJ, Lopez J, Navarro-Perez ML, Perez-Bote JL, Ortega-Olivencia A. 2015. Evolution of the staminode in a representative sample of *Scrophularia* and its role as nectar safeguard in three widespread species. Science Naturwissenschaften. 102:37.

Sankaran M, Dinesh MR, Chaitral N, Ravishankar KV. 2018. Morphological, cytological, palynological and molecular characterization of certain *Mangifera* species. Current Science. 155:1379–1386.

Scholefield PB. 1982. A scanning electron microscope study of flowers of avocado, litchi, macadamia and mango. Scientia Horticulturae. 16(3): 263–272.

Singh I, Shono M. 2005. Physiological and molecular effects of 24-epibrassinolide, a brassinosteroid on thermotolerance of tomato. Plant Growth Regulation. 47(2–3):111.

Singh S. 1961. Studies on the morphology and viability of the pollen grains of mango. Horticultural Advances. 5:121–144.

Smets EF. 1986. Localization and systematic importance of the floral nectarines in the Magnoliatae (Dicotyledons). Bulletin du Jardin Botanique National de Belgique. 56(1/2):51–76.

Subbarayudu S, Shankar Naik B, Sunitibala Devi H, Bhau BS, Shaik Sha Valli Khan P. 2014. Microsporogenesis and pollen formation in *Zingiber officinale* Roscoe. Plant Systematics and Evolution. 300(4): 619–632.

Takhtajan A. 1997. Diversity and classification of flowering plants. New York (NY): Columbia University Press.

Tolke ED, Bachelier JB, Lima EA, Galetto L, Demarco D, Carmello-Guerreiro SM. 2018. Diversity of floral nectary secretions and structure, and implications for their evolution in Anacardiaceae. Botanical Journal of the Linnean Society. 187:209–231.

Weryszko-Chmielewska E, Masierowska M, Konarska A. 2003. Characteristics of floral nectarines and nectar in two species of *Crataegus* (Rosaceae). Plant Systematics and Evolution. 238(1–4): 33–41.

Weryszko-Chmielewska E, Sulborska A. 2011. Staminodial nectar structure in two *Pulsatilla* (L.) species. Acta Biologica Cracoviensia Series Botanica. 53:94–103.

Wunnachit W, Jenner C, Sedgley M. 1992. Pollen vigour and composition in relation to andromonoecy in cashew (*Anacardium occidentale* L.: Anacardiaceae). Sexual Plant Reproduction. 5(4):264–269.

Yonemori K, Honsho C, Kanzaki S, Eiadthong W, Sugiura A. 2002. Phylogenetic relationships of *Mangifera* species revealed by ITS sequences of nuclear ribosomal DNA and a possibility of their hybrid origin. Plant Systematics and Evolution. 231(1–4):59–75.

Downloaded From: https://bioone.org/journals/Palynology on 09 Jan 2025
Terms of Use: https://bioone.org/terms-of-use