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Floral biology, pollen morphology, and reproductive behaviour of three tropical *Nymphaea* cultivars

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Abstract

A study was conducted to examine the flowering phenology, floral biology, and pollen morphology of three tropical *Nymphaea* cultivars *viz*. 'Blue Whistle', 'Carla's Sonshine', and 'Poonsup' under Kerala conditions. Anthesis behavior varied significantly among cultivars and across seasons, with flowers opening between 07:00 and 09:15 h and closing between 16:00 and 18:15 h. Temperature exerted a stronger influence than light intensity and resulting in extended anthesis during cooler months (November to February) and shortened durations during monsoon (June to October). All cultivars were protogynous, exhibiting a female phase on the first day and a male phase over the following three days. Stigma receptivity lasted for 22-25 h and showed cultivar-specific variation in cup diameter and number of appendages. Pollen viability ranged from 42.16 % to 80.92 %, with 'Poonsup' recording the highest viability. Scanning electron microscopy revealed oblate-spheroidal, medium-sized, zonosulcate, and psilate pollen grains consistent among cultivars. The results highlight the combined influence of genetic and environmental factors on reproductive behavior in *Nymphaea* and provide valuable insights for breeding programs aimed at enhancing floral longevity, fertility, and ornamental potential in tropical waterlilies.

Keywords *Nymphaea*, waterlily, pollen morphology, stigma receptivity, floral biology, SEM, hybridization

Introduction

Waterlilies (*Nymphaea* spp.) are among the most striking representatives of the family *Nymphaea*ceae, comprising nearly 70 species distributed across six genera and inhabiting tropical and temperate freshwater ecosystems worldwide, except Antarctica and major deserts (Choudhury *et al*., 2013). These perennial aquatic plants are valued for their aesthetic, ecological, and economic significance, serving as ornamental staples in aquatic landscaping and contributing to sectors such as medicine, food, cosmetics, and bio-remediation. Species of *Nymphaea* exhibit extraordinary morphological and genetic diversity, differing in flower shape, size, fragrance, pigmentation, and temperature tolerance. Their adaptability and the wide range of floral morphotypes make them ideal models for studies in reproductive biology, evolutionary systematics, and genetic improvement. Tropical waterlilies, including *Nymphaea caerulea, N. rubra, and N. micrantha*, exhibit diverse flowering rhythms and ecological adaptations that distinguish them from temperate species. Their anthesis is typically synchronized with early-morning pollinator activity, and the duration of floral opening is strongly influenced by temperature and light intensity (Kong *et al*., 2019). Recent studies on tropical *Nymphaea* hybrids have highlighted wide variation in pollen viability, stigma receptivity, and floral longevity, underscoring the role of genetic and environmental factors in shaping reproductive success under tropical conditions (Zhang *et al*., 2021). Despite the horticultural appeal of tropical waterlilies, systematic documentation of their reproductive morphology and pollen viability remains limited, constraining both breeding efficiency and phylogenetic inference.

Pollen viability is a key determinant of reproductive success in flowering plants (Madhavi *et al*., 2021). Low viability often results in reduced fertilization efficiency and seed set (Deng *et al*., 2017). Studies in *Nymphaea* indicate wide variability among species and cultivars: for instance, the percentage of viable pollen grains was reported as 17.3 % in the cultivar *'Peter Slocum'*, 19.3 % in *N. colorata*, 10.3 % in *N. micrantha*, and 17.6 % in *N. gigantea* (Sun *et al*., 2018). Such data underline the need for detailed palynological and viability assessments, especially in breeding and hybridization programs.

Scanning electron microscopy (SEM) has emerged as a powerful tool for fine-scale palynological studies, providing detailed visualization of exine architecture, apertures, and sculpturing patterns (Ridgway and Skyvarla, 1969; Prejith *et al.,* 2025). Bodhipadma *et al*. (2013) observed, via SEM, that *Nymphaea nouchali* var. *versicolor* pollen is ellipsoidal and heteropolar, with a ring-like sulcus and psilate surface. Zhang *et al*. (2022) compared pollen morphology of hardy and tropical *Nymphaea* hybrids under SEM and found clear structural correlations with cross-compatibility and floral phenology, demonstrating the value of such analyses for both taxonomy and breeding.

