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RESEARCH ARTICLE

Morpho-anatomy and mycorrhiza of epiphytic orchids of Tripura, Northeast India

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Abstract

In the present study, eight epiphytic orchids were collected from three different districts of Tripura to investigate the morphological and anatomical attributes of leaves and roots, as well as mycorrhizal morphology and colonization. The observations revealed that leaf morphological traits varied among the orchids. Additionally, root length and root collar diameter showed significant variation among the epiphytic orchids. Four types of stomata *viz.,* anomocytic, tetracytic, diacytic and paracytic were observed. The stomatal index ranged from 14.89 in *Rhynchostylis retusa* to 32.27 in *Rhynchostylis retusa* (L.) Blume. The maximum root length was observed in *Acampe praemorsa*, while minimum root length was noted in *Rhynchostylis retusa* C.E.C.Fisch. *Dendrobium transparens* exhibited the highest root hair density, whereas *R. retusa* had the lowest. All the species examined showed mycorrhizal colonization percentages ranging from 36.77 in *D. lindleyi* to 71.66 in *A. praemorsa*. The percentage of root length with intact pelotons ranged from 12.91% in *D. lindleyi* to 42.41% in *A. praemorsa*, while the percentage with lysed pelotons ranged from 23.86% in *D. lindleyi* to 37.55% in *D. aphyllum*. The cortical cells of the epiphytic orchids contained both intact and lysed pelotons, with their ratio varying among the studied species. The morphological and anatomical characteristics, along with their mycorrhizal colonization patterns, firmly support the adaptive features of orchids as epiphytes.

Keywords: Orchidaceous mycorrhiza; pelotons; root hairs; root morphology; stomata

Introduction

Around 10% of all flowering plants belong to the Orchidaceae family (1), leading to significant variations in floral traits and life cycle pattern (2). Orchids produce tiny seeds with low nutrient reserves, making them highly dependent on mycorrhizal fungi for seed germination and seedling growth (3). However, their reliance on these fungi decreases as they mature (4). While some orchids are terrestrial, they are primarily epiphytic (5). The aerial roots of epiphytic orchids originating from the rhizome covered with velamen, plays a crucial role in the accumulation of organic substances (6) and they thrive in a wide range of habitat complexities and diversities (7).

The orchids and mycorrhizal fungi share a symbiotic relationship, although the degree and complexity of this symbiosis vary with age of the orchid (5, 8) and the stage of root development (9). Orchid mycorrhiza is characterized by the formation of distinct pelotons, which may be either loosely or rigidly orientated in the root cells (10). The absorption of fungal carbohydrates is attributed to the regulated digestion of pelotons within host cells (11).

India is home to 1331 orchid species from 186 genera, with 900 species found in the northeastern region across 165 genera (12). In Tripura, the flora includes 33 orchid species belonging to 22 genera were (13). The region has recently added a total of twenty-five new orchid species (14-17).

Reports are available on the occurrences of mycorrhizal fungi in the epiphytic orchids (18-23).However, there is lack of studies on the morphology, anatomy and mycorrhizal colonization in epiphytic orchids from north eastern states of India, although the region harbours the most robust flora of these species. Therefore, the main focus of this research is to study the mycorrhizal status, morphology and anatomy of the root and leaf stomatal features of epiphytic orchids in Tripura.

Materials and Methods

Site of collection

The epiphytic orchids for this research were collected from five different locations across three distinct districts of Tripura, northeastern India. The geographic coordinates of the collection sites and the relative locations of the host plant were documented. Tripura experiences a warm and humid

tropical climate with four distinct seasons *viz*., pre-monsoon, monsoon, post-monsoon and winter. During the winter, the average temperature, humidity, precipitation and wind speed were 15.8°C, 73%, 6 mm and 1 kmph, respectively. In the pre-monsoon and monsoon seasons, these values were 21.5°C, 65%, 343 mm, 3kmph and 23.6°C, 76%, 362 mm, 3 kmph, respectively. During post monsoon average temperature, humidity, precipitation and wind speed was recorded as 28.6° C, 82%, 33 mm and 1 kmph, respectively.

Ecological attributes of the epiphytic orchids

The ecological characteristics of each selected orchid were documented through consultations with the local communities, forest residents and elderly individuals. These findings were then compared with the available established literature. The IUCN status was determined using the IUCN Red List of Threatened Species. Additionally, the ethnobotanical applications of each orchid were documented based on verified information and insights gathered from local sources.

Morphological characteristics of orchids

To compare the morphometric characteristics of various epiphytic orchid species, measurements of various plant sections, including roots and leaf lamina, were taken into consideration. Root length and root collar diameter were measured, while the leaves were measured for length, breadth and leaf area. Leaf area was calculated using graph paper.

Root hair morphology

To evaluate root hair characteristics, 10 randomly chosen root segments, each measuring about 1 cm in length, were placed in distilled water on microscope slides and covered with cover slips. Using a dissecting microscope at 10x magnification, the number of root hairs per centimetre of root length was counted. Measurements of root hair length and breadth were taken using compound microscope equipped with an ocular scale. The root segments used for mycorrhizal colonization assessment were also utilized for root hair density measurements. Root segment counts in three dimensions were performed by adjusting focus of a microscope's plane (24).

