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Anatomy of Some Terete-Leaved Orchid Species

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ABSTRACT

Leaf anatomy was investigated in six epiphytic orchid species in five genera—*Cleisostoma filiforme* (Lindl.) Garay, *Eria pannea* Lindl., *Oberonia oklongensis* Chowlu *et al., Papilionanthe teres* (Roxb.) Schltr, *Papilionanthe vandarum* (Rchb. f.) Garay and *Schoenorchis gemmata* (Lindl.) J. J. Smith. The aim of the study was to determine if these species exhibited any visible special xeromorphic characters. Freehand transverse section and peeling techniques were used. Most species had a thick cuticle with a cuticular ledge and were amphistomatic. However, cuticular papillae were present only in *O. oklongensis, P. vandarum* and *S. gemmata*. Specialised water-storage cells were present within the mesophyll tissue in all the species except *E. pannea*. Both xylem and phloem found in *S. gemmata* consisted of fibrous caps. Raphide bundles were present abundantly in the mesophyll and epidermal cells of *E. pannea* and *O. oklongensis.* Sphaeraphides were present in the epidermal cells along with stomata of *P. teres*. Guard cells were cuticularized in almost all species except *E. pannea.* Wax-secreting cells were observed in *E. pannea* and *O. oklongensis*. Paracytic stomata were found in *C. filiforme, E. pannea, P. teres, P. vandarum* and *S. gemmata.* Only *O. oklongensis* possessed actinocytic stomata.

Keywords: anatomy, terete leaf, xeromorphic, epiphyte, orchid, India

INTRODUCTION

Terete-leaved orchids are characterized by cylindrical, fleshy leaves and are adapted to dry periods. They do not possess pseudobulbs or fleshy stems but do possess succulent, drought resistant leaves. The epiphytes studied here were small (*Cleisostoma filiforme* (Lindl.) Garay, *Eria pannea* Lindl., *Oberonia oklongensis* Chowlu *et al.* and *Schoenorchis gemmata* (Lindl.) J. J. Smith), medium (*Papilionanthe vandarum* (Rchb. f.) Garay) and tall (*Papilionanthe teres* (Roxb.) Schltr).

Leaf traits are key factors in terms of reflecting the influence of the environment on the plant and the adaptation of a plant to the environment (Dunbar-Co *et al.*, 2009). Leaf anatomical structures are the foundations of leaf physiological functions and consequentially, changes in leaf anatomical structures greatly affect plant growth and metabolism (Pandey *et. al.*, 2009). Plants with xeromorphic features usually grow in an environment where leaf photosynthesis is limited by water availability (Haworth and McElwain, 2008).

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The morphology of these terete species shows many xerophytic features. Keeping this in mind, their anatomy can be studied to see whether they exhibit special xeromorphic features as well. Thus, in the present paper, the leaf anatomy of six species from five genera of terete-leaved orchid species was studied.

MATERIALS AND METHODS

Six epiphytic terete-leaved orchids—*Cleisostoma filiforme* (Lindl.) Garay, *Eria pannea* Lindl., *Oberonia oklongensis* Chowlu *et al., Schoenorchis gemmata* (Lindl.) J. J. Smith, *Papilionanthe vandarum* (Rchb. f.) Garay and *Papilionanthe teres* (Roxb.) Schltr—were collected from different districts of Manipur, India (Table 1) and grown in the Orchidarium of the Centre for Orchid Gene Conservation of Eastern Himalayan Region at Hengbung, India. After flowering, the taxonomical and anatomical characters of the plants were critically examined.

Mature leaves were collected and freehand, transverse sections were made with a sharp razor blade. The thin sections were stained with safranin and mounted on glass slides. The sections were examined and photographed at 100× and 400× magnification under a light microscope (Model-CX31; Olympus Corp.; Tokyo, Japan). Selected parameters—the thickness of cuticle, epidermis and water-storage cells—were measured at the midpoint of each transverse section with a standardized ocular micrometer scale.

The adaxial and abaxial epidermis of middle leaf parts of mature leaves were peeled from fresh leaves and photographed at 100× and 400× magnification under the light microscope. Digital images were manually analyzed using the Photoshop software package (version 7.0; Adobe; Noida, Uttar Pradesh, India). The length and width of stomata were recorded. The stomatal apparatus area (A_s) and stomatal index percentage (SI %) were also recorded. The stomatal apparatus area was calculated using the formula of Shelley and David (2001): $A_s = ¼ \times \pi \times l \times w$, where l is the length and w is the width. The methodology followed was the same as that of Guan *et al*. (2010). For leaf histological observations, five leaves were examined for each species and more than 15 images per leaf were analyzed.

Statistical analysis was carried out using a post hoc test (Guan *et al*., 2010). Results were presented as mean ± SE. Significant levels were defined at $P < 0.05$ using one way analysis of variance.

Table 1 Ecological traits of six terete-leaved orchid species collected in India.

Species	Source		Flowering	Habitat
	Locality	Altitude (m)		
Cleisostoma filiforme	Tamenglong (Kahulong – 1)	1,287	May–Aug	Epiphyte
	Ukhrul (Kamjong)	1,460		
Eria pannea	Tamenglong (Kahulong – 3)	1,191	May–Aug	Epiphyte
	Senapati (Willong Khunou)	1,028		
	Chandel (Kwatha – 3)	470		
Oberonia oklongensis	Senapati (Oklong)		May–Jun	Epiphyte
Papilionanthe teres	Jiribam (Kazigram, Makru)	17–163	May–Jul	Epiphyte
Papilionanthe vandarum	Senapati (Yangkhulen)	1,740	Feb–Mar	Epiphyte
Schoenorchis gemmata	Tamenglong (Longku – 5)	1,355	May–Sep	Epiphyte

Numbers 1, 3 and 5 in the locality show sub-localities with different altitude.

RESULTS

Transverse section of leaf

The leaf outline was circular with a single V-shaped groove in *E. pannea* (Figure 1b) and *O. oklongensis* (Figure 1c) whereas it had two distinct shallow grooves in *P. teres* and *P. vandarum* (Figures 1d and 1e) and was wavy with multiple shallow grooves in *C. filiforme* (Figure 1a). However, it was broadly V-shaped in *S. gemmata* (Figure 1f). The cuticle was not smooth but ridged in all species (Figures 3a–3f). The adaxial cuticle was thicker than the abaxial in all species. The cuticle thickness was greatest in *P. vandarum* (adaxial = 19.00 µm; abaxial = 17.5 µm) and least in *S. gemmata* (adaxial = 13.5 µm; abaxial = 11.8 µm). Epidermal cells were polygonal in *C. filiforme* (Figure 2a) and *E. pannea*, rectangular in *O. oklongensis* (Figure 2b) and *P. vandarum* (Figure 2d) and spherical to polygonal in *P. teres* and *S. gemmata* (Figures 2c and 2e). Epidermal thickness tended to be greater on the adaxial surface than the abaxial in all species except in *P. vandarum* and *P. teres* (Table 2). The adaxial epidermal thickness was greatest in *S. gemmata* (47.0 µm) and least in *E. pannea* (31.5 µm). The abaxial epidermal thickness was greatest in *P. teres* (58.5 µm) and least in *E. pannea* (29.5 µm).