While palynological studies have been conducted on selected *Nymphaea* species, comprehensive comparative analyses of hybrid cultivars remain scarce. The ornamental hybrids of *Nymphaea* 'Blue Whistle', 'Carla's Sonshine', and 'Poonsup' represent tropical day-blooming cultivars with distinct floral morphologies. However, detailed documentation of pollen exine features, aperture morphology, and viability in these cultivars is lacking. The present investigation aimed to study the performance of three tropical *Nymphaea* cultivars for their morphological and floral characters, pollen morphology and surface ornamentation using SEM and correlate structural features with reproductive performance.

Materials and methods

Plant material: Three tropical day-blooming *Nymphaea* cultivars *viz*. 'Blue Whistle', 'Carla's Sonshine' and 'Poonsup' were selected for the study (Fig. 1). The plants were maintained in circular plastic tubs of diameter 45 cm for a period of two years at the Instructional Farm, College of Agriculture, Vellayani (8°25′N, 76°59′E, 29 m above MSL), Kerala, India. All the plants were grown under identical cultural and management practices, ensuring uniform nutrition and pest management regimes throughout the study period. The experiment was conducted in a completely randomized block design (CRD) with five replications.

Flowering phenology and floral traits: Floral behaviour was observed on five tagged plants per cultivar under natural conditions. Three buds from each replication were tagged when they first appeared at the bottom of the plant on the surface of the mud. The tagged buds were observed until they reached the water surface, and the number of days taken was recorded. The time and duration of flower opening and closing were recorded at 15-minute intervals on the first day of anthesis. Field life was noted as the number of consecutive days each flower remained open. The duration from bud emergence at the water surface to full bloom was recorded in days, while the time taken for bud emergence from mud to the surface was also measured. Monthly flower production per plant was recorded. Stigma receptivity was determined by noting the presence of stigmatic exudate in the stigmatic cup, with duration recorded in minutes from the pre-anthesis stage onwards.

Qualitative floral traits (inner and outer petal colour, pedicel and stamen colour, sepal shape, petal apex form) and quantitative characters (flower bud length, flower diameter, pedicel perimeter, number and size of sepals and petals, number and length of stamens, number of stigmatic appendages, and stigmatic cup diameter) were recorded. For the assessment of colour characteristics, the Royal Horticultural Society (RHS) colour chart was used.

Pollen viability assessment: Freshly dehisced anthers were collected during the second day of anthesis between 07:30 and 09:00 h. Pollen grains were acetolysed following the standard protocol of Nair (1970) and stained with 2% acetocarmine in a 1:1 glycerin-water mixture. Two slides were prepared per cultivar, and four random microscopic fields per slide were examined under a compound microscope at 40 x magnification. Pollen grains that were small, unfilled, shriveled, or partially stained were classified as sterile, while those that were uniformly stained and turgid were considered fertile. Pollen viability (%)

Fig.1. The *Nymphaea* varieties used for the study (a) Blue Whitsle, (b) Carla's Sonshine, (c) Poonsup

was calculated as:

Fertility (%) = [(Number of fertile pollen grains /Total number of pollen grains observed) x 100]

The mean values from replicate slides were used for statistical comparison among cultivars.

Scanning Electron Microscopy (SEM): Pollen microstructural analysis was carried out at the Central Laboratory for Instrumentation and Facilitation (CLIF), University of Kerala, Karyavattom Campus, Thiruvananthapuram. Flowers were collected at full anthesis (second day) when anther dehiscence was complete. Pollen grains were prepared for scanning electron microscopy following Erdtman (1953) with minor modifications.

Dried pollen grains were mounted on aluminium stubs using double-sided conductive carbon tape and sputter-coated with gold (Quorum SC7620) for 90 s. Imaging was performed using a ZEISS EVO-18 Scanning Electron Microscope operated at 15 kV. For each cultivar, 20 randomly selected pollen grains were measured for polar axis length (P) and equatorial diameter (E) using the in-built measurement software. The shape index (P/E ratio) was calculated following Wang (1955), and pollen size was expressed as P × E (Erdtman, 1969). Observations were recorded from representative polar and equatorial views, with attention to exine sculpturing and aperture morphology.

Data analysis: All recorded data were statistically analyzed using one-way analysis of variance (ANOVA) to test for differences among cultivars. Mean values were expressed as mean±standard deviation (SD).

Results and discussion

Flowering phenology and floral biology: Anthesis timing and duration varied significantly among cultivars and across seasons (Table 1). Flowers commenced opening between 07:00 and 09:15 h and closed between 16:00 and 18:15 h. The duration of anthesis was strongly influenced by environmental factors, particularly temperature, which exerted a greater effect than light intensity. During June to October, persistent rainfall and overcast conditions delayed flower opening and hastened closure. Conversely, the most extended anthesis durations were recorded during November to February, with mean values of 675 min in 'Blue Whistle', 465 min in 'Carla's Sonshine', and 680 min in 'Poonsup'. Similar durations were maintained during March to May, whereas monsoon conditions reduced the duration to 465, 285, and 375 min, respectively. The distinct seasonal trend in anthesis duration was therefore evident among the cultivars, with extended flowering periods during cooler months and shortened durations during the monsoon. These results affirm strong environmental regulation of floral opening and closing. Similar findings were reported by Sridith *et al*. (2007) and Bodhipadma *et al*. (2013), who demonstrated that temperature, humidity, and light intensity collectively determine anthesis patterns in *Nymphaea*. The relatively prolonged anthesis observed in 'Blue Whistle' and 'Poonsup' compared with'Carla's Sonshine' reflects cultivar-specific thermal sensitivity. Such photothermal responsiveness is well recognized in *N. rubra* and *N. caerulea*, wherein cooler night temperatures promote prolonged floral longevity (Kong *et al*., 2019). Recent molecular evidence further supports the influence of auxin-mediated signaling and cell wall remodeling

Table 1. Anthesis time of the three *Nymphaea* cultivars

Cultivar	November-February	Total duration (Minutes per day)	March-May	Total duration (Minutes per day)	June-October	Total duration (Minutes per day)
'Blue Whistle'	7:00 am to 6:15 pm	675	7:00 am to 6:15 pm	675	9:00 am to 4:45 pm	465
'Carla's Sonshine'	9:15 am to 5:00 pm	465	9:15 am to 5:00 pm	465	11:15 am to 4:00 pm	285
N 'Poonsup'	7:15 am to 6:35 pm	680	7:15 am to 6:15 pm	660	9:45 am to 4:00 pm	375

in controlling circadian flower movements in waterlilies (Ki *et al*., 2018).

All three cultivars exhibited a floral life span of 3-4 days under field conditions. The flowers were functionally protogynous, remaining in the female phase on the first day, characterized by visible stigmatic exudation, and shifting to the male phase over the subsequent three days, as indicated by pollen dehiscence. This diurnal rhythm of early-morning opening and late-evening closure conforms to the day-blooming behaviour typical of tropical *Nymphaea* species (Bhunia and Mondal, 2012). The synchronization of anthesis with pollinator activity, particularly of bees and beetles (Osborn *et al*., 1991; Dkhar *et al*., 2013), suggests adaptive evolution for maximizing reproductive success.

The flowers produced abundant nectar (1-2 mL per flower), thereby attracting a high level of pollinator activity. However, the window of effective pollination was narrow, with stigma receptivity lasting only 22-25 h. Comparable receptivity periods have been recorded in *N. pubescens* and *N. rubra*, where stigmatic secretion begins prior to anthesis and ceases within 24-30 h (Murthy, 2000; Gandhi, 2014). The variation observed in stigma cup diameter and number of stigmatic appendages among cultivars indicates genetic differences that may affect female fertility and compatibility.

The timing and duration of anthesis in these tropical *Nymphaea* cultivars not only reflect environmental regulation but also carry important ecological implications. Early-morning flower opening coincides with the peak activity of insect pollinators such as bees and beetles, facilitating efficient pollen transfer and enhancing reproductive success. Such temporal synchronization between floral anthesis and pollinator foraging is a key adaptive trait in aquatic habitats, where temperature, humidity, and light strongly influence pollinator dynamics. However, shifts in environmental factors like rainfall or temperature could disrupt this synchrony, potentially reducing pollination efficiency and seed set (Lawson *et al*., 2019).

Significant variability was observed among the three cultivars for both floral quantitative (Table 2) and qualitative traits (Table 3). All cultivars produced solitary, hermaphroditic flowers arising from the leaf axils. Buds were broad and rounded, distinct from conical leaf primordia. The progression of buds from rhizome emergence to the water surface paralleled leaf development, taking 7.08±0.17 days in 'Blue Whistle', 10.31±0.39 days in 'Carla's Sonshine', and 9.24±0.13 days in 'Poonsup'. The interval from surface emergence to full anthesis ranged between 1.53±0.15 and 2.57±0.21 days, consistent with the 12-day bud-to-bloom period reported for *N. thermarum* (Povilus *et al*., 2015).

Stamens were arranged spirally, each comprising a filament, an introrse anther, and a terminal sterile appendage. The outermost stamens were the longest, with gradually decreasing length toward the inner whorls. Filaments in the outer whorl were dorsiventrally flattened and slightly dilated at the base. Prior to anthesis, stamens were incurved over the receptacle; at maturity, they became sickle-shaped. Dehiscence initiated in the outer stamens, releasing limited pollen, while stigma receptivity was indicated by colourless, tasteless nectar on the stigma disc. On the second day, dehiscence advanced centripetally, producing abundant pollen, and the inner stamens and carpellary appendages converged to shield the stigma. By the final day, petal margins contracted, stamens darkened and wilted, and the floral head bent below the water surface, marking senescence.

Distinct stigma receptivity patterns were observed among the three *Nymphaea* cultivars. In 'Blue Whistle', the stigma remained receptive from 15:30 h on the previous day of flower opening (Day 0) until 17:00 h on the following day (Day 1), lasting for approximately 25 h 30 min. The cultivar exhibited a relatively large stigmatic cup with a diameter of 17.41±0.76 mm and possessed 19.83±0.25 appendages. In contrast, 'Carla's Sonshine' showed a shorter receptive period of 22 h 15 min, extending from 17:30 h (Day 0) to 16:45 h (Day 1), with a smaller stigmatic cup measuring 11.88±0.11 mm and 17.73±0.49 appendages. 'Poonsup' displayed an intermediate pattern, remaining receptive from 15:15 h (Day 0) to 14:45 h (Day 1) for about 23 h 30 min, and was characterized by a stigmatic cup diameter of 11.79±0.43 mm with 19.84±0.51 appendages. These differences in the duration and morphology of stigma receptivity indicate cultivar-specific regulation of female fertility, reflecting inherent genetic variation in floral physiology among the tropical *Nymphaea* hybrids.

Table 2. Floral quantitative characters of three *Nymphaea* cultivars

Cultivar	Days to appearance of flower bud	Days to flower opening	Bud length (cm)	Flower diameter (cm)	Sepal length (cm)	Sepal width (cm)	Number of petals	Number of stamens	Diameter of stigmatic cup (mm)	Number of stigmatic appendages
'Blue Whistle'	7.08±0.17[c]	1.56±0.20[b]	5.07±0.23[a]	10.14±0.12[a]	4.81±0.14[a]	2.10±0.04	24.46±0.94[b]	66.13±1.81[c]	17.41±0.76[a]	19.83±0.25[a]
'Carla's Sonshine'	10.31±0.39[a]	1.53±0.15[b]	4.65±0.25[b]	7.41±0.73[b]	4.15±0.09[c]	2.13±0.11	19.09±0.26[c]	106.62±1.39[a]	11.88±0.11[b]	17.73±0.49[b]
'Poonsup'	9.24±0.13[b]	2.57±0.21[a]	4.26±0.09[b]	9.92±0.28[a]	4.47±0.18[b]	1.83±0.21	28.04±0.45[a]	96.62±1.89[b]	11.79±0.43[b]	19.84±0.51[a]
SE(m)	0.15	0.11	0.12	0.26	0.08	0.08	0.36	0.99	0.29	0.25
CD(5%)	0.51	0.37	0.41	0.91	0.28	-	1.24	3.42	1.02	0.87

Values are mean of five replications; SE (m): Standard error of mean; CD: Critical difference; Superscripts with the same alphabet indicate on par values and those in different alphabets indicate significant difference at 5 per cent level of significance.

The observed inter-cultivar differences in stigma receptivity and floral longevity may be attributed to variations in endogenous hormonal regulation, particularly auxin and ethylene signaling pathways that modulate flower opening, stigma secretion, and senescence in *Nymphaea* and other angiosperms (Ke *et al*., 2018; van Doorn and Kamdee, 2014). Genetic differentiation influencing floral morphogenesis and ovule development, as demonstrated in *Nymphaea thermarum*, may also underlie the cultivar-specific timing of anthesis and duration of receptivity (Povilus *et al*., 2015).

The cultivars displayed distinct petal colours and morphologies (Table 3). 'Blue Whistle' exhibited vivid violet petals (RHS 86D) with lighter centres and acute apices, while 'Carla's Sonshine' and 'Poonsup' produced light yellowish-green petals (RHS 2C), the latter exhibiting pale purplish-blue tips (RHS 112B). Although all cultivars shared fundamental features like hermaphroditic, cup-shaped buds and dish-shaped mature corollas variations were observed in floral dimensions, sepal morphology, and petal count . These observations are congruent with those reported for tropical hybrids of *N. nouchali* and *N. colorata* (Choudhury *et al*., 2013; Zhang *et al*., 2022). The violet pigmentation of 'Blue Whistle' and the yellow-green to bluish hue of 'Poonsup' correspond to anthocyanin and carotenoid biosynthesis regulated by ANS and CHS gene expression (Ma *et al*., 2019). Colour variation among these cultivars thus reflects the genetic plasticity of pigment biosynthetic pathways-a hallmark of modern *Nymphaea* breeding (Xiong *et al*., 2023).

Table 3. Floral qualitative characters of three *Nymphaea* cultivars

Qualitative characters	'Blue Whistle'	'Carla's Sonshine'	*N* 'Poonsup'
Flower colour	Brilliant violet petals (RHS 86D) with light violet centre	Light Yellowish Green (RHS 2C)	Light yellowish green petal (RHS 2C)
Petal apex	Acute	Obtuse	Obtuse
Sepal shape	Linear lanceolate	Linear lanceolate	Ob lanceolate

Pollen viability: Pollen viability plays a crucial role in ensuring successful hybridization and seed set (Hu *et al*., 2017). Viable pollen grains in the examined *Nymphaea* cultivars appeared spheroidal, evenly stained, and turgid, whereas sterile grains were collapsed, shriveled, and lightly stained (Fig. 1). The mean pollen viability varied among cultivars, being 60.00 % in 'Blue Whistle', 42.16 % in 'Carla's Sonshine', and 80.92 % in 'Poonsup' (Table 4). The relatively higher fertility of 'Poonsup' indicates superior male gametophytic performance, suggesting its potential suitability as a pollen parent in future breeding programs. Comparable fertility ranges (10-40%) have been reported in *N. colorata* and *N. gigantea* (Sun *et al*., 2018). Environmental stress factors such as high humidity and fluctuating temperature during pollen maturation are known to adversely affect viability (Deng *et al*., 2017). Therefore, the observed inter-cultivar variation likely results from combined genetic and microclimatic influences. The uniformity in pollen shape and smooth exine texture observed here is typically associated with entomophilous (insect-mediated) pollination systems (Walker, 1974; Bhunia and Mondal, 2012).

Morphologically, pollen grains across all cultivars were monads, single, and oblate in shape, with a triangular outline in polar view

Table 4. Pollen characters and fertility percentage of three *Nymphaea* cultivars

Cultivar	'Blue Whistle'	'Carla's Sonshine'	N. 'Poonsup'	SE(m)	CD
Length of Polar Axis/ (µm)	18.99 ±2.10	21.64 ±3.40	19.66 ±1.65	1.02	-
Length of Equatorial Axis (µm)	20.62 ±1.86b	25.18 ±2.76a	22.14 ±1.47b	0.86	2.58
Polar Axis/ Equatorial Axis	0.92 ±0.05	0.86 ±0.09	0.89 ±0.04	0.03	-
Pollen Size (Polar Axis × Equatorial Axis) (µm^2)	394.30 ±79.94b	550.35 ±124.06a	436.76 ±61.79ab	37.71	113.68
Pollen Shape	Oblate spheroidal	Oblate spheroidal	Oblate spheroidal	-	-
Pollen fertility (percentage)	60.00±00b	42.16 ±1.90c	80.92 ±0.91a	0.7	2.43

Values are mean of five replications; SE (m): Standard error of mean; CD: Critical difference; Superscripts with the same alphabet indicate on par values and those in different alphabets indicate significant difference at 5 per cent level of significance.

and elliptical to boat-shaped contour in equatorial view. The equatorial axis ranged from 20.62 to 25.18 µm, and the polar axis from 18.99 to 21.64 µm, classifying them as medium-sized pollen according to Erdtman (1969). Based on Wang's (1955) shape classification, all pollen grains were oblate-spheroidal (P/E ratio < 1). Among cultivars, 'Carla's Sonshine' possessed significantly larger equatorial dimensions (P< 0.05), while no significant differences were observed in polar axis length. This suggests that lateral expansion, rather than elongation, represents the principal morphological axis of variation, potentially reflecting evolutionary adaptation to differing pollination ecology or hybrid ancestry (Volkova and Shipunov, 2007).

Scanning electron microscopy (SEM) revealed consistent exine ornamentation among the three cultivars (Fig. 2). Pollen grains were ellipsoidal, heteropolar, and zonosulcate, exhibiting a distinct equatorial sulcus encircling the grain. The exine surface was psilate, characterized by a smooth, low-relief texture devoid of spines, muri, or reticulation on both distal and proximal faces. Such psilate ornamentation, typical of tropical *Nymphaea* species, was also reported in *N. caerulea*, *N. micrantha*, and *N. stellata* (Murthy, 2000; Dkhar *et al*., 2013; Bhowmik and Datta, 2023). The absence of sculpturing elements suggests a derived palynological trait distinguishing *Nymphaea* from primitive genera such as *Barclaya*, which exhibit reticulate exine patterns (Walker, 1974; Osborn *et al*., 1991).

The mean dimensions recorded in this study (polar axis 18.99-21.64 µm; equatorial axis 20.62-25.18 µm) are consistent with *N. nouchali* var. versicolor (Bodhipadma *et al*., 2013) and fall within the medium-sized class. The uniform oblate form (P/E ratio < 1) is interpreted as an adaptation to aquatic environments, where efficient pollen deposition on the stigmatic surface is crucial for fertilization (Hu *et al*., 2017). The shared psilate exine

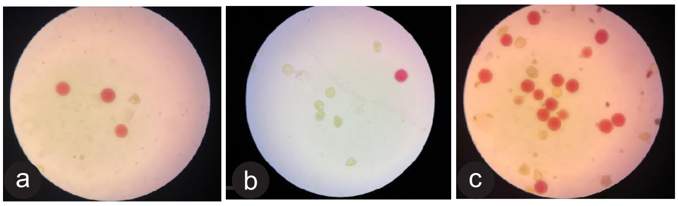

Fig 1. Light micrograph of pollen grains (40x) : (a) *Nymphaea* 'Blue Whitsle' (b) *Nymphaea* 'Carla's Sonshine' (c) *Nymphaea 'Poonsup'*

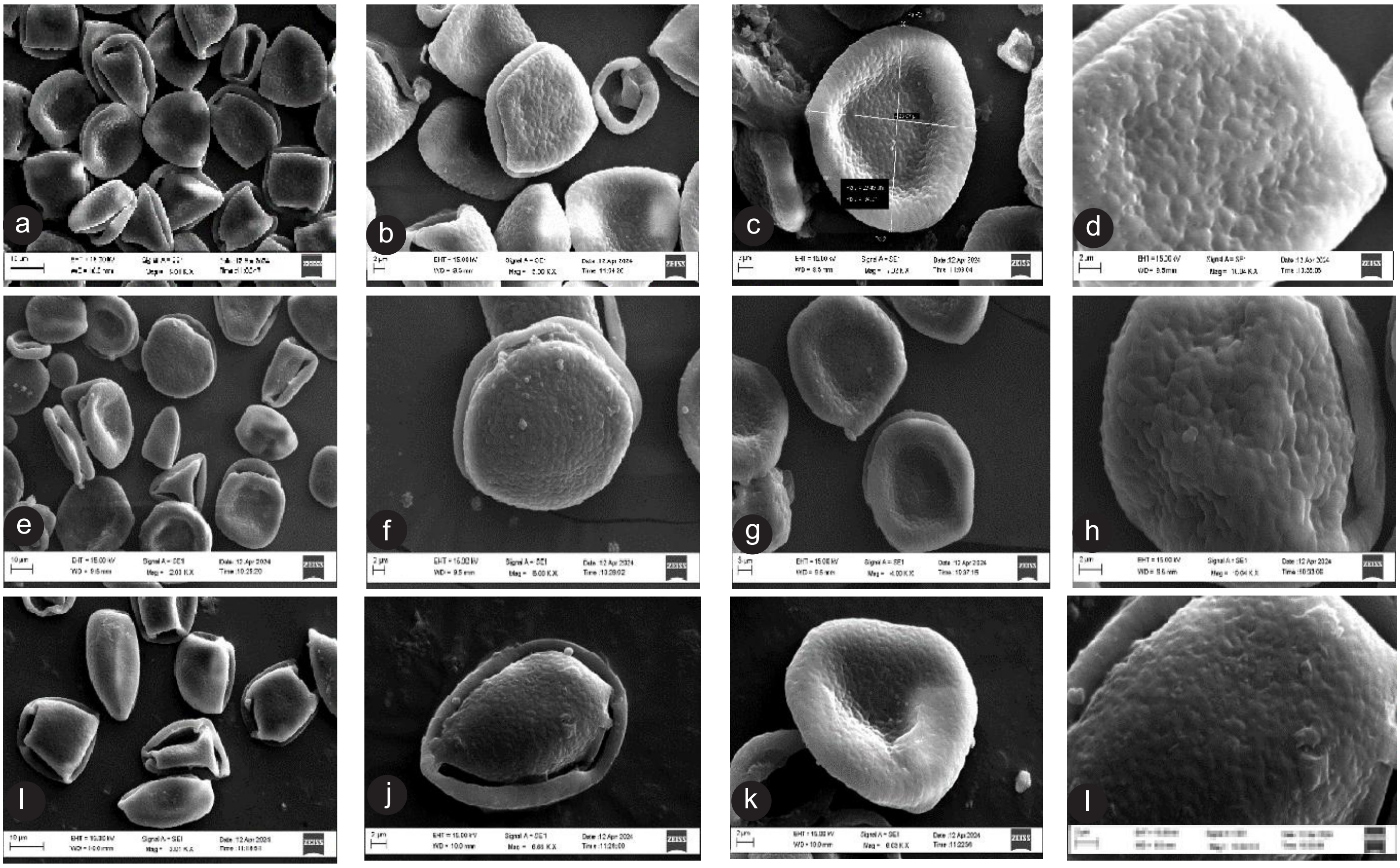

Fig. 2. Scanning Electron Microscopy (SEM) micrographs of the pollen grains: (a) to (d) SEM micrographs of the pollen grains of *Nymphaea* 'Blue Whitsle' (a) pollen population (3000x), (b) Equatorial view(5000x), (c) Polar view (7000x), (d) Surface ornamentation, (10000x), (e) to (f) SEM micrographs of the pollen grains of *Nymphaea* 'Carla's Sonshine', (e) pollen population (2000x), (f) equatorial view (5000x), (g) polar view (4000x), (h) surface ornamentation (10000x), (i) to (l) SEM micrographs of the pollen grains of *Nymphaea* 'Poonsup', (i) pollen population (3000x), (j) equatorial view (6660x), (k) polar view (8000x), (l) surface ornamentation(10000x).

and zonosulcate aperture across cultivars indicate phylogenetic affinity and reinforce their close genetic relationship, consistent with their common tropical, day-blooming habit.

The documented variability in pollen viability, stigma receptivity, and anthesis duration among the cultivars provides practical cues for synchronizing parental lines during controlled hybridization and enhancing cross-compatibility in breeding programs. Moreover, the characterization of floral and palynological traits in these tropical hybrids establishes baseline data essential for conserving genetically distinct cultivars and guiding the selection of parental genotypes with superior reproductive efficiency and ornamental traits.

The present study revealed significant cultivar-specific and seasonal variation in the flowering phenology, floral biology, and pollen characteristics of three tropical *Nymphaea* cultivars- 'Blue Whistle', 'Carla's Sonshine', and 'Poonsup'. Anthesis timing and duration were strongly influenced by temperature, with flowers opening earlier and remaining open longer during the cooler months (November to February) and showing reduced duration under monsoon conditions (June to October). 'Blue Whistle' and 'Poonsup' exhibited longer anthesis periods, reflecting greater adaptability to environmental fluctuations. All cultivars were protogynous, with flowers remaining in the female phase on the first day and transitioning to the male phase thereafter. The brief stigma receptivity (22 to 25 h) and variation in stigmatic morphology indicate genetic control of female fertility. Pollen viability ranged from 42.16 % to 80.92 %, with 'Poonsup' showing superior viability, suggesting its potential as a pollen parent in breeding programs. Pollen grains were oblate-spheroidal, medium-sized, and psilate with a zonosulcate aperture -traits typical of tropical *Nymphaea* species. These findings highlight the combined role of genetic and environmental factors in determining reproductive success. The cultivar-specific differences documented provide valuable insights for hybridization, selection, and conservation of *Nymphaea* germplasm, contributing to the development of hybrids with enhanced floral longevity and ornamental value.

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