

# SEED MORPHOMETRY AND ECOLOGICAL ADAPTATIONS IN A FEW ORCHID SPECIES FROM EASTERN GHATS, ANDHRA PRADESH, INDIA

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## Abstract

The present study examines the seed and embryo morphometry of eight orchid taxa occurring across diverse ecological habitats of Andhra Pradesh, India, so as to understand their adaptive strategies. Significant interspecific variations were observed in seed size and shape, testa cell dimensions, and embryo volume, indicating ecological specialization amongst terrestrial and epiphytic orchids. Seed length ranged from 0.0745 mm in *Eulophia graminea* to 0.6933 mm in *Cymbidium aloifolium* while seed width varied between 0.0184 mm and 0.2042 mm. The length-width ratio showed marked diversity, with the highest value (4.82) in *Rhynchosstylis retusa* and the lowest (2.40) in *Oberonia ensiformis* reflecting habitat-driven seed shape plasticity. Seed volume varied over 100-fold (0.0000544-0.0062444 mm<sup>3</sup>), indicating differences in dispersal potential. The testa cell measurements also differed considerably, from 44.63 µm in *Vanda testacea* to 243.62 µm in *R. retusa*. Embryo length ranged from 0.0653 to 0.2756 mm, with embryo volume spanning 0.000012-0.000300 mm<sup>3</sup>. Seed-to-embryo volume ratios revealed low embryo occupancy (1.11-20.81%), except in *C. aloifolium*, indicating high air-space content in most taxa. Air space ranged from 9.53% to 95.20%, highlighting contrasting dispersal and germination strategies. Overall, terrestrial species tended to possess smaller seeds with high air space enhancing wind dispersal, whereas epiphytic orchids such as *Acampe ochracea* and *R. retusa* exhibited larger embryos and thicker testa cells, reflecting adaptation to humid canopy habitats. These morphometric insights provide essential baseline data for conservation, propagation, and habitat-specific restoration of native orchids.

## Introduction

THE FAMILY Orchidaceae represents one of the largest and most diverse families of angiosperms, comprising nearly 29,481 orchid species worldwide (WFO, 2023) distributed across 693 genera (POWO, 2025). In India, the family is represented by 1256 species belonging to 155 genera (Singh *et al.*, 2019) which accounts for nearly 10% of the Indian flora (Kumar and Manilal, 1994). Orchid seeds are extremely minute, light-weighted, and non-endospermic. These show extensive variations in size, shape, colour, and surface morphology-features that carry considerable ecological importance. For most of the orchid species, seed size typically ranges between 300-800 µm (Molvray and Kores, 1995). These seeds also display a remarkable diversity, varying from 0.1 mm in *Oberonia* to nearly 6 mm in *Epidendrum*, and often possess complex seed coat architecture with intricate, hierarchical sculpturing (Barthlott *et al.*, 2014). The taxonomic importance of orchid seed morphology was first emphasized by Clifford and Smith (1969). Seed characters are not only valuable as taxonomic markers, but these also assist in inferring phylogenetic relationships (Barthlott, 1976) and identifying hybrids (Arditti *et al.*, 1979). Verma *et al.* (2014) analysed the seed traits of 32 threatened orchids from the Western Himalayas through light and scanning electron

microscopy, underscoring the usefulness of seed characters in clarifying taxonomic and phylogenetic affinities. They documented a wide diversity of seed shapes including fusiform, spathulate, ovoid, and filiform forms; their study revealed that epiphytic species may contain up to 79% air space within the seed, greatly enhancing dispersal ability. They further observed that terrestrial orchids typically possess higher seed volume-to-embryo volume ratios. Previous researchers have highlighted the significance of seed morphology in species delimitation and in defining subgeneric groupings (Augustine *et al.*, 2001; Mathews and Levins, 1986; Vij *et al.*, 1992). Molvray and Kores (1995) observed a wide array of seed shapes in orchids, ranging from filiform and fusiform to clavate and ellipsoidal forms, sometimes with conspicuous wings. Detailed studies on structure of seed testa in orchids were conducted by Barthlott and Ziegler (1981) and they identified 20 distinct seed types using various morphological traits.

The importance of seed attributes in orchid taxonomy was first recognized by Clifford and Smith (1969), and later by Dressler (1981). Dressler (1993) proposed classification system based on micro morphological characters. According to Larry (1995) and Mathews and Levins (1986), seed micromorphology provides

systematic insights useful for defining subgeneric boundaries and hypothesizing relationships amongst congeneric species. Based on Scanning Electron Microscope (SEM) studies, Barthlott (1976) indicated that seed morphology provides valuable clues to phylogenetic relationships. Arditti (1979) indicated that seed volume reflects seed size, and Arditti *et al.* (1980) reported that the seed length-to-width ratio (L/W) yields important data regarding the degree of truncation. Healey *et al.* (1980) studied the morphometry of orchid seeds from California, providing detailed measurements of seed size, embryo dimensions, and testa cell structure. Their findings highlighted notable interspecific variations and these demonstrated how seed micromorphological traits reflect ecological adaptations to the diverse climatic and habitat conditions of California's orchid flora. Augustine *et al.* (2001) further observed that testa cells often show reticulation, which varies amongst different orchid genera.

Barthlott and Ziegler (1981) classified orchid seeds into 20 distinct categories based on parameters, such as seed shape, dimensions of testa cells, surface sculpturing, presence of intercellular gaps, and beading patterns. Several comprehensive reviews on orchid seed micromorphology and its taxonomic relevance were later provided by Arditti and Ghani (2000); Chase and Pippen (1988, 1990); Barthlott (1976); Thoda (1986); Kurzweil *et al.* (1991). Vij *et al.* (1992) demonstrated that variations in testa thickening and sculpturing are closely linked to habitat type, with terrestrial orchids generally showing thinner testa thickenings whereas, epiphytic species exhibit more pronounced thickenings. Garg *et al.* (1992) carried out a detailed morphometric investigation of orchid seeds from the Western Himalayas and recorded considerable interspecific diversity in seed size, testa cell organization, and embryo dimensions. Their findings revealed that seed micromorphological traits are strongly associated with ecological adaptations, including dispersal efficiency, habitat preference, and altitude-related environmental stresses. This study emphasized the importance of seed characters not only in taxonomy but also in understanding ecological specialization amongst Himalayan orchids.

Through SEM studies on South African orchids, Kurzweil (1993) recognized two principal seed types: the *Satyrium* type, characterized by straight or slightly undulating anticlinal cell walls with marked thickening, and the *Disa uniflora* type, which possesses convex testa cells with sinuous anticlinal walls. Molvray and Kores (1995) described the orchid seed coat as a rigid outer layer made up of testa cells that encloses a loosely arranged, papery embryo. Further micromorphological

investigations using SEM were conducted by Augustine *et al.* (2001) on species of *Bulbophyllum*. Swamy *et al.* (2004), in their SEM-based investigations of orchids from Karnataka, documented rope-like twisted testa cells in *Aerides maculosum* and *Xenikophyton smeantum*, suggesting a close taxonomic affinity between these species. These authors also observed that relative seed truncation was highest in *Coelogyne breviscapa* and lowest in *Eria dalzelli*. Additionally, they noted that embryo volume changes during different developmental stages and that seeds with larger internal air spaces possess greater dispersal potential. Sharma *et al.* (2004) reported spindle-shaped seeds in *Paphiopedilum*.

Gamarra *et al.* (2007) studied seed micromorphology within the tribe Neottinae and reported that all examined species possess fusiform seeds with transverse ridges on the inner periclinal walls; these features are considered diagnostic for the group. Subsequent studies by Gamarra *et al.* (2008, 2012) on members of the subtribe Orchidinae further confirmed the taxonomic significance of testa sculpturing patterns. Aytasakcin *et al.* (2009), while studying Turkish orchids, emphasized the diagnostic value of seed length, testa reticulation, seed volume-to-embryo volume ratios, and internal air space. Chaudhary *et al.* (2014), in a comparative study of *Dendrobium* species, reported that orchids from temperate regions tend to have larger seeds and higher seed volume-to-embryo volume ratios than those from tropical and subtropical regions; whereas, tropical and subtropical taxa generally exhibit higher air space percentages. Hariyanto *et al.* (2020) highlighted that most contemporary studies on orchid seed micromorphology rely on light microscopy, scanning electron microscopy, and simple geometric morphometry using parameters, such as seed length, width, and length-to-width ratios. Seed and embryo volumes are typically estimated using sphere or ellipsoid formulae (cf. Arditti and Ghani, 2000). However, some authors have pointed out limitations of these formulae when seeds do not conform to ideal geometric shapes and have suggested alternative methods, such as direct measurements between testa and embryo or image-based volume estimation. Many studies also compute the percentage of free air space as an indicator of dispersal potential, along with embryo-to-seed volume or area ratios to assess germinability and ecological strategy. Collier *et al.* (2023) identified a consistent pattern across multiple regional studies showing that terrestrial orchids generally have larger seeds and embryos with lower percentages of air space. In contrast, epiphytic and other wind-dispersed dust-seed orchids tend to possess smaller seeds, higher air space, and morphologies highly adapted for long-

distance wind dispersal. This life-form related pattern has been confirmed in orchids from Hawaiian, tropical, temperate, Neotropical, and SouthEast Asian regions, although certain exceptions occur, particularly amongst species with specialized dispersal mechanisms or mycoheterotrophic life histories. According to Lee *et al.* (2023), SEM and developmental studies indicate that orchid seed coats are typically extremely thin, often consisting of only one to a few cell layers, and may display reticulations or surface ornamentation that vary amongst orchid taxa. Seed coat structure including thickness and surface sculpture plays a crucial role in regulating water uptake, dormancy, and germination, as well as in interactions with soil and microbial environments necessary for mycorrhizal colonization. The authors also emphasized that seed coat chemistry, including phenolic and lipid components, can significantly influence seed dormancy and viability.

According to Oikonomidis *et al.* (2024), per cent free air space is widely used as an indicator of buoyancy and wind dispersal potential. Studies showed that species with high free air space tend to have broader geographical ranges and greater dispersal ability; low air space is often found in endemics or species with restricted distributions. However, some authors are of the view point that calculating air space using simple formulae can give misleading (even negative) values when seed shape or embryo placement deviates from assumed geometry, prompting calls for more direct measures (micro-CT, image stacks). They explained and compiled datasets and comparative analysis indicate the E:S ratio is a useful predictor of germination behaviour: species with relatively large embryos (high E:S) are more likely to germinate asymbiotically on nutrient media; while, tiny-embryo species frequently require specific mycorrhizal partners or fail to germinate in asymbiotic tests. Meta-analyses across ~200 species have formalized this link and recommended integrating E:S into conservation propagation planning.

Hariyanto *et al.* (2020) reviewed orchids from various tropical and temperate regions (Indonesia, India, Japan, Neotropics, Hawaii, Europe) and multiple genera, including *Bulbophyllum*, *Dendrobium*, *Habenaria*, *Lycaste*, *Ophrys*, among others. However, the coverage remains uneven, with a notable bias toward epiphytic tropical genera and readily accessible temperate floras. In contrast, many understory terrestrial orchids from tropical forests have been poorly sampled.

## Material and Methods

### Collection of Mature Capsules and Seeds

Seeds of eight wild orchid species (3.0-4.5 months after pollination) were collected from different regions of Andhra Pradesh during 2022-2024 for the present study. The species included *Acampe ochracea* (Lindl.) Hochr., *Cymbidium aloifolium* (L.) Sw., *Eulophia graminea* (Lindl.), *Luisia zeylancia* (Lindl.), *Oberonia ensiformis* (Sm.) Lindl., *Rhynchostylis retusa* (L.) Blume., *Vanda testacea* (Lindl.) Rchb.f., and *V. tessellata* (Roxb.) Hook. ex G. Don. (Table 1). Freshly harvested seeds were air-dried for a minimum of two wks and subsequently stored in sealed tubes at 5°C under dry conditions.

### Seed Morphology and Micromorphology

Seed morphology and micromorphological features were documented using a Stereomicroscope, Light microscope (LM), and Scanning Electron Microscope (SEM). Morphological traits recorded were seed shape (SS), seed colour (SC), seed length (SL), seed width (SW), seed length-to-width ratio (SL/SW), and seed volume (SV). Embryo characteristics included embryo shape (ES), embryo colour (EC), embryo length (EL), embryo width (EW), embryo length-to-width ratio (EL/EW), embryo volume (EV), the ratio of seed volume to embryo volume (SV/EV), and percentage air space (AS) (Tables 2-3). Seed shape, embryo shape, seed colour, and embryo colour were observed using compound

Table 1. Orchid species collected from different places of Eastern Ghats of Andhra Pradesh, India.

Species	Localities	Habit	Time of fruiting
<i>Acampe ochracea</i> (Lindl.) Hochr.	Lothu gadda, Alluri Sitharama Raju district, A.P	Epiphyte	Mar-May
<i>Cymbidium aloifolium</i> (L.) Sw.	Banda veedhi, Alluri Sitharama Raju district, A.P	Epiphyte	Jan-Feb
<i>Eulophia graminea</i> Lindl.	Rampachodavaram, Alluri Sitharama Raju district, A.P	Terrestrial	Jan-Feb
<i>Luisia zeylancia</i> Lindl.	Pedagaruvu, Alluri Sitharama Raju district, A.P	Epiphyte	Jun-July
<i>Oberonia ensiformis</i> (Sm.) Lindl.	Bandabayalu, Alluri Sitharama Raju district, A.P	Epiphyte	Sept-Oct
<i>Rhynchostylis retusa</i> (L.) Blume.	Pedabayalu, Alluri Sitharama Raju district, A.P	Epiphyte	July-Aug
<i>Vanda testacea</i> (Lindl.) Rchb.f.	Lotugadda, Alluri Sitharama Raju district, A.P	Epiphyte	Mar-Apr
<i>V. tessellata</i> (Roxb.) Hook. ex G. Don.	P.G Madugula, Alluri Sitharama Raju district, A.P	Epiphyte	Apr-May

Table 2. Seed characters and quantitative data.

Species	Seed colour	Seed length (mm)	Seed Width(mm)	L/W	Seed volume (mm <sup>3</sup> ×10 <sup>-3</sup> )	Length of testa cells	Width of testa cells	number of testa cells
<i>Acampe ochracea</i> (Lindl.) Hochr.	Light Yellow	0.2230	0.0523	4.26	0.0002317 (0.2317 mm×10 <sup>-3</sup> )	96.72	13.67	3.98
<i>Cymbidium aloifolium</i> (L.) Sw.	Yellow	0.6933	0.2042	3.39	0.0062444 (6.2444 mm×10 <sup>-3</sup> )	100.45	37.73	6.95
<i>Eulophia graminea</i> Lindl.	Yellow	0.0745	0.0184	4.03	0.0000544 (0.0544 mm×10 <sup>-3</sup> )	45.52	17.34	8.35
<i>Luisia zeylancia</i> Lindl.	Yellow	0.1680	0.0695	2.41	0.0001752 (0.1754 mm×10 <sup>-3</sup> )	80.63	12.11	2.63
<i>Oberonia ensiformis</i> (Sm.) Lindl.	Light Yellow	0.1356	0.0563	2.40	0.0000912 (0.0912 mm×10 <sup>-3</sup> )	102.17	20.49	3.65
<i>Rhynchostylis retusa</i> (L.) Blume.	Light Yellow	0.2465	0.0500	4.82	0.0001831 (0.183 mm×10 <sup>-3</sup> )	243.62	73.69	4.64
<i>Vanda testacea</i> (Lindl.) Rchb.f.	Light Yellow	0.1926	0.0525	3.66	0.0001846 (0.1846 mm×10 <sup>-3</sup> )	44.63	11.28	4.32
<i>V. tessellata</i> (Roxb.) Hook. ex G. Don.	Yellow	0.2192	0.0663	3.30	0.0002081 (0.2081 mm×10 <sup>-3</sup> )	72.36	12.85	4.31

L, Seed length; W, Seed width

microscope. The colour descriptions were based on visual assessment. Measurements of SL, SW, EL, and EW at their maximum longitudinal and lateral axes were obtained using a light microscope (Olympus) fitted with a calibrated ocular micrometer.

The colours ranged from light yellow to green to brownish to orange. The measurement of embryo is done through light microscope, measuring its length and breadth of the embryo. As embryos are elliptical in cross section, their volume is calculated with the Arditti *et al.* (1979).

$$\text{Seed volume: } V_s = (W^2 \times L \times \pi) / 6$$

Where

L: Seed length (measured along the longest axis).

W: Seed width (measured along the widest axis).

$\pi$ : Pi, often approximated as 22/7 or 1.047 for  $\pi r^2 \pi / 3$  in the original formula text.

$$\text{Embryo volume} = 4/3 (1.047) (L/2) (W/2)$$

L: Embryo length (measured along the longest axis).

W: Embryo width (measured along the widest axis).

Table 3. Embryo characters and quantitative data.

Taxa	Colour	Length (mm)	Width (mm)	L/W	Embryo Volume mm <sup>3</sup> × 10 <sup>-3</sup>	Seed Volume to Embryo Volume	Air Space (%)
<i>Acampe ochracea</i> (Lindl.) Hochr.	Light yellow	0.1598	0.0562	2.84	0.000264 (0.264 mm×10 <sup>-3</sup> )	1.26	24.36
<i>Cymbidium aloifolium</i> (L.) Sw.	Light yellow	0.2756	0.0456	6.04	0.000300 (0.300 mm×10 <sup>-3</sup> )	20.81	95.20
<i>Eulophia graminea</i> Lindl.	Yellow	0.0653	0.0186	3.51	0.000012 (0.0120 mm×10 <sup>-3</sup> )	4.53	77.94
<i>Luisia zeylancia</i> Lindl.	Yellow	0.1015	0.0465	2.18	0.000115 (0.115 mm×10 <sup>-3</sup> )	1.52	34.36
<i>Oberonia ensiformis</i> (Sm.) Lindl.	Light yellow	0.0865	0.0195	4.43	0.000017 (0.0170 mm×10 <sup>-3</sup> )	5.37	81.42
<i>Rhynchostylis retusa</i> (L.) Blume.	Light yellow	0.1783	0.0378	4.71	0.000133 (0.133 mm×10 <sup>-3</sup> )	1.38	27.34
<i>Vanda testacea</i> (Lindl.) Rchb.f.	Light yellow	0.1167	0.0523	2.23	0.000167 (0.167 mm×10 <sup>-3</sup> )	1.11	9.53
<i>V. tessellata</i> (Roxb.) Hook. ex G. Don.	yellow	0.1532	0.0364	4.20	0.000106 (0.106 mm×10 <sup>-3</sup> )	1.96	49.06

L, Embryo length; W, Embryo width

Air space is calculated using the formula

$$\text{Air space} = (\text{Seed Volume} - \text{Embryo volume} / \text{Seed volume}) \times 100$$

The measurements for SL, SW, EL, and EW were taken from approximately 30 seeds for each species. Air space (%) was determined using the formula (seed volume - embryo volume) / seed volume)  $\times$  100%, following the method of Arditti and Ghani (2000). All quantitative data obtained in the present study were statistically analyzed using analysis of variance (ANOVA), followed by *Duncan's* multiple range test, performed with SPSS version 21.0 for Windows.

### SEM Analysis

For the SEM examination, seed samples were mounted on aluminium stubs and sputter-coated with a palladium/gold layer using a SC 7620 Mini Sputter Coater. The detailed surface morphology of the seed coat (testa cells) was observed using a Generation FEI QUANTA 200F SEM operating at an accelerating voltage of 15 kV. The main parameters assessed included testa cell sculpturing patterns and wall thickenings.

## Results

### Seed Shape and Testa Cell Characteristics

The presently investigated orchid species exhibited considerable interspecific variations in seed shape and testa cells morphology (Fig. 1 A-I). Most seeds were cylindrical to fusiform, with species such as *Acampe ochracea*, *Eulophia graminea*, and *Rhynchosstylis retusa*, and displaying elongated spindle-shaped seeds (Fig. 1 C, F, H), as reflected by their high length-width (L/W) ratios of 4.03, 4.82, and 4.26 respectively. In contrast, *Luisia zeylanica* and *Oberonia ensiformis* showed comparatively broader seeds with lower L/W ratios of 2.41 (Fig. 1G) and 2.40 (Fig. 1D) respectively. Testa cell structure also varied markedly amongst taxa (Fig. 2A-H). The longest testa cells were recorded in *R. retusa* (243.62  $\mu\text{m}$ ) (Fig. 2D) followed by *Cymbidium aloifolium* (100.45  $\mu\text{m}$ ) (Fig. 2C) and *O. ensiformis* (102.17  $\mu\text{m}$ ) (Fig. 2B). In contrast, *Vanda testacea* (Fig. 2G) exhibited the shortest testa cells (44.63  $\mu\text{m}$ ). Testa cell width ranged from 11.28  $\mu\text{m}$  (*V. testacea*), *V. tessellata* 12.85  $\mu\text{m}$  (Fig. 2H) to 73.69  $\mu\text{m}$  (*R. retusa*) (Fig. 2D).

The average number of testa cells per seed ranged between 2.63 (*L. zeylanica*) (Fig. 2E) and 8.35 *E. graminea* (Fig. 2A), indicating species-specific variation in seed surface patterning. These observations suggest that testa cell length and density are useful diagnostic features with potential taxonomic significance amongst the studied orchids.

### Seed Size and Seed Volume

Seed size differed significantly across species (Table 3). The longest seeds were recorded in *C. aloifolium* (0.6933 mm), followed by *R. retusa* (0.2465 mm) and *A. ochracea* (0.2230 mm). *Eulophia graminea* possessed the smallest seeds (0.0745 mm). Seed width followed a similar trend, with *C. aloifolium* (0.2042 mm) being the broadest, and *E. graminea* (0.0184 mm) being the narrowest. Seed volume was highest in *C. aloifolium* ( $6.2444 \times 10^{-3} \text{ mm}^3$ ), nearly 30 times higher than in *E. graminea* ( $0.0544 \times 10^{-3} \text{ mm}^3$ ) Table 2. Intermediate seed volumes were observed in *A. ochracea* ( $0.2317 \times 10^{-3}$

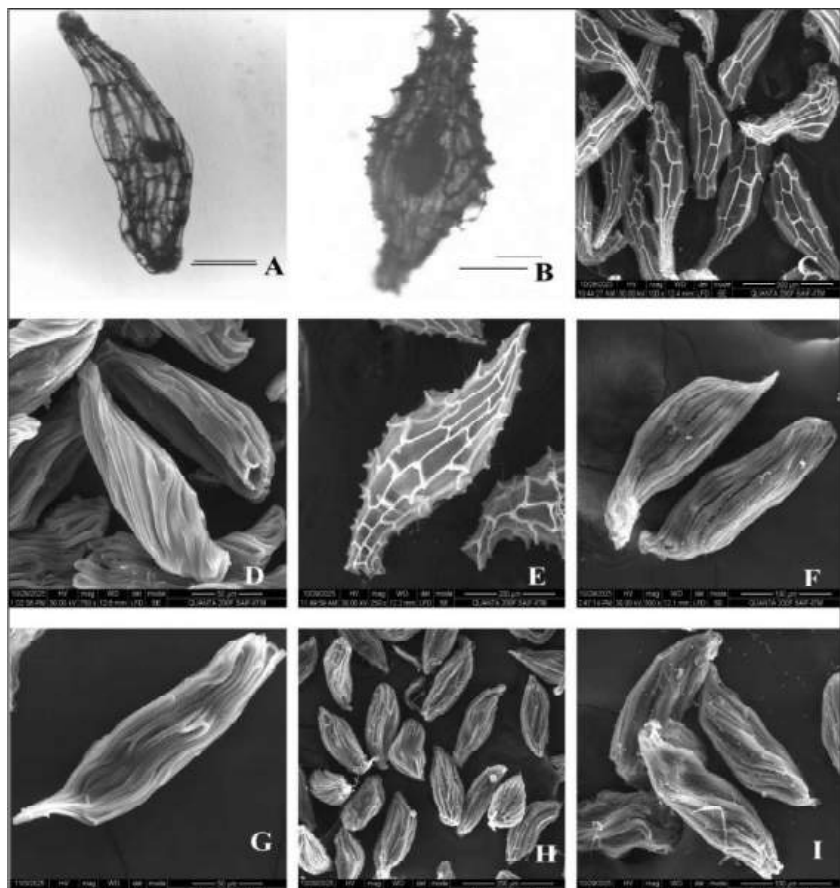


Fig. 1A-I. Seed morphometry in the studied orchid species: A, *Eulophia graminea* seed under high power microscope, Bar : 200  $\mu\text{m}$ ; B, *Cymbidium aloifolium* seed under high power microscope, Bar : 200  $\mu\text{m}$ ; C, *Acampe ochracea* seeds under SEM; D, *Oberonia ensiformis* seeds under SEM; E, *Cymbidium aloifolium* seeds under SEM; F, *Eulophia graminea* seeds under SEM; G, *Luisia zeylanica* seed under SEM; H, *Rhynchosstylis retusa* seeds under SEM; I, *Vanda testacea* seeds under SEM.

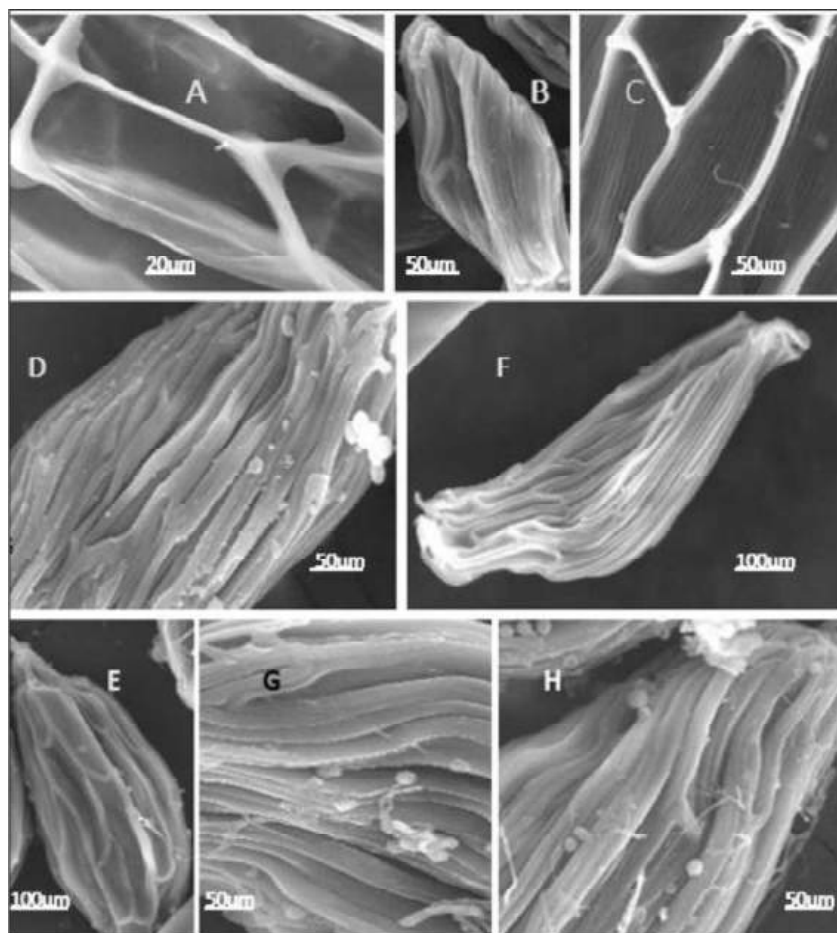


Fig. 2. Seed morphometry in the studied orchid species. Orchid seed testa cells: A, *Eulophia graminea*; B, *Oberonia ensiformis*; C, *Cymbidium aloifolium*; D, *Rhynchostylis retusa*; E, *Luisia zeylanica*; F, *Acampe ochracea*; G, *Vanda testacea*; H, *Vanda tessellata*.

mm<sup>3</sup>) and *V. tessellata* ( $0.2081 \times 10^{-3}$  mm<sup>3</sup>). The wide disparity in seed size and volume indicates adaptive differences among terrestrial (*Eulophia*) and epiphytic genera (*Acampe*, *Rhynchostylis*, *Vanda*), likely related to dispersal strategies and fruit dehiscence mechanisms.

#### Embryo Size and Embryo Volume

Embryo dimensions showed substantial variation. The largest embryos were recorded in *C. aloifolium* ( $0.2756$  mm  $\times$   $0.0456$  mm) (Fig. 1B) (Table 3) with a volume of  $0.300 \times 10^{-3}$  mm<sup>3</sup>, followed by *R. retusa* ( $0.133 \times 10^{-3}$  mm<sup>3</sup>). The smallest embryo volume was seen in *E. graminea* ( $0.012 \times 10^{-3}$  mm<sup>3</sup>) (Fig. 1A). The embryo L/W ratio ranged from 2.18 in *L. zeylanica* to 6.04 in *C. aloifolium*, showing that some species possessed more elongated embryos whereas others exhibited relatively rounded forms. Species of *Vanda* and *Acampe* possessed moderate embryo volumes ( $0.106$ - $0.264 \times 10^{-3}$  mm<sup>3</sup>), reflecting the generalized epiphytic orchid embryo architecture.

#### Seed Volume to Embryo Volume Ratio and Air Space Percentage

A notable feature of orchid seeds is the presence of substantial air space. The highest proportion of air space was observed in *C. aloifolium* (95.20%), followed by *O. ensiformis* (81.42%) and *E. graminea* (77.94%). In contrast, *V. testacea* exhibited the lowest air space (9.53%), indicating a relatively large embryo in proportion to seed size. The seed volume to embryo volume ratios reflected similar trends. *C. aloifolium* registered the highest ratio (20.81), whereas species such as *A. ochracea* (1.26), *L. zeylanica* (1.52), and *V. testacea* (1.11) showed lower ratios, implying more compact embryos with minimal air cavities. These variations suggest differential adaptations for seed dispersal. Species with higher air space likely possess seeds capable of enhanced buoyancy and extended wind dispersal. Species with lower air space percentages may exhibit shorter dispersal ranges or specialized dispersal strategies.

#### Fruiting Period and Seed Colour

Fruiting periods differed amongst the studied taxa, ranging from January-February in *E. graminea* and *C. aloifolium* to July-August in *R. retusa* and April-May in *V. tessellata*. (Fig. 1H). Seed colour ranged from yellow to light yellow, which is typical of mature orchid seeds.

## Discussion

The present study revealed significant interspecific variations in seed and embryo morphology among eight orchid species occurring in Andhra Pradesh. Seed size, testa cell architecture, embryo dimensions, and air-space percentage exhibited wide variability, emphasizing the complex evolutionary adaptations of orchids to their respective ecological niches.

#### Seed Shape, Testa Cell Patterning and Taxonomic Implications

The results showed that seed shape and testa morphology are useful diagnostic features. Species such as *A. ochraceae*, *E. graminea* and *R. retusa* possessed elongated, fusiform seeds with high L/W ratios, whereas *Luisia zeylanica* and *Oberonia*

*ensiformis* showed shorter, broader seeds. Such seed-shape differences are commonly reported as taxonomically significant in orchids. The importance of testa patterning and seed shape as diagnostic characters has been emphasized in some earlier SEM-based studies, including those by Güler (2016) and Lee *et al.* (2023), who reported that testa sculpture, periclinal wall shape, and cell number are robust markers for species delimitation within the family Orchidaceae. Light and scanning-electron microscopic studies on testa cell patterning (shape and ridging of periclinal walls, anticlinal wall height, and the presence of openings) provide reliable diagnostic characters and correlates with seed buoyancy and dispersal potential in orchids (Arditti and Ghani, 2000; Diantina *et al.*, 2020; Verma *et al.*, 2014). Testa cell characteristics also varied remarkably. *R. retusa* contained the longest and widest testa cells, indicating a highly ornamented seed coat typical of certain epiphytic taxa. In contrast, *V. testacea* possessed the smallest testa cells, suggesting a simpler seed coat pattern. These findings support earlier findings that testa cell size and number are useful characters in distinguishing genera within Orchidaceae. The wide range in testa cell number (2.63-8.35 per seed) further strengthens their potential for systematic classification and species delimitation.

#### *Seed Size Variation and its Ecological Significance*

Seed size varied dramatically, with *C. aloifolium* producing the largest seeds and *E. graminea* the smallest. Terrestrial orchids (*Eulophia*) generally produced smaller seeds, possibly as an adaptation for wider wind dispersal across forest floors, whereas epiphytic orchids such as *C. aloifolium* and *R. retusa* exhibited comparatively larger seeds. Larger seed volumes may provide increased mechanical protection to embryos or related to fruit dehiscence behavior in canopy habitats. The large seed volume of *C. aloifolium* may also be associated with enhanced buoyancy due to very high air space percentages, facilitating long-distance dispersal from elevated host trees. Verma *et al.* (2014) noted that epiphytic species tend to produce relatively small-sized seeds as compared to terrestrial, lithophytic or mycoheterotrophic species.

#### *Embryo Size, Volume, and Early Developmental Strategies*

Embryo size differed significantly amongst the studied taxa with *C. aloifolium* exhibited the largest embryo volume, while *E. graminea* possessed the smallest. Orchid embryos are typically undifferentiated, yet the variations observed here suggest species-specific developmental strategies. Larger embryos, such as those of *R. retusa* and *Acampe*, may possess slightly

greater nutrient reserves than extremely minute embryos like *E. graminea*. The embryo L/W ratios showed that some species have elongated embryos (*C. aloifolium*, *O. ensiformis*), whereas others (*L. zeylanica*, *V. testacea*) had more rounded embryos. These differences might influence germination success and protocorm formation, as embryo shape is known to affect water absorption and initial swelling during germination.

#### *Air Space Percentage and Seed Dispersal Mechanisms*

Air space is a critical parameter influencing seed buoyancy and dispersal. The exceptionally high air space (95.20%) in *C. aloifolium* suggests an evolutionary adaptation for wind-mediated dispersal from high forest canopies. Species such as *O. ensiformis* (81.42%) and *E. graminea* (77.94%) also showed high air space, indicating light, dust-like seeds adapted for effective wind dispersal. Conversely, *V. testacea* displayed the lowest air space (9.53%), coupled with a high embryo proportion, suggesting denser seeds with possibly shorter dispersal distances. Such seeds may depend on specific microsites, host tree bark cracks, or humidity-mediated dispersal patterns. Lower air space may also reflect adaptation to more stable or localized habitats. This is supported by studies such as those of Arditti and Ghani (2000). Diantina *et al.* (2020), specifically explained that orchid seeds possess large air spaces, extremely low mass, and thin testa walls, all of which enhance wind dispersal efficiency. This shows that species with a higher proportion of internal air volume tend to be more buoyant and are adapted for long-distance wind dispersal.

#### *Relationship between Seed and Embryo Volumes*

The seed volume to embryo volume ratio is an important indicator of the relative investment in structural tissues versus embryonic tissues. In *C. aloifolium*, the ratio reached 20.81, showing that the seed coat and air cavity dominate its architecture. In contrast, *V. testacea* and *A. ochracea* had very low ratios (1.11-1.26), implying comparatively embryo-rich seeds. Such variations reflect a fundamental ecological trade-off: species with large air spaces favour dispersal efficiency, whereas species with larger embryos may favour improved early germination and establishment. Arditti and Ghani (2000) calculated SV/EV (seed volume / embryo volume) for several orchid species and related this feature to the growth habit (terrestrial vs. epiphytic). Overall, the differences observed across different species highlight the remarkable diversity in reproductive strategies within Orchidaceae and underline how seed micromorphology is shaped by habitat, life form (epiphytic vs. terrestrial), and dispersal mechanisms.

The data provided in this study indicates that there is an urgent need for future research in biodiversity assessment, propagation, and conservation efforts for wild orchids fostering more informed conservation strategies. In this connection it is worthwhile to mention that some successful attempts have been made to conserve some of the Indian orchid species of diverse habits and habitats by developing mass propagation protocols using different explants (Anuprabha *et al.*, 2017; Arora *et al.*, 2014, 2016; Bhowmik and Rahman, 2023; Dhillon and Pathak, 2023; Dhiman *et al.*, 2007; Hossain *et al.*, 2009, 2012; Jaryal *et al.*, 2025ab; Kaur *et al.*, 2006, 2017; Kirti *et al.*, 2023; Kumari and Pathak, 2021; Pathak *et al.*, 2016, 2017, 2022, 2023; Sembi *et al.*, 2011; Thakur and Pathak, 2020, 2021; Tripura *et al.*, 2022; Vasundhra *et al.*, 2019, 2021; Verma *et al.*, 2013). However, there is still much to be learned about the nutritional requirements of many orchid species, as the existing data remains limited.

### Conclusion

This present study highlighted the significant interspecific variations in seed and embryo morphology amongst eight orchid species of Andhra Pradesh, shedding light on the relationship between seed structure and ecological adaptation. The observed differences in seed shape, testa cell characteristics, embryo size, and air-space percentage underlined the role of micro morphological traits in orchid dispersal strategies and ecological niche adaptation. The findings reported that the seed traits, such as testa cell length, number of testa cells, embryo volume, and seed-to-embryo volume ratio, can serve as valuable taxonomic markers within orchid genera. Additionally, the variations in air-space percentage demonstrated their critical influence on dispersal potential, with species like *C. aloifolium* exhibiting high dispersal capacity due to lightweight, air-filled seeds, while *V. testacea* showed limited dispersal ability due to denser seeds with less air-space. These results contribute to a deeper understanding of reproductive strategies and ecological diversity in orchids.

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