The mesophyll was differentiated into spongy and palisade parenchyma only in *E. pannea* (Figures 1a–1f). Specialized water-storage cells with thick walls and some with banded thickenings were present within the mesophyll tissue in all species with the exception of *E. pannea* (Figures 1a–f). Water-storage cells were elongated and barrel-shaped with heavily thickened walls in *C. filiforme* (Figure 2f), bee-hive-like, with smaller cells present within a bigger cell in *O. oklongensis* (Figure 2k), globular-to-barrel-shaped with thick, discontinuous inner walls and some with a groove and with thick walls in *P. teres* (Figures 2g and 2h), irregular- or star-shaped with banded thickenings and discontinuous inner walls in *P. vandarum* (Figure 2i) and globular-to-barrel-shaped with discontinuous inner walls and with banded thickenings in *S. gemmata* (Figure 2j). The size of water-storage cells was largest in *O. oklongensis* (length = 269 µm, width = 194 µm) and smallest in *C. filiforme* (length = 93 µm, width = 54.5 µm).

A phloem cap was present in all species (Figures 1a-1f). Both xylem and phloem had fibrous caps in *S. gemmata* (Figure 1f). Vascular bundles were in a single ring with large and small bundles alternating in *C. filiforme* and in two rings—the outer with small bundles and the inner with large bundles—in *E. pannea*, *O.oklongensis* and *P. vandarum* (Figures 1a–1c and 1e). However, the vascular bundles were in three rings with their sizes increasing closer to the centre in *P. teres* (Figure 1 d). In *S. gemmata*, the vascular bundles were in a V-shaped curve with both small and large bundles distributed together (Figure 1f). Another interesting feature observed in *S. gemmata* was that two separate, small vascular bundles were joined by a connecting tissue (Figures 1n–1o).

Multicellular glandular hairs were observed in *E. pannea* and *O. oklongensis* (Figure 2l). Cuticular ledges were present in all species (Figures 1a–1e). However, cuticular papillae were present only in *O. oklongensis, P. vandarum* and *S. gemmata* (Figures 1i, 1k and 1l). Raphide bundles were abundantly present in the epidermal and mesophyll cells of *E. pannea* and *O.oklongensis* (Figures 2m and 2n).

Stomata

All the species studied were amphistomatic. Stomatal size (length × width) and stomatal apparatus area was largest in *P. teres* (67.5 × 58.5 µm and 3098.79 µm², respectively) and smallest in *E. pannea* (42.5 × 37.5 µm and 1259.93 µm², respectively). The stomatal shape was elliptic in *E. pannea*, orbiculate in *O. oklongensis* and *P. vandarum* and orbiculate to sub-orbiculate in *C. filiforme*, *P. teres* and *S. gemmata* (Figures 3a–3f). The stomatal index percentage was highest in *P. vandarum* (9.18%) and lowest in *E. pannea* (3.07%). Guard cells were cuticularized in all

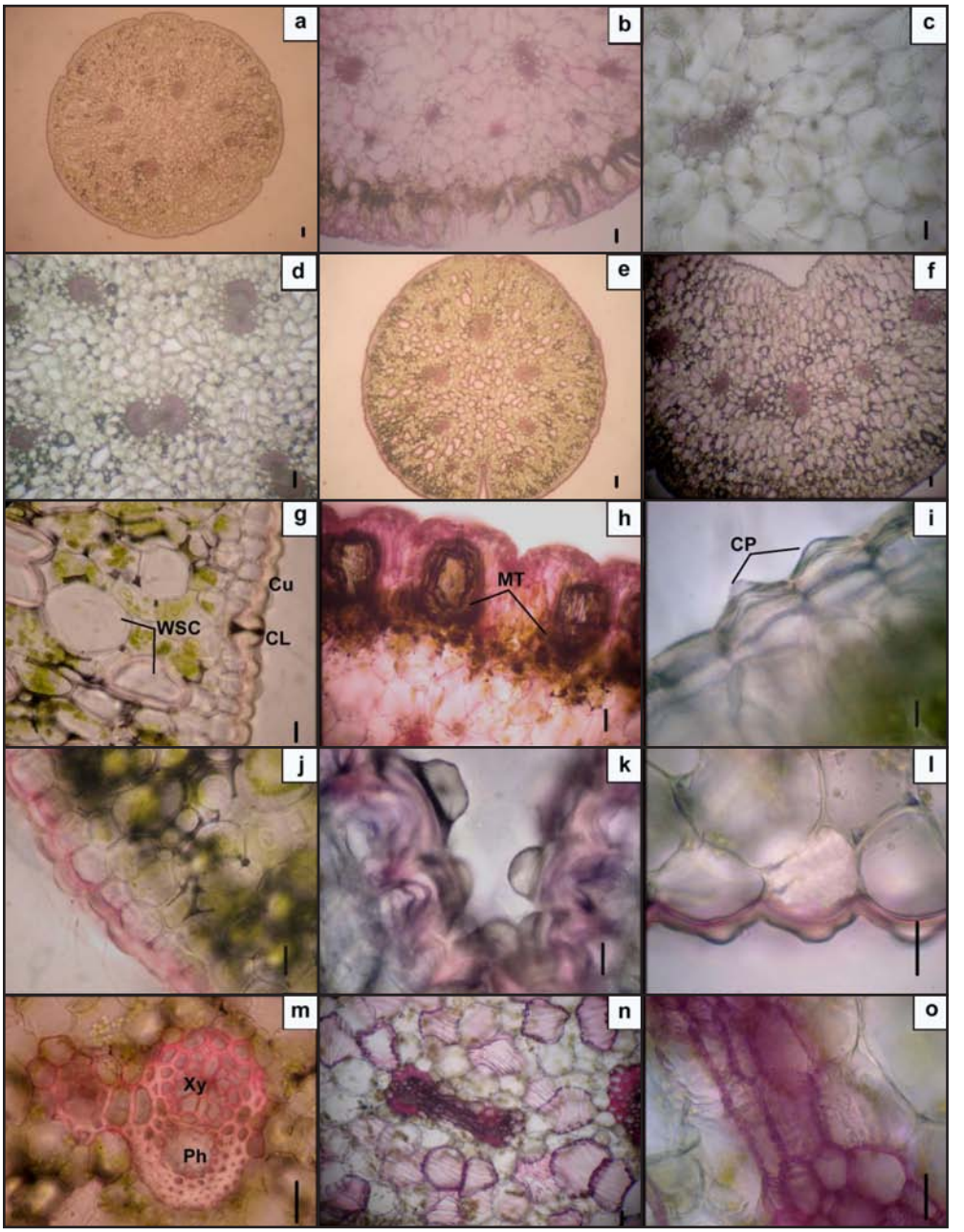

Figure 1 Transverse sections of leaf (a–f) outline at 100×: a) *C. filiforme*; b) *E. pannea*; c) *O. oklongensis*; d) *P. teres*; e) *P. vandarum*; f) *S. gemmata*; Transverse section of leaf cuticle (g–l) at 400×: g) *C. filiforme*; h) *E. pannea*; i) *O. oklongensis*; j) *P. teres*; k) *P. vandarum*; l) *S. gemmata*; Transverse section of leaf vascular bundle at 100×: m) *C. filiforme*; Transverse section of leaf joined vascular bundles at 100×: n) *S. gemmata*; Magnified view of vascular bundle connecting tissue at 400×: o) *S. gemmata*. Cu =Cuticle; E = Epidermis; EC = Epidermal cell; GC = Guard cell; Ph = Phloem; PC = Phloem cap; R = Raphide; SC = Subsidiary cell; VB = Vascular bundle; VBC = Vascular bundle connecting tissue; WSC = Water-storage cell; Xy = Xylem. Scale bars = 50 µm.

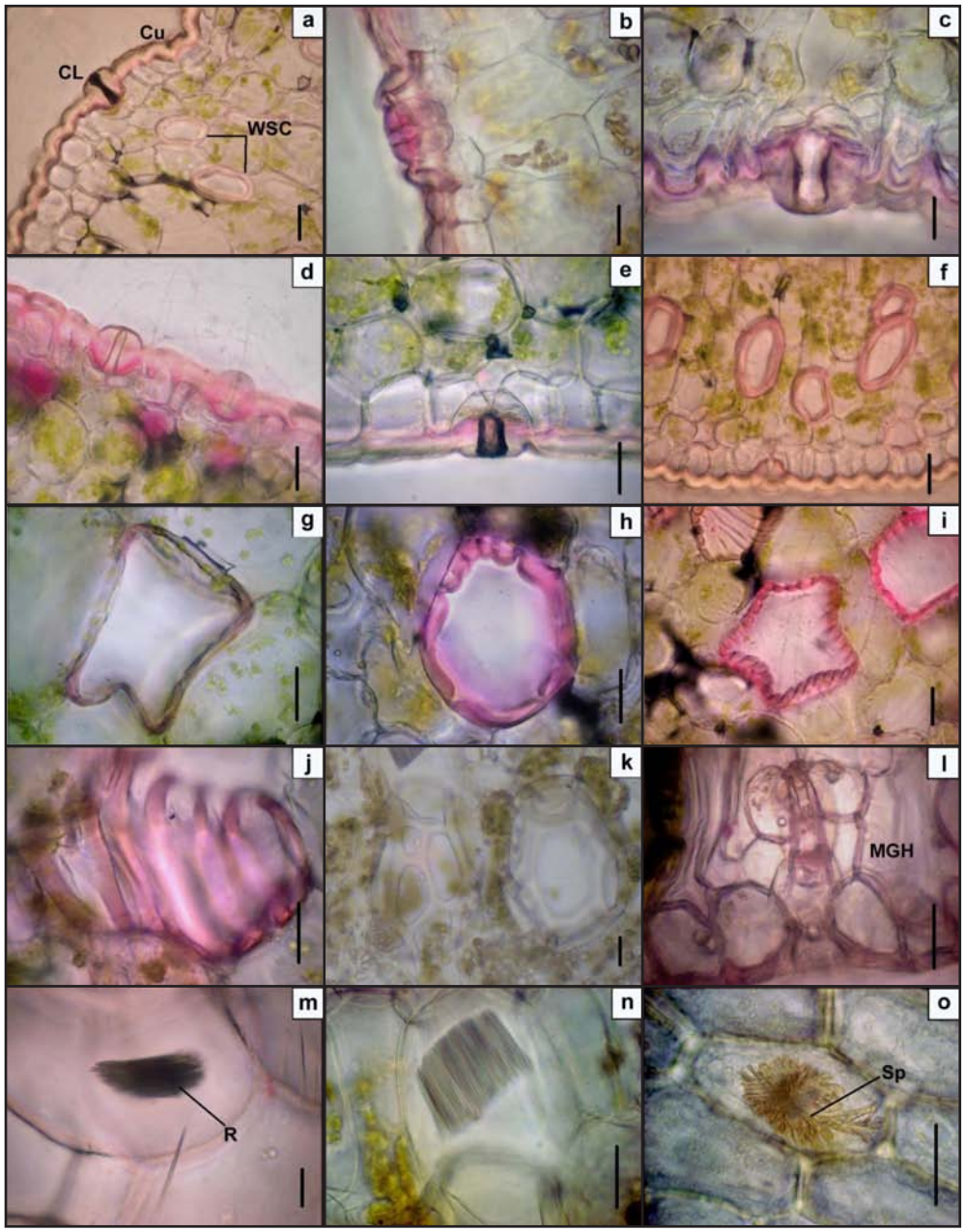

Figure 2 Transverse section of leaf cuticular ledges (a–e) at 400×: a) *C. filiforme*; b) *O. oklongensis*; c) *P. teres*; d) *P. vandarum*; e) *S. gemmata*; Transverse section of leaf water-storage cells (f–k) at 400×: f) *C. filiforme*; g) and h) *P. teres*; i) *P. vandarum*; j) *S. gemmata*; k) *O. oklongensis* (400×); Multicellular glandular hair at 400×: l) *E. pannea*; Transverse section of leaf raphides (m–n) at 400×: m) *E pannea*; n) *O. oklongensis*; Transverse section of leaf sphaeraphides at 400×: o) *P. teres*; Cu = Cuticle; CL = Cuticular ledges; MGH = Multicellular glandular hair; R = Raphide; Sp = sphaeraphide; WSC = Water-storage cell. Scale bars = 50 µm.

species except *E. pannea*. Guard cell chloroplasts were observed in all species (Figures 3a–3f). Wax-secreting cells were observed in *E. pannea* and *O. oklongensis.* Raphide bundles were present along with stomata in the epidermal cells of *E. pannea* and *O. oklongensis.* Sphaeraphides or sphaerites were observed in the epidermal cells of *P. teres* (Figure 2o). Stomata were paracytic in *C. filiforme, E. pannea*, *P. teres*, *P. vandarum* and *S. gemmata* and actinocytic in *O. oklongensis* (Figures 3a–3f).

DISCUSSION

In all species studied, the cuticle was not smooth on any leaf surfaces. The cuticle thickness was greatest in *P. vandarum* and least in *S. gemmata* (Table 2). The cuticle is a thin, hydrophobic and flexible membrane composed of a polymer matrix (cutin) and associated solvent-soluble lipids (cuticular waxes) (Rasmussen, 1987). The major function of cuticle is to efficiently prevent water loss from the leaf interior (Mill and Stark Schilling, 2009). The presence of a thick cuticle on the leaf surface is an indicator of aridity (Haworth and McElwain, 2008). Adaxial

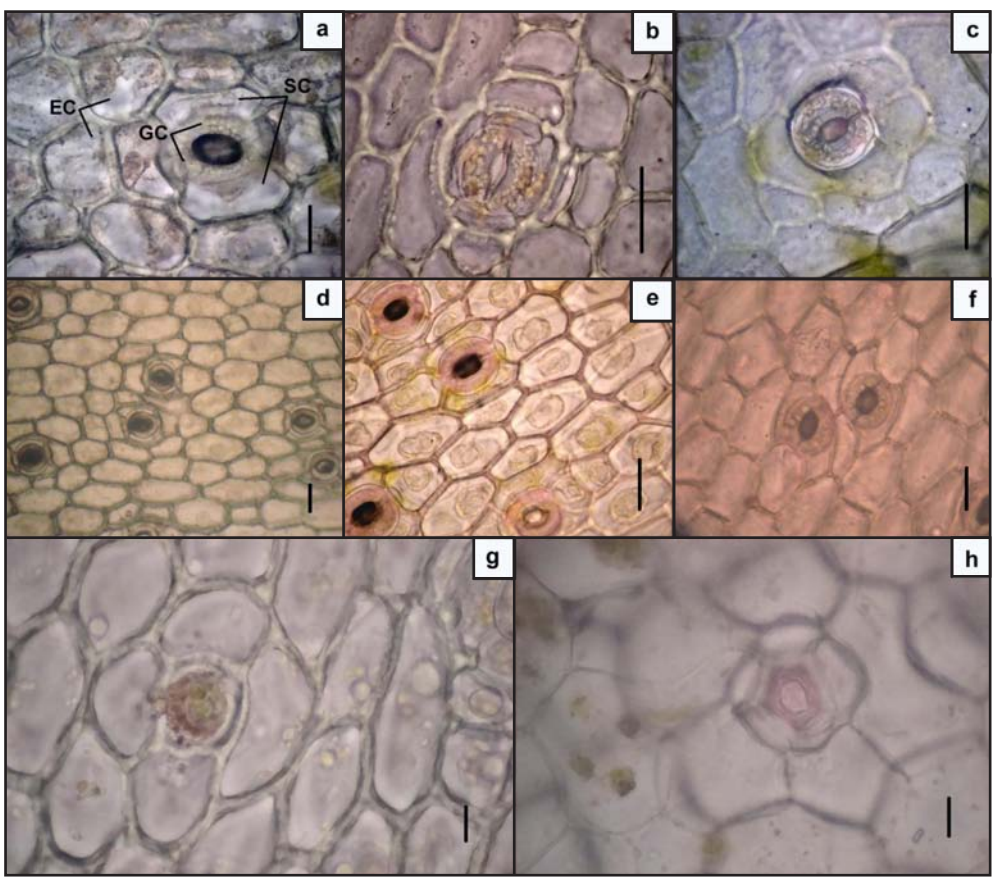

Figure 3 Epidermal leaf anatomy of stomata (a–f) at 400×: a) *C. filiforme*; b) *E. pannea*; c) *O. oklongensis*; d) *P. teres*; e) *P. vandarum*; f) *S. gemmata*; Epidermal leaf anatomy of wax-secreting cells (g–h) at 400×: g) *E. pannea*; h) *O. oklongensis.* Cu = Cuticle; CL = Cuticular ledges; CP = Cuticular papillae; MT = Mesophyll tissue; WSC = Water-storage cell. Scale bars-30 µm.

epidermal thickness was greatest in *S. gemmata* and least in *E. pannea*. However, the abaxial epidermal thickness was greatest in *P. teres* and least in *E. pannea* (Table 2). Overall the epidermal thickness was least in *E. pannea*. Large epidermal cells in many orchid species serve as water-storage cells. In some species of orchids, the water stored in epidermal cells can account for up to 80% of the entire leaf volume (Pridgeon and Stern, 1982). The water-storage cells were largest in *O. oklongensis* and smallest in *C. filiforme* (Table 2). Club-shaped water-storage cells were reported in *P. vandarum* by Kaushik (1983) but were not observed in the present study. The water-storage cells observed were irregular or star-shaped with banded thickenings and discontinuous inner walls. Xeromorphic traits such as cuticular ledges, cuticular papillae and phloem caps were observed in all the species studied. Raphide bundles were observed in the mesophyll cells of *E. pannea* and *O. oklongensis.* However, they were not observed in *P. vandarum* in the present study though they have been reported by Kaushik (1983) for this species. Raphides are calcium oxalate crystals and are regarded as non-nitrogenous waste products of plants, being needle-like, long slender crystals usually lying parallel to each other in a bundle, which is sometimes found in a special sac-like cell (Pandey, 2001). Certain raphides cause irritation and afford protection to the plants from animals (Pandey, 2001).

The stomatal size and stomatal apparatus area were largest in *P. teres* and smallest in *E. pannea* (Table 3). The exchange of gases—most importantly of water vapour and CO_2—between the interior of the leaf and the atmosphere is controlled by the stomata (Buckley, 2005). Stomatal distribution, size, density, morphology and behavior are closely associated with plant transpiration (Willmer and Fricker, 1996). Larger stomata are slower to close and have a greater potential for hydraulic dysfunction under conditions of drought (Aasamaa *et al.*, 2001). Paracytic stomata were observed in *C. filiforme, E. pannea*, *P. teres*, *P. vandarum* and *S. gemmata* and actinocytic stomata were observed in *O. oklongensis*. Paracytic stomata were also reported in *P. vandarum* (*Aerides vendarum*) and *S. gemmata* (*Cleisostoma gemmatum*) in earlier studies (Kaushik, 1983). The presence of subsidiary cells is very common in Orchidales and this condition is more widespread than the absence of subsidiary cells, that is, anomocytic stomata (Williams, 1979). All the species studied

Table 2 Leaf anatomical characteristics (mean±SD, n = 5) of the terete-leaved orchid species studied.

Species	CT_{ad} (µm)	CT_{ab} (µm)	ET_{ad} (µm)	ET_{ab}(µm)	WSC_L (µm)	WSC_W (µm)
Cleisostoma filiforme	15.50±0.50^{ab}	14.40±0.86^{ab}	32.00±1.84^{a}	30.14±2.01^{a}	93.00±14.97	54.50±6.91
Eria pannea	17.50±1.12^{ab}	11.50±1.00^{a}	31.50±3.12^{a}	29.50±2.78^{a}	-	-
Oberonia oklongensis	13.50±1.00^{a}	12.50±1.12^{a}	41.50±4.72^{ab}	40.50±5.39^{a}	269.00±0.00	194.00±0.00
Papilionanthe teres	18.50±1.87^{b}	16.50±1.00^{bc}	41.50±1.5^{ab}	58.50±2.69^{b}	144.50±0.00	68.50±0.00
Papilionanthe vandarum	19.00±1.27^{c}	17.50±1.58^{ab}	37.50±2.24^{ab}	38.50±2.69^{a}	149.00±0.00	104.50±0.00
Schoenorchis gemmata	13.50±1.00^{a}	11.80±1.32^{a}	47.00±8.15^{b}	39.00±4.00^{a}	161.00±0.00	123.00±0.00

$^{a–c}$ = Different lowercase superscript letters in the same column indicate statistical difference $P < 0.05$ using analysis of variance.

CT_{ad} = Adaxial cuticle thickness; CT_{ab} = Abaxial cuticle thickness; ET_{ad} = Adaxial epidermis thickness; ET_{ab} = Abaxial epidermis thickness; WSC_L = Length of water-storage cell; WSC_W = Width of water-storage cell.

Table 3 Dermal anatomical characteristics (mean±SD, n = 5) of the terete-leaved orchid species studied.

Species	A_s (μm²)	SI (%)	L_s (μm)	W_s (μm)
Cleisostoma filiforme	2141.29 ± 132.35^{b}	4.76 ± 0.57^{bc}	58.50 ± 1.87^{c}	46.50 ± 1.87^{b}
Eria pannea	1259.93 ± 122.97^{a}	3.07 ± 0.17^{a}	42.50 ± 1.12^{a}	37.50 ± 2.74^{a}
Oberonia oklongensis	1970.35 ± 124.21^{b}	3.31 ± 0.53^{ab}	50.00 ± 1.58^{b}	50.00 ± 1.58^{b}
Papilionanthe teres	3098.79 ± 63.32^{c}	7.48 ± 0.88^{d}	67.50 ± 1.12^{d}	58.50 ± 1.00^{c}
Papilionanthe vandarum	2345.19 ± 230.008^{b}	9.18 ± 0.47^{e}	57.50 ± 2.50^{c}	51.50 ± 3.32^{b}
Schoenorchis gemmata	1487.58 ± 54.24^{a}	5.03 ± 0.33^{c}	50.50 ± 0.50^{b}	37.50 ± 1.12^{a}

$^{a–e}$ = Different lowercase superscript letters in the same column indicate statistical difference $P < 0.05$ using analysis of variance.

A_s = Stomatal apparatus area; SI (%) = Stomatal index percentage; L_s = Stomatal length; W_s = Stomatal width.

were amphistomatic. In terete or ensiform leaves, stomata naturally occur on the whole surface (Rasmussen, 1987). An additional factor related to stomatal distribution is the existence of a crassulacean acid metabolism in many orchids. This pathway of carbon fixation is correlated with thick leaves which are associated with amphistomaty (Mott *et al.,* 1982). Cuticularized guard cells and wax-secreting cells which are xeromorphic characters were also observed. Sphaeraphides or sphaerites, which are aggregates of calcium oxalate crystals forming a star-like compound structure, were present in the epidermal cells of *P. teres*. They act as a defense mechanism against predators in some plants (Pandey, 2001).

CONCLUSION

All the species studied possessed both morphological (terete and fleshy leaves) and anatomical features (thick cuticles with cuticular papillae and cuticular ledges, water-storage cells, multicellular glandular hairs, wax-secreting cells and phloem cap) which are xeromorphic in nature. Hence, it can be concluded that these species are efficient in water use and can adapt very well even under water stress conditions.

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