Root sampling

Eight different epiphytic orchid species were selected and roots from five individuals of each species were carefully uprooted from the tree stem and preserved in Formaldehyde Acetic Acid (FAA) solution in labelled plastic bottles. The FAA solution was prepared by adding 10% formaldehyde to a mixture containing 50% ethanol, 5% acetic acid and 35% distilled water. Since orchids roots are firmly connected to host plants; collecting root samples required utmost caution. Upon returning to the laboratory, the root samples were divided into two portion; one half were used for the morphological and anatomical characterization, while the other half was reserved for the mycorrhizal investigation.

Root processing and quantification of orchid fungal colonization

The preserved root samples were carefully remove and repeatedly rinsed with running tap water, followed by washing with double-distilled water. Manually sectioned root segments that floated on the surface of water in petri plates were transferred to beakers, treated with 10% NaOH and stained using recommended protocol (25). A total of 20 manually sectioned, stained root samples were mounted on glass slides filled with lactoglycerol for observation under compound microscope (Olympus CX21i). The magnified intersection method (26) was employed to determine root length colonization. Quantitative assessment included total root length colonization, intact and lysed pelotons and fungal hyphae-colonized root length.

Processing of leaves of epiphytic orchids for stomatal characteristics

Harvested leaves were thoroughly washed under running water to remove any dirt or debris before analysis of stomatal features. Using a sterilized blade, the abaxial and adaxial surfaces of the leaf samples were peeled. The peeled leaves samples were immersed in 1.5% NaOH for an hour and then rinsed repeatedly with distilled water. The leaf samples were then mounted to a grease-free slide containing lactoglycerol and stained with 5% malachite green. Observations were conducted under a light microscope and microphotographs were captured, using a compound microscope connected to a computer.

Determination of Stomatal index

The stomatal index measures the proportion of stomata in relation to the total number of epidermal cells, within each stoma counted as a single cell. For the assessment of stomatal index, the following formula is used (27).

Stomatal index (SI) = S/E+S x 100, where, S = No. of stomata/ microscopic field, E = No. of epidermal cells / microscopic field.

Anatomy of the roots of the selected epiphytic orchids

The preserved root samples were removed and cleaned under running water to eliminate adhered organic matter. They were then sectioned manually. The root segments that floated on the water's surface in Petri plates after sectioning were transferred to a beaker and treated with 10% NaOH at a temperature of 80°C for 15–20 min. Subsequently, the treated root samples were thoroughly washed with distilled water followed by running tap water.

The washed root segments were then stained with diluted Safranin (0.2%), mounted on lactoglycerol and examined under a light microscope. Microphotographs were captured and observations on the fundamental anatomical characteristics were recorded. For the study of anatomical properties of root, 10 manually sectioned root segments were analysed.

Data analysis

Morphometric traits, stomatal characteristics, mycorrhizal colonization and root morphology of the epiphytic orchids, were analysed using one-way analysis of variance (ANOVA) with mean. All the analysis was performed using Statistica (ver. 9.0., Statsoft).

Results

Epiphytic orchids and their respective hosts

For the present assessment, a total of eight epiphytic orchids were selected and collected from three different districts of Tripura (Table 1 and Fig.1). The orchids were obtained from seven different hosts plants, each belonging to a distinct plant family. These host plants exhibited diverse bark characteristics, which may play a role in the host selection by the epiphytic orchids (Table 2).

Ecological attributes

The ecological attributes of the selected epiphytic orchids are presented in Table 2. Many of these selected epiphytic orchids have ethnomedicinal potential, while some are valued for their ornamental uses in decoration. The epiphytic orchids thrive in shady environment for their optimal growth and development and their flowering seasons vary.

Table 1. Collection sites of selected epiphytic orchids with their respective hosts and location

Name of the collected plants	Vernacular name	Host plant	Host family	Collection site	District	GPS location
Acampe praemorsa (Roxb.) Blatt. & McCann	Rasna (Bengali)	*Artocarpus heterophyllus* (L)	Moraceae	Chourangi Para	West Tripura	N23°48'.00.''E 091°.15'.21'' 74 masl
Bulbophyllum affine Wall. ex Lindl.	-	*Mangifera indica* (L)	Anacardiaceae	Barpathari	South Tripura	N23°.17'.42''E 091°.24'.04'' 169masl
Coelogyne viscosa Rchb.f.	-	*Schima wallichi* (DC) Korth	Theaceae	Suryamaninagar	West Tripura	N23°.45'.52.09"E 091°.15'.37.64" 71 masl
Cymbidium aloifolium (L.) Sw.	Pragasa (Bengali)	*Shorea robusta* (Roth)	Dipterocarpaceae	Paratia	Gomti	N23°.29.930'E 091°.29.053" 92 masl
Dendrobium aphyllum(Roxb.) C.E.C.Fisch	Fasia mach (Chakma)	*Tectona grandis* (L)	Lamiaceae	Amarpur	Gomti	N23°.29.930'E 091°.37.834" 91 masl
Dendrobium lindleyi Steud	-	*Syzyzium cumini* (L)	Myrtaceae	Suryamaninagar	West Tripura	N23°.45'.52.09"E 091°.15'.37.64" 71 masl
Dendrobium transparens Wall. ex Lindl.	-	*Artocarpus chaplasha* Roxb.	Moraceae	Suryamaninagar	West Tripura	N23°.45'.52.09"E 091°.15'.37.64" 71masl
Rhynchostylis retusa (L) Blume	Sita pushpa (Bengali)	*Tectona grandis* (L)	Lamiaceae	Amarpur	Gomti	N23°.29'.930'E 091°.37.834" 91masl

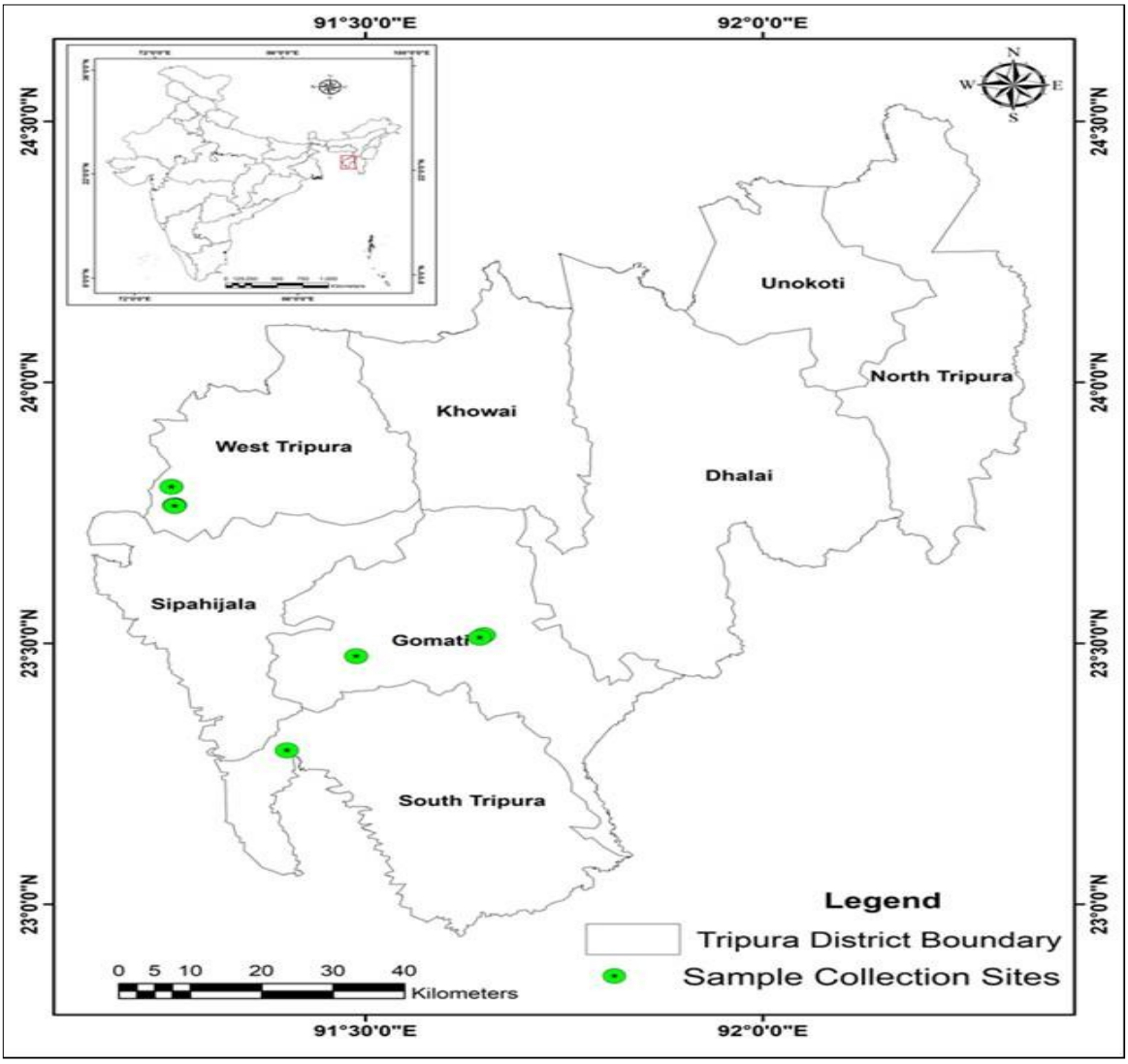

Fig.1. Map location of sampling sites of selected epiphytic orchids.

Table 2. Ecological attributes and ethnobotanical uses of epiphytic orchids of Tripura

Plant species	Flowering Time	IUCN Status	Habitat	Host bark characteristics	Ethnobotanical uses
Acampe praemorsa	March-April	Yet to be assessed	Moss covered tree trunks	Bark greyish black in colour, smooth and compact vertical cracks in certain areas	Rheumatism and arthritis (43)
Bulbophyllum affine	June-August	Yet to be assessed	Shady places of tree trunks	Bark brown in colour, thick, fragile with uneven surface comprising distinct protuberance	Antipyretic, anti-phlegm (44)
Coelogyne viscosa	October-December	Yet to be assessed	Moist areas of tree trunks	Bark brownish grey with longitudinal fissures	Ornamentation and decoration
Cymbidium aloifolium	April-May	Yet to be assessed	Semi-evergreen and deciduous forests	Bark hard and compact with vertical cracks	Anti-paralysis (43) .
Dendrobium aphyllum	March-April	LC (Ver3.1)	Shady places of tree trunks	Thick, soft and fragile bark divided into thin fibrous strips, grey in colour	Normal shape of deformed head structure of newly borne baby (43)
Dendrobium lindleyi	March-April	Yet to be assessed	Shady branches of trees	Bark rough and dark grey in colour	Ornamentation and decoration
Dendrobium transparens	April-May	Yet to be assessed	Shady places of tree trunks	Bark greyish brown in colour, latex milky	Fractured and dislocated bones (46)
Rhynchostylis retusa	June-July	Yet to be assessed	Moist deciduous forests	Thick, soft and fragile bark divided into thin fibrous strips, grey in colour	Blood dysentery (43)

Morphological and anatomical characteristics of leaf

Leaf characters were recorded revealing significant morphological variations among the orchids. Root length and root collar diameter also varied significantly among some of the epiphytic orchids (Table 3).

Stomatal properties, such as stomatal type (adaxial and abaxial surface), stomatal index, stomatal length and width, guard cell dimensions were noted down (Table 4). Four different types of stomata *viz.,* anomocytic (*A. praemorsa, B. affine, D. aphyllum* and *D. lindleyi*) tetracytic (*C. graminifolia*), diacytic (*R. retusa*) and paracytic (*D. transparens*) were observed (Fig. 3.). Stomatal index ranged from 14.89±0.62 (*D. lindleyi*) to 32.27±1.28 (*R. retusa*). The maximum and minimum stomatal length including guard cell was noted in *R. retusa* (52.34±0.24) and minimum *C. graminifolia* (26.46±0.36), respectively. Similarly, maximum and minimum stomatal width including guard cell, was noted in *R. retusa* (28.69±0.29) and the minimum in *C. graminifolia* (11.28±0.21), respectively.

The length and breadth of cortical cells, endodermal cells, water cells, exovelamen and pith differed significantly among the selected epiphytic orchids. The maximum cortical cell length was recorded in *B. affine,* while minimum was recorded in *C. aloifolium*. The maximum cortical breadth was recorded in *D. transparens*, with minimum in *C. aloifolium. B. affine* exhibited the largest endodermal and water cell length and breadth. The smallest endodermal cell length and breadth were recorded in *D. transparens,* while smallest water cell length and breadth were recorded in *C. graminifolia*. *A. praemorsa* had the longest exovelamen and pith length, while smallest exovelamen and pith length was recorded in *D. lindleyi* and *D. aphyllum* respectively. The maximum and minimum pith breadth was recorded in *B. affine* and *D. transparens*, respectively (Table 5).

Morphological and anatomical properties of root

Root length, root collar diameter, root hair length, root hair breadth and root hair density were determined among the selected epiphytic orchids, revealing significant differences across majority species (Table 3 and 6). The longest root length was observed in *A. praemorsa*, while the shortest root length was noted in *D. aphyllum.* The maximum and minimum root collar diameter was observed in *R. retusa* and *A. praemorsa*, respectively. *D. transparens* and *A. praemorsa* exhibited the longest and shortest root hair length respectively. *D. transparens* exhibits maximum root hair density, while *R. retusa* showed the least.

Table 3. Morphological characteristics of leaf and root of epiphytic orchids

Plant species	LL (cm)	LB (cm)	LA (cm²)	RL (cm)	RCD (mm)	Leaf characters
Acampe praemorsa	10.92±0.12a	1.32±0.09a	4.6±0.03a	5.36±1.14a	0.43±0.03a	Leaves distichous, linear, sheathing at base, apex unequally 2-lobed, thick and coriaceous
Bulbophyllum affine	11.52±0.04a	2.31±0.16b	5.9±0.01b	2.81±1.08b	0.53±0.07a	Leave single, narrowly oblong, obtuse, the base narrowed
Coelogyne viscosa	10.84±0.33a	1.65±0.12c	4.2±0.03a	3.46±0.33b	0.55±0.03a	Leaves lanceolate, plicate, finely nerved, acute with a tapering
Cymbidium aloifolium	46.62±0.02b	1.76±0.22c	8.4±0.01c	2.82±0.35b	0.53±0.03a	Leaves coriaceous, obtuse, fleshy, linear-oblong, slightly notched at the apex with the base expanded
Dendrobium aphyllum	5.18±0.26c	1.02±0.11a	3.2±0.04d	2.14±0.52c	0.60±0.06b	Leaves oblong or ovate-lanceolate, acute and deciduous after one growing season
Dendrobium lindleyi	11.60±0.79a	1.96±0.13c	3.2±0.02d	3.36±0.26b	0.57±0.03a	Leaves solitary, oblong, notched at the top
Dendrobium transparens	11.94±0.60a	1.97±0.16c	4.34±0.03a	2.89±0.33b	0.57±0.03a	Leaves linear to lanceolate, acute
Rhynchostylis retusa	37.20±0.14b	2.27±0.20b	9.7±0.03c	3.70±0.60b	0.67±0.03b	Leaves are lorate, obliquely bilobed at the apex with a mucro in between, lobules rounded

Different alphabets differ significantly at $p<0.05$

LL=Leaf length, LB=Leaf breadth, LA=Leaf area, RL=Root length, RCD=Root collar diameter

Table 4. Stomatal characters of the selected epiphytic orchids

Plant species	Leaf surface	Type of stomata		Stomatal index (%)	Stomatal length with guard cell (µm)	Stomatal width with guard cell	Established reports
		Adaxial	Abaxial				
Acampe praemorsa	Amphistomatic	Anomocytic	Anomocytic	18.36±1.47a	32.58±0.20a	17.62±0.27a	(47)
Bulbophyllum affine	Amphistomatic	Anomocytic	Anomocytic	22.49±1.31ba	46.69±0.20b	24.50±0.29b	(47)
Coelogyne graminifolia	Amphistomatic	Tetracytic	Tetracytic	16.51±0.90 c	26.46±0.36c	11.28±0.21c	-
Cymbidium aloifolium	Amphistomatic	Diacytic	Diacytic	25.81±1.01d	37.50±0.22d	16.46±0.25d	(48)
Dendrobium aphyllum	Amphistomatic	Anomocytic	Anomocytic	18.68±1.74 c	31.26±0.10e	18.33±0.25e	(47)
Dendrobium lindleyi	Amphistomatic	Anomocytic	Anomocytic	14. 89±0.62c	32.20±0.14f	19.18±0.15f	-
Dendrobium transparens	Amphistomatic	Paracytic	Paracytic	15.27±0.71c	28.67±0.22g	12.21±0.11g	-
Rhynchostylis retusa	Amphistomatic	Diacytic	Diacytic	32.27±1.28e	52.34±0.24h	28.69±0.29h	(48)

Table 5. Anatomical characters of the root segments of the epiphytic orchids

Plant species	Cortical cell (µm)		Endodermal cell (µm)		Water cell (µm)		Exovelamen (µm)		Pith (µm)	
	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth
Acampe praemorsa	53.44±0.12 a	45.58±0.24a	26.73±0.15a	21.99±1.31a	19.46±0.64a	14.34±3.71a	28.48±0.70a	23.30±0.70a	25.51±1.35a	13.80±1.35a
Bulbophyllum affine	67.85±0.12 b	53.03±0.02b	37.12±0.18b	36.61±0.26b	18.70±0.87a	7.88±0.69b	26.44±0.69a	21.44±0.29b	24.01±1.89a	18.10±1.45b
Coelogyne graminifolia	45.32±0.24 c	38.29±0.03c	22.49±0.11c	18.4±1.72c	14.31±1.08a	3.88±0.76b	25.56±0.45b	21.72±0.22b	23.88±0.91a	13.90±1.69a
Cymbidium aloifolium	40.93±0.29 dc	34.41±0.25c	23.29±0.60c	19.22±0.28c	15.35±1.52a	4.91.±0.63b	25.86±0.95b	22.18±0.39a	23.04±1.33a	13.61±0.79a
Dendrobium aphyllum	47.60±1.14ec	32.53±1.40c	26.52±0.03a	25.67±0.22d	12.16±1.16b	4.52±1.12b	25.78±0.71b	21.86±0.57b	16.7±1.02b	11.42±1.52a
Dendrobium lindleyi	66.11±1.30 b	57.59±0.78b	26.19±0.08a	22.44±0.18a	15.16±1.01a	6.96±1.48b	23.68±0.73c	21.60±0.65b	23.6±1.13a	13.82±1.35a
Dendrobium transparens	66.42±0.82 b	60.58±1.28d	18.75±0.24d	16.51±0.02e	16.21±1.37a	7.11±1.46b	26.38±0.31a	22.02±0.23b	21.18±1.74a	10.91±1.39c
Rhynchostylis retusa	42.60±1.08cd	35.40±0.24c	31.29±0.10b	26.46±0.12d	17.52±1.04a	6.54±1.06b	27.8±0.86a	22.6±0.92a	22.08±1.30a	11.12±1.76c

Different alphabets differ significantly at *p<0.05*

Mycorrhizal colonization pattern

All the selected epiphytic orchids in the present study were associated with the accumulation of organic debris and mosses. Aerial roots were colonized by mycorrhizal fungi only when in contact with the plant substratum. The colonization percentage among the examined species ranged from 36.77±4.03 (*D. lindleyi*) to 71.66±2.08 (*A. praemorsa*). Colonization zones were typically observed in the cortical region of the roots, adjacent to the substrate. Cortical cells were characterized by the presence of peloton at various stages of development and lysis. Root hairs in some epiphytic orchids possess hyphae and microsclerotia-like structures. Fungal hyphae were also observed on the root surface at the point of contact with the substratum. The percentage of root length with intact pelotons ranged from 12.91±1.85 (*D. lindleyi*) to 42.41±1.80 (*A. praemorsa*), while with lysed pelotons ranged from 23.86±2.67 (*D. lindleyi*) to 37.55±2.30 (*D. aphyllum*). The mycorrhizal colonization pattern is illustrated in the Fig 2.

Ratio of intact and lysed peloton

The ratio of intact to lysed pelotons varied significantly among the selected epiphytic orchid species (Table 6). The highest intact-to-lysed peloton ratio was observed in *A. praemorsa* (1.51±0.09), while lowest was recorded in *R. retusa* (0.45±0.08), respectively.

Discussion

This study represents one of the first efforts from northeast India to examine the morpho-anatomical attributes and the extent of mycorrhizal colonization. However, the mycorrhizal colonization in *Acampe praemorsa, Cymbidium aloifolium* and *Rhynchostylis retusa* has been reported earlier (28). Contrary, the remaining three orchids selected for this study, were reported for the first time to possess orchidaceous mycorrhizal fungal structures.

The findings confirm that all of the epiphytic orchids included for this investigation were mycorrhizal highlighting their dependence on these organisms (3). The percentage of

Table 6. Root morphology and mycorrhizal colonization in selected epiphytic orchids of Tripura

Plant species	Root hair morphology			Mycorrhizal colonization (%)			RILP	% COIP
	RHL(µm)	RHB (µm)	RHD	RLIP	RLLP	RLTC		
Acampe praemorsa	59.7±1.12a	6.75±0.36a	3.35±0.15a	42.41±1.80a	29.25±1.33a	71.66±2.08a	1.51±0.09a	69.16±3.07a
Bulbophyllum affine	59.85±1.51a	7.35±0.28a	3.40±0.05a	39.24±1.03a	26.99±1.13a	67.01±1.45a	1.49±0.05a	62.30±2.53a
Coelogyne viscosa	65.5±1.19b	7.65±0.24b	3.54±0.04a	24.93±1.73b	28.81±2.49a	53.74±3.73b	0.89±0.09b	67.69±2.23a
Cymbidium aloifolium	84.8±0.80c	7.7±0.22b	3.39±0.55a	38.09±2.23a	30.82±1.87ad	68.91±3.03a	1.30±0.10a	61.54±2.75a
Dendrobium aphyllum	72.55±1.23d	7.05±0.35a	3.44±0.04a	23.95±1.59b	37.55±1.94b	61.50±2.35ab	0.68±0.07c	63.89±1.99a
Dendrobium lindleyi	83.3±1.09c	7.85±0.21a	3.33±0.06a	12.91±1.85c	23.86±2.67ac	36.77±4.03c	0.54±0.08c	60.49±2.67ba
Dendrobium transparens	89.3±1.75e	8.1±0.18ab	3.55±0.02a	20.43±2.11bc	37.54±2.30b	57.97±3.60ab	0.56±0.04c	66.73±2.36a
Rhynchostylis retusa	64.35±0.99b	7.25±0.32a	3.29±0.06ab	15.74±2.07c	35.03±3.06ab	50.78±4.78b	0.45±0.08d	58.93±2.32ba

Different alphabets differ significantly at *p<0.05,* RHL=Root hair length, RHB=Root hair breadth, RHD=Root hair diameter, RLIP=Root length with intact peloton, RLLP=Root length with lysed peloton, RLTC=Root length with total colonization, RILP=Ratio of intact and lysed peloton, % COIP=Percentage of cortical cell occupied by intact peloton

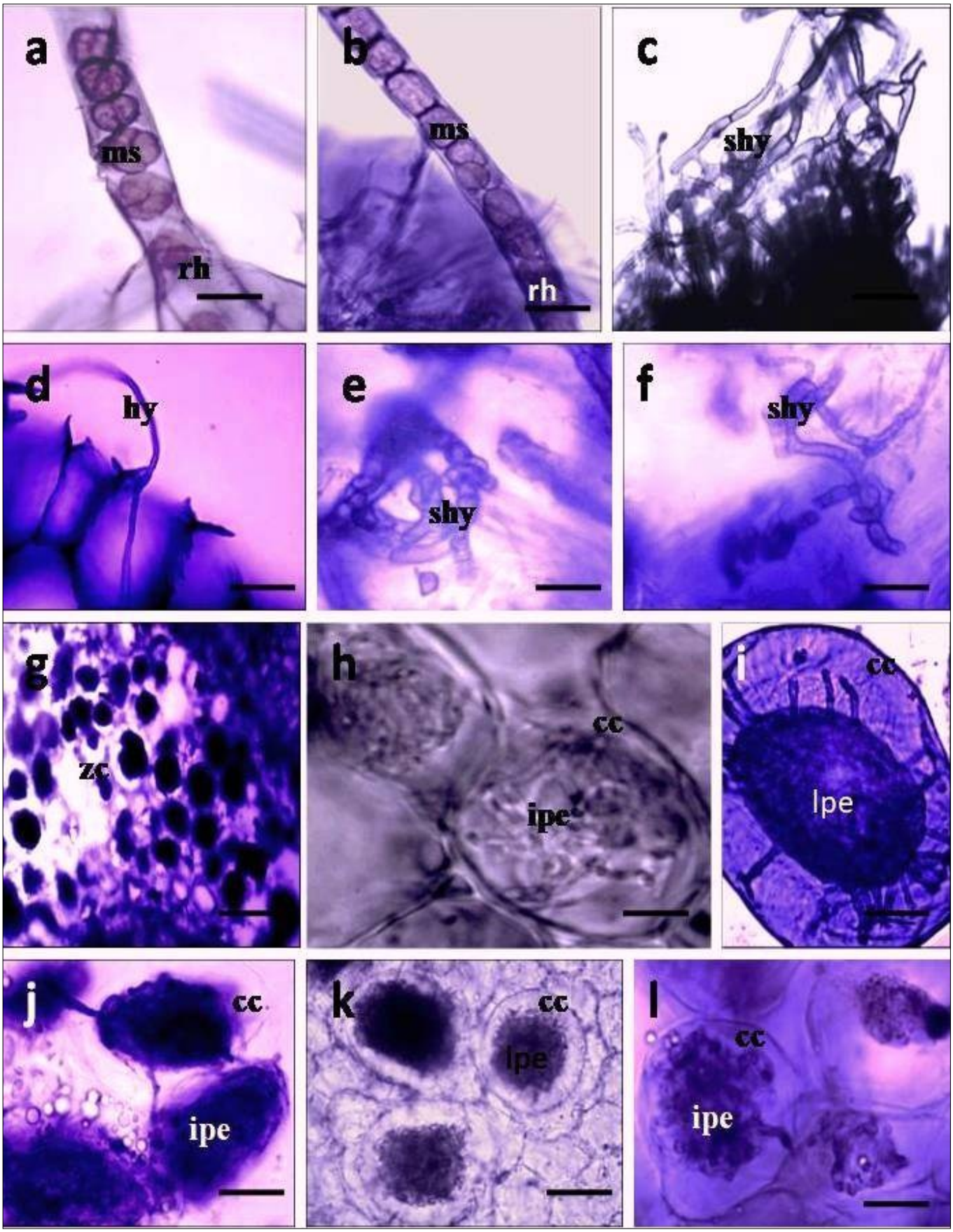

Fig. 2. Light microscopic images of mycorrhizal association in epiphytic orchids.(a) Microsclerotia like structures (ms) within root hairs (rh) of *Bulbophyllum affine*, (b) Microsclerotia like structures (ms) within root hairs (rh) of *Acampe praemorsa*, (c) Septate hyphae (shy) in the root region of *Cymbidium aloifolium,* (d) Hyphae (hy) entering into the root cortex of *Cymbidium aloifolium*,(e-f) Septate hyphae (shy) in the root cortex of *Dendrobium transparens*,(g) Transverse section of *Dendrobium aphyllum* epiphytic root showing zone of colonization (zc),(h) Intact pelotons (ipe) in root cortical cells(cc) of the *Acampe praemorsa*, (i and k) Lysed pelotons in root cortical cells(cc) *Coelogyne viscosa* and *Coelogyne viscosa* (j and l) Intact pelotons (ipe) and cortical cells (cc) *Rhynchostylis retusa* and *Dendrobium lindleyi*. (Scale bars: a, b, h-l=50 µm; c-f=100 µm; g=200 µm).

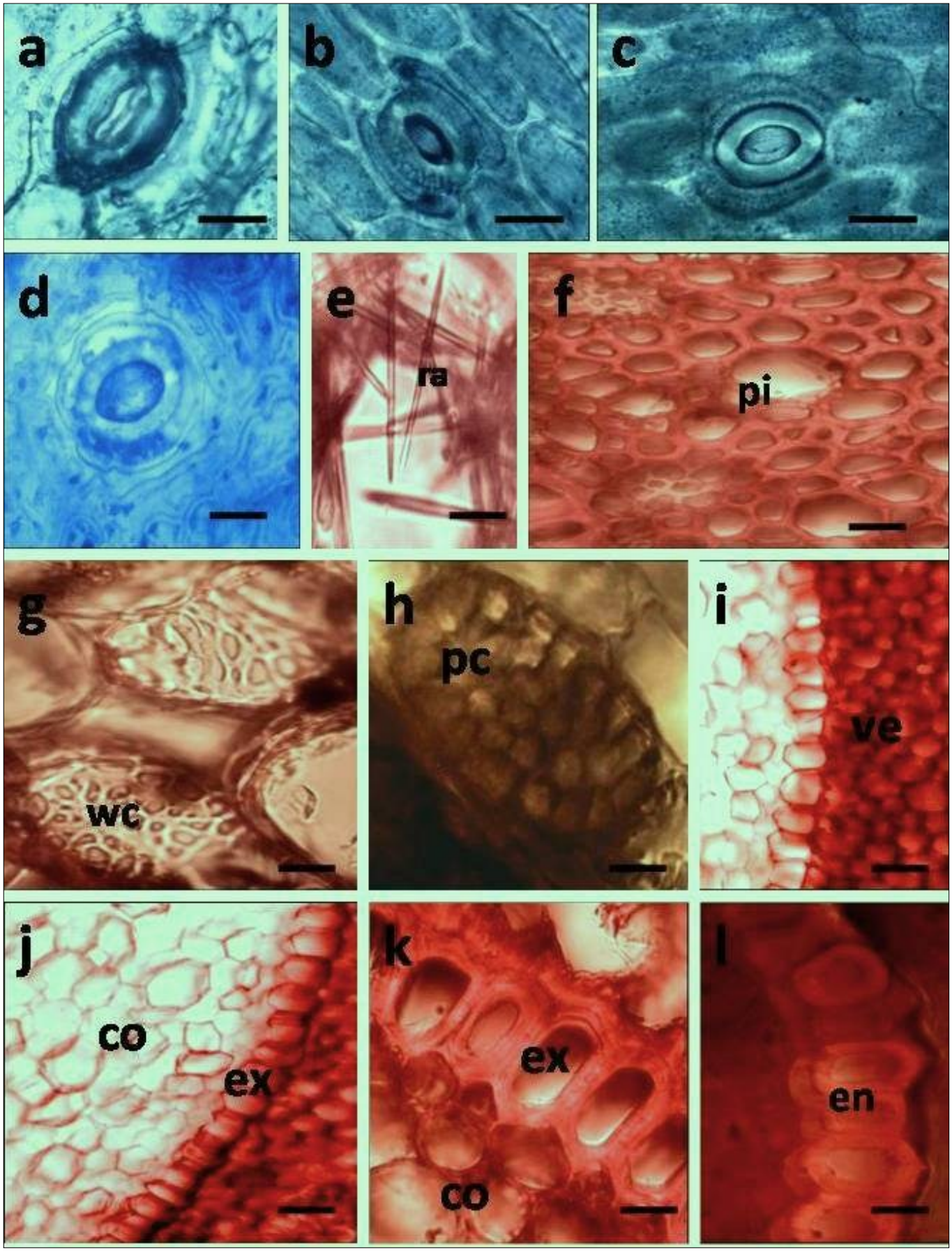

Fig. 3. Light microscopic images of leaf stomata and anatomical features root. (a) Leaf segment showing anomocytic stomata of *Acampe praemorsa*,(b) Leaf segment showing diacytic stomata of *Cymbidium aloifolium*, (c) Leaf segment showing diacytic stomata of *Rhynchostylis retusa*, (d) Leaf segment showing anomocytic stomata *Bulbophyllum affine*, (e-f) Transverse section (TS) of root segment of *Cymbidium aloifolium* showing raphides(ra) and pith, (g-h) TS of root segment of *Rhynchostylis retusa* showing banded water storage cell (wc) and passage cell (pc). (i-j) TS of root segment of *Acampe praemorsa* showing velamen(ve), cortex(co) and exodermis (ex), (k-l) TS of root segment of *Dendrobium aphyllum* showing cortex(co) and exodermis (ex) and endodermis (en). (Scale bars: a-d, k, l=50 µm; f-h=100 µm; e, i, j=150 µm).

mycorrhizal colonization across the eight selected species ranged from 36.77±4.03 (*D. lindleyi*) to 71.66±2.08 (*A. praemorsa*). The cortical cells were characterized by the presence of peloton in various stages of development and lysis. The percentage of root length with intact pelotons ranged from 12.91±1.85 (*D. lindleyi*) to 42.41±1.80 (*A. praemorsa*), while lysed pelotons ranged from 23.86±2.67 (*D. lindleyi*) to 37.55±2.30 (*D. aphyllum*). Fungal hyphae penetrated the roots of the epiphytic orchids via their root hair (29). The hyphae often penetrate root hairs near the tips, although instances of penetration further away from the tip were also observed. Typically, a single hypha penetrated each root hair, however in some cases multiple hyphae entered single root hairs as well (29). (29), proclaimed that when the fungal hyphae reach the root hair cells, the root hair cells become bent and inaccurate to varying degrees. The bending of root hair cells marked the presence of fungi in the root hair (30). Fungal hyphae formed pelotons, highly coiled structures, after entering the cortical cells. In line with the earlier findings (19,28), root cortical cells in the current investigation showed both lysed and intact pelotons. The ratio of intact to lysed pelotons varied significantly among the selected epiphytic orchid species. The maximum and minimum ratio of intact to lysed peloton were observed in *A. praemorsa* (1.51±0.09) and *R. retusa* (0.45±0.08), respectively.

According to a theory put forward (31), intact pelotons are associated with the flow of nutrients between fungi and orchids. The growth of cortical pelotons and their subsequent lysis follow a time scale pattern (3) and the proportion of intact to lysed pelotons vary depending on the species and the plant's environment. Fungal-mediated lysis in orchid mycorrhizae is considered a sign of plant defense against invasion and is linked to the transfer of nutrients to the host (4). However, the appearance of a growth response to colonization before the lysis of the pelotons contradicts in this hypothesis (32).

The penetration of cellular fungal hyphae did not result in any cell wall thickening or distortion that would indicate local hydrolysis (33). In sections of the root close to the substrate in epiphytic orchids, mycorrhizal colonization was frequently observed consistent with prior observations (18,34). In contrast to past results (33), the selected epiphytic orchid roots showed a moderate amount of colonization (minimum 36.77% to maximum 71.66%). Unlike arbuscular mycorrhizal (AM) colonization, where root properties significantly influence the extent and dependency of the host mycorrhizal fungus (35), the root hair properties of epiphytic orchids in this analysis were not correlated with the degree of fungal colonization. The kinds of fungi engaged in the relationship may be to attributed to the disparity between the two mycorrhizal forms (36). The presence of mycorrhizal fungi in the roots of epiphytic orchids is supported (37), but there is no conclusive evidence to support their role in the direct uptake of nutrients (36).

The outermost layer of the velamen is mostly composed of cellulose, with varying amounts of lignin and suberin (19,39). (20,38), hypothesized that velamens are linked to mechanical protection, water and nutrient absorption, reduced transpiration and water loss and infrared reflection. (40) stated that orchids living in wet settings lack velamen or have single layer velamen, while orchids residing in desert and dry habitats have multilayer velamen. All of the epiphytic orchids that were chosen for this research exhibit thickened cell walls in the velamen, supporting prior results (21). The thickening of the velamen cell wall provides mechanical support for the prevention of water loss (39).

The exodermis, an outer layer of the cortex located beneath the velamen, exhibits secondary cell wall thickenings that become void and dead at maturity (39). Lignin and suberin impregnation contribute to mechanical protection against water evaporation, aid in cortical moisture retention and regulate the entrance of mycorrhizal fungal into cortical cells (21,39). In the coastal forest with high light intensity, the thickening of the velamen and exodermis serves as a structural adaptation (21). Beneath the exodermis lies the thin-walled parenchymatous cortex, which varies in diameters.

In this study, water-storage cells with banded thickening were also noted, which were previously reported (40). The rooting zones of epiphytic orchids are limited because they are anchored to small patches of organic matter on branches and depend on rainwater. During rainfall, these patches become wet but soon dry out. Uniformly thickened water storage cells not only ensures the selective advantages for the preservation of constant water content, but are also associated with enhanced vascular apoplastic transport of material (22). Furthermore, the thickened endodermal cell walls also serve as an apoplastic barrier, shielding vascular bundles from pathogens (21).

Stomatal properties such as stomatal type (adaxial and abaxial surface), