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SEED MORPHOMETRY OF SOME INDIAN ORCHIDS WITH SPECIAL REFERENCE TO THEIR INTER-RELATIONSHIPS AND ECOLOGICAL SIGNIFICANCE

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Abstract

SEM (Scanning Electron Microscope) studies on seed morphometry of nine orchid species, such as *Acampe praemorsa* (Roxb.) Blatt. & Mc. Cann., *A. rigida* (Buch.-Ham.ex J. E.Sm.) P.F. Hunt, *Calanthe triplicata* (Willem.) Ames, *Luisia zeylanica* Lindl., *Malaxis densiflora* (A. Rich.) Ktze, *Oberonia arnottiana* Wight., *O. ensiformis* (J.E. Sm.) Lindl., *Vanda testacea* (Lindl.) Reichb. f., and *V. tessellata* (Roxb.) W. J. Hook. ex Don have been carried out. The present data deals with quantitative data related to the length and width of the seed and embryo, seed and embryo volume, percentage of air space, and number of testa cells. The seed truncation character can be used to differentiate between species in the genera such as *Acampe, Calanthe, Luisia, Malaxis, Oberonia,* and *Vanda*. These data indicate that the seeds of *Calanthe* species are more truncated than those of the other studied taxa. Seeds with higher ratio of seed volume/embryo volume (more than 2.2) especially in *C. triplicata, V. tessallata,* and *V. testacea* are expected to be more buoyant than those with a lower ratio of seed volume/embryo volume. These are widely spread out species in Western Ghats of South India. The buoyancy of seeds could be attributed to the dispersal of seeds to vast areas as well as wide distribution of the species.

Introduction

THE ORCHIDACEAE is one of the largest families of flowering plants; it comprises about 779 genera and 22,500 species (Mabberley, 2008). In India, with 1331 species spreading over 184 genera, it represents second largest flowering plant family and contributes about 10% of Indian Flora (Kumar and Manilal,1994). Orchid seeds are light in weight and the tiniest amongst the seeds produced by flowering plants and these are non-endospermic, vary considerably in their size, morphology, color, and minute details. In majority of orchid species, seed size shows variation from 300–800 μm (Molvray and Kores, 1995). The taxonomic significance of the seed characteristics was first reported by Clifford and Smith (1969). Besides, serving as taxonomic markers, the morphological characters of seeds can be used to deduce phylogenetic relationship (Barthlott, 1976) and to identify their involvement in hybrid genotypes (Arditti *et al.,* 1979).

Seed morphology has got importance in delineation of species within the genus and also delineation of subgeneric groups (Augustine *et al.,* 2001; Larry, 1995; Mathews and Levins 1986; Ness, 1989; Pathak *et al.*, 2011; Verma *et al*. 2014; Vij *et al.*,1992). Molvary and Kores (1995) also reported that the orchid seed varies in shape from filiform to fusiform, clavate to ellipsoidal and often prominently winged. Barthlott and Ziegler (1981) worked elaborately on the seed coat structure of orchids and recognized 20 different seed types by taking varying seed characteristics.

In South India, about 250 species spreading to 70 genera have been reported (Abraham and Vatsala, 1981). Except for a few detailed reports (Augustine *et al.*, 2001; Pathak *et al.*, 2011; Swamy *et al.*, 2007; Verma *et al*. 2014; Vij *et al.*, 1992), not much work has been done on seeds of Indian orchids. The present investigation deals with the Scanning Electron Microscopic (SEM) studies on seed characters of nine orchid species belonging to six orchid genera *i.e., Acampe, Calanthe, Luisia, Malaxis, Oberonia,* and *Vanda.*

Materials and Methods

Seeds of nine orchid species belonging to subfamily Epidendroideae were collected from different parts of Eastern and Western Ghats of India (Table 1). The mature capsules were freshly collected during 2010-2012. Seeds were separated from capsules and collected in petri dishes. Optical photomicroscope (Motic 2.0, 5 Megapixel) was used to measure the length and width of seeds.

The seeds of all the above species were fixed in 2.5% glutaraldehyde prepared in 0.2 M cacodylate buffer (pH 7.2) and kept in room temperature for two hours; the seed samples were then dehydrated in graded ethyl alcohol: acetone series. Subsequently, these were

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Table 1. *List of species presently investigated for Scanning Electron Microscope (SEM) studies.

Species	Place of collection and elevation	Habitat & host tree	Accession Number
Sub family - Epidendroideae			
Tribe - Malaxideae			
Malaxis densiflora (A.Ri.ch.) O. Kuntze.	Pallode (KE), 900 m	Te	ANUH 1010
Oberonia arnottiana Wight.	Paderu (AP), 910 m	Epi & *Proteum serratum*	ANUH 1011
O. ensiformis Lindl.	Paderu (AP), 910 m	Epi & *Pterocarpus morsupium*	ANUH 1012
Tribe - Arethuseae			
Subtribe - Bletiinae			
Calanthe triplicata Lindl.	Pallode (KE), 900 m	Epi & *Oozenia ozenensis*	ANUH 1017
Tribe- Vandeae			
Subtribe - Aeridinae			
Acampe praemorsa Blatt. & Mc. C.	Chintapalli (AP), 839 m	Epi & *Terminalia chebula*	ANUH 1018
A. rigida Lindl.	TBGRI, Pallode (KE), 900 m	Epi & *Albezia lebbeck*	ANUH 1019
Luisia zeylanica Lindl.	Giddalur (AP), 300 m	Epi & *Terminalia alata*	ANUH 1020
Vanda testacea (Ldl.) Reich. F.	Lothugadda (AP), 750 m	Epi & *Artocarpus heterophyllus*	ANUH 1021
V. tessellata Hk. F.	Lothugadda (AP), 750 m	Epi & *Artocorpus heterophyllus*	ANUH 1022

*Arranged according to Dressler (1993)

Epi, Epiphyte; Te, Terrestrial; AP, Andhra Pradesh; KE, Kerala; TN, Tamil Nadu; TBGRI, Tropical Botanical Garden and Research Institute.

dried in critical point dryer. After the critical drying, these samples were mounted on to copper stubs and were gold coated for five min. The processed specimens were examined and photographed on a

Table 2. Seed characters and quantitative data.

Taxa	Time of fruiting	Colour	Length (mm)	Width (mm)	L/W	Seed volume mm^3x10^{-3}	Average length of tests cells (mm)	Average width of Testa cells (mm)	Average no. of testa cells
Malaxis densiflora	Mar-Jun	White	0.3289 ± 0.0497	0.0985 ± 000983	3.33	0.0008355 0.355 mm^3x10^{-3}	37.81	13.69	11.62
Oberonia arnotiana	Sept-Oct	Yellow	0.27398 ± 0.004986	0.09012 ± 0.004733	3.03	0.0005805 0.5605 mm^3x10^{-3}	105.03	17.88	3.62
O. ensiformis .	Sep-Oct	Light yellow	0.2657 ± 0.00546	0.08009 ± 0.00434	3.31	0.,000443 0.443 mm^3x10^{-3}	107.5	20.91	3.79
Calanthe triplicata	Apr-May	White	0.9474 ± 0.1701	0.0992 ± 0.0227	9.55	0.002440 2.440 mm^3x10^{-3}	140.54	31.18	9.87
Acampe praemorsa	Mar-Jun	Light brown	0.1847 ± 0.06906	0.06906 ± 0.00345	2.67	0.0002306 (0.2306 $mm^3x10^{-3)}$	68.56	11.19	3.66
Acampe rigida	Mar-Jun	Light brown	0.2402 ± 0.003910	0.0633 ± 0.00452	3.79	0.0002520 (0.2520 $mm^3x10^{-3)}$	79.22	13.24	5.42
Luisia zeylanica	Jun-Jul	Yellow	0.2545 ± 001553	0.07445 ± 0.003838	3.39	0.00037045 (0.37045 mm^3x10^{-3}	84.52	12.29	3.1
Vanda testacea	Mar-Apr	Light yellow	0.2185 ± 0.0344	007232 ± 0.0004432	4.87	0.00029855 (0.2985 mm^3x10^{-3}	47.82	13.91	4.42
V. tessellata	Apr-May	Yellow	0.1892 ± 0.021051	0.06829 ± 0.000453	2.77	0.0002308 (0.2308 $mm^3x10^{-3)}$	69.50	11.06	4.81

HITACHI, S3000 N Model Scanning Electron Microscope in IICT (Indian Institute of Chemical Technology, Hyderabad, India). Under light microscope with micrometer, at the longest and widest axis of the seed, the width and length of seeds were measured clearly. Seeds exhibited different forms, therefore, seed volumes were calculated using the formula $Vs = 2 [(Ws/2)^2 (1/2Ls) (1.047)]$, where Vs = seed volume, Ws/2 = half of seed width, Ls = seed length, 1.047 = ð/3 (Arditti *et al.*, 1980). Orchid embryos were elliptical in cross section and therefore their volume was calculated by using the formula $Ve = 4/3\ LeWe^2$, where Ve = embryo volume, Le = half of the embryo length, and We = half of the embryo width. Percentage of airspace was calculated by using the formula, seed volume – embryo volume/seed volume × 100. Standard deviation was also calculated for each character of seed and embryo.

Results and Discussion

SEED CHARACTERS

Seed Colour

The colour of the seeds in all investigated species was pale yellow to yellow and light brown to white.

Seed Shape

Scanning Electron Microscope (SEM) photographs showed the fine details of the *M. densiflora* seeds. Seeds were quadrilateral-shaped with blunt ends (Fig. 1A, B) with an ellipsoidal embryo (Fig. 1A). The seeds were with opening at the base, *i.e.*, at the chalazal or suspensor end (Fig. 1B). The seeds of *O. arnottiana* were short and spindle shaped with blunt ends (Fig. 1 E, F, G, H). In *O. ensiformis* also, seeds were spindle shaped but with a bulged central part having ellipsoidal embryo (Fig. 2A, B). The seeds were with openings at chalazal end (Figs. 1 G H; 2 C, D). In *C. triplicata,* seeds were filamentous shaped with visible embryo located in the centre (Fig 2 I, J, K). The SEM studies revealed that the seeds of *A. praemorsa were* ovoid (Fig. 2E, F, G) or spathulate; whereas in *A. rigida,* these are fusiform with slight curvature. All seeds were with an ellipsoidal embryo with blunt ends (Fig. 2F). The seeds of *V. tessellata* and *V. testacea* were spindle shaped or oblong (Figs. 2 O, P, Q). Testa cells were elongated and longitudinally oriented in *V. tessellata*; in case of *V. testacea,* testa cells were spirally arranged, giving a characteristic rope-like appearance to seeds (Fig. 2 O, P, Q, R).

Length/Width (L/W) Ratio of Seed

Length/width ratio of seeds gives some interesting information on relative degree of truncation of orchid seeds (Arditi *et al.*, 1980; Augustine *et al.*, 2001). The maximum L/W ratio was observed in *Calanthe triplicata* (9.55) whereas minimum L/W ratio was observed in *A. praemorsa* (2.67). The L/W ratio in other investigated taxa was, 4.87 in *V. testaceae,* 3.79 in *A. rigida*, 3.39 in *L. zeylanica,* 3.31 in *O. ensiformis,* and 2.77 in *V. testellata.* The present data is in agreement with studies of Vij *et al.* (1992) and Swamy *et al.* (2004). The seed truncation character can be used to differentiate between species in the genera such as *Acampe, Calanthe, Luisia, Malaxis, Oberonia,* and *Vanda*. The present data indicate that the seeds of *Calanthe* species are more truncated than those of the other studied taxa.

Seed Volume

In the present study, seed volume ranged from 0.2306 $mm^3 \times 10^{-3}$ to 2.44 $mm^3 \times 10^{-3}$ (Table 2). The highest seed volume was observed in *Calanthe triplicata* (2.44 $mm^3 \times 10^{-3}$) followed by *M. densiflora, O. arnottiana, O. ensiformis*, followed by *L. zeylanica*. In *V. testaceae* and *V. tessellata,* the seeds were of lesser volume and small sized. In the species of *Bulbophyllum* and *Cymbidium,* the higher seed volume is the result of long width to some extent than length of testa (Augustine *et al.,* 2001; Swamy *et al.,* 2004). Healey *et al.,* (1980) and Augustine *et al.* (2001) were justified in selectively using seed morphometry to find out phylogenetic relationship in orchids.

Average Number of Testa Cells

The average number of testa cells in the long axis of the seeds was 11.2 in *Malaxis densiflora* followed by *A. rigida, C. triplicata, L. zeylanica, O. ensiformis, O. arnottiana, V. tessellata,* and *V. testacea* (Table 2). The least number of testa cells were found in *L. zeylanica i.e.,* 3.1.

The longest testa cell was observed in *C. triplicata* (140.54 μm) and the testa cell with greatest width was also observed in *C. triplicata* (31.18 μm). The testa cells of smallest width were found in *V. tessellata* (11.06 μm).

Vij *et al.* (1992) categorized the orchid seed into three types based on the length of testa cells; those that are greater than 200 μm were categorized as long ones, less than 200 μm to 100 μm as intermediate, and below 100 μm as short ones. The presently investigated taxa namely *O. ensiformis, O. arnottiana,* and *C. triplicata* are the group with intermediate cells and other studied species are the group with short testa cells because of short length of their testa cells.

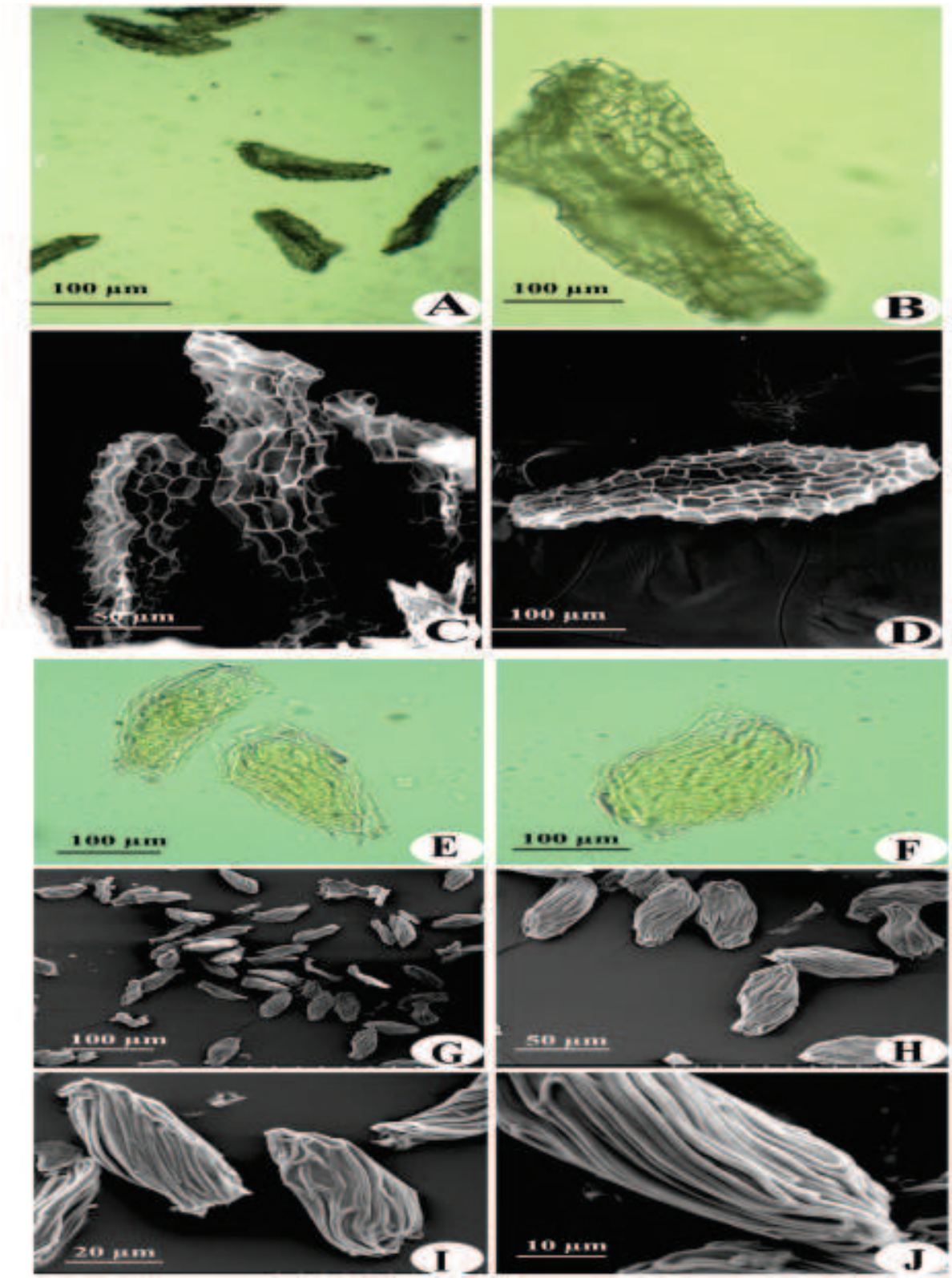

Fig. 1. Light microscopic and scanning electron microscopic (SEM) photographs of *Malaxis densiflora*. (A-D) and *Oberonia arnottiana* (E-J): A, Few seeds with embryo under the light microscope; B, Transparent seed under the light microscope; C, A few seeds under SEM; D, A seed under SEM; E, Few seeds under the light microscope with embryos; F, Seed under the light microscope with embryo; G, Few seeds under SEM; H, Enlarged view of seeds under SEM; I, Enlarged view of seed with high magnification under SEM; J, Part of the testa under high magnification of SEM.

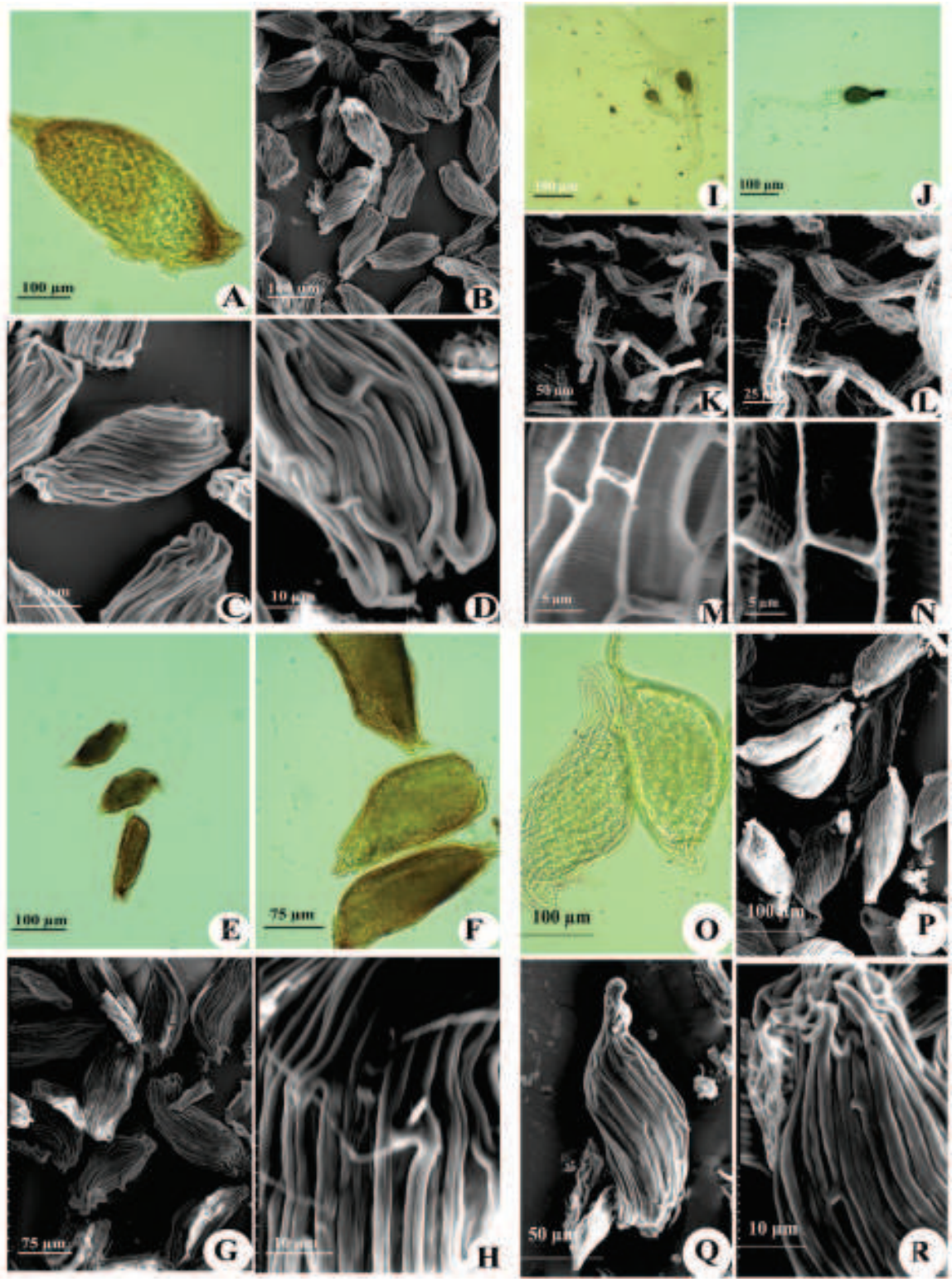

Fig. 2. Light microscopic and SEM photographs of *Oberonia ensiformis* (A-D), *Acampe praemorsa* (E-H*), Calanthe triplicata* (I-N*), and Vanda testacea* (O-R): A, A seed under the light microscope with embryo; B, A few seeds under SEM; C, Enlarged view of seeds under SEM; D, Part of the testa under SEM; E, Few seeds with embryo under the light microscope; F, Seeds under the high power of light microscope; G, Seeds under SEM; H, Testa cells; I, Seeds under the light microscope; J, Enlarged view of a seed with embryo under light microscope; K, A few seeds under SEM; L, A few seeds under SEM; M, Testa cells with transverse walls under high magnification of SEM; N, Testa cells with pores, transverse walls under high magnification of SEM; O, Seeds with embryos under the light microscope; P, Few seeds under SEM; Q, Seed under SEM; R, Chalazal pore of seed under SEM.

Table 3. Embryo characters and quantitative data.

Taxa	Colour	Length (mm)	Width (mm)	L/W	Embryo Volume mm^3x10^{-3}	Seed volume to embryo volume	Airspace (%)
Malaxis densiflora	White	0.1621 ± 0.002952	0.0628 ± 0.02501	2.58	0.0003339 (0.3339 mm^3x10^{-3})	2.50	60.50
Oberonia arnottiana	Yellow	0.09975 ± 0.00769	0.00937	1.20	0.00003505 (0.03505 mm^3x10^{-3})	1.65	39.62
O. ensiformis	Light yellow	0.09689 ± 0.01744	0.07394 ± 0.007629	1.31	0.0002756 (0.2756 mm^3x10^{-3})	1.60	38.19
Calanthe triplicata	White	0.1413 ± 0.0591	0.07513 ± 0.0251	1.88	0.00041661 (0.4166 mm^3x10^{-3})	5.85	42.92
Acampe praemorsa	Light brown	0.1073 ± 0.00295	0.0515 ± 0.00654	2.08	0.0001486 (01486 mm^3x10^{-3})	1.55	35.53
A. rigida	Light brown	0.1703 ± 0.02150	0.04215 ± 0.002150	4.09	0.0001579 (0.1579 mm^3x10^{-3})	1.59	37.34
Luisia zeylanica	Yellow	0.1212 ± 0.01217	0.05333 ± 0.00321	2.27	0.0001791 (0.179 mm^3x10^{-3})	2.06	51.63
Vanda testacea	Light yellow	0.1250 ± 0.0150	0.0452 ± 0.00264	2.76	0.0001334 (0.1334 mm^3x10^{-3})	2.23	55.31
V. tessellata	Yellow	0.1452 ± 0.001829	0.0340 ± 0.01252	4.26	0.000087734 (008773 mm^3x10^{-3})	2.63	62.00

EMBRYO CHARACTERS

The colour of the embryo in the presently investigated taxa varied from, light yellow to yellow and white to brown. In seeds, the embryos generally occupied a very small portion. According to Augusteine *et al.,* (2001), orchid embryos occupied small portion in seeds but in *Bulbophyllum* embryos, it occupied a large portion in the seed and the maximum embryo length and width was observed in *A. rigida* is (0.1703).

In this present investigation, maximum L/W ratio was found in *V. tessellata* (4.26) followed by *A. rigida* (4.09) and minimum in *O. arnottiana* (1.20). L/W ratio of embryos in *A. praemorsa, L. zeylanica, M. densiflora* and *V. testacea r*anged from 2.08 to 2.76 (Table. 3). According to Healey *et al*. (1980), the volume of embryo differs from genus to genus. In the present investigation, highest seed volume was observed in *C. triplicata* (0.4166$mm^3 \times 10^{-3}$) and minimum in *V. tessellata* (0.08773 mm^3x10^{-3}) (Table. 3).

Seed Volume to Embryo Volume (Vs/Ve) Ratio

Some observations of seed volume to embryo volume ratio are very interesting in present investigation. This value was maximum in *C. triplicata* (5.85) followed by *V. tessellata, M. densiflora,* and *V. testacea.* (Table 3). According to Arditti *et al.* (1980), the species showing greater variation in seed and embryo volumes and percentage of air space could survive amongst their different populations.. Young seeds have small undifferentiated embryos where as the mature seeds from the dehisced capsules have embryos of a larger volume.

Air Space

In the present investigation, the seeds with maximum percentage of airspace were noticed in *C. triplicata* (82.92%) followed by *V. tessellata, M. densiflora, V. testacea* and *L. zeylanica.* (Table 3). These orchids with more airspace are said to be widely distributed in Eastern and Western Ghats whereas *A. praemorsa, O. ensiformis,* and *O. arnottiana* with low air space are restricted and endemic to Southern India.

From the above data, direct correlation has been drawn between the seed/embryo volume ratio, the percentage of air space and the buoyancy of the seeds. The seeds having larger Vs/Ve ratio are expected to be more lighter than those with smaller ratio (Arditti *et al.*, 1979; Garg *et al.,* 1992). The buoyancy of seeds could be attributed to the distribution of seeds to vast areas.

Ecological Significance

Seeds with higher ratio of seed volume/embryo volume (more than 2.2) especially in *C. triplicata, V. tessellata,* and *V. testacea* are expected to be more buoyant than those with a lower ratio of seed volume/embryo volume. These are widely spread out species in Western Ghats of South India. Higher percentage of

airspace was also noticed in these orchid taxa. In general, the dust- like minute seeds are suitable for long distance dispersal by wind. Many scientists [Clifford and Smith (1969); Pathak *et al.*, (2011); Rasmussen (1995); Swamy *et al.,* (2004); Verma *et* al., (2014; Vij *et al.,* (1992)] opined that the seed size also has direct correlation with plant habit (epiphytes with smaller seeds than the terrestrials). In the other studied orchid taxa such as *Acampe rigida, A. praemorsa, O. arnottiana, O. ensiformis*, its value was less than three and air space was also reduced indicating thereby that their distribution is restricted (localized) to Western and Eastern Ghats of Southern India.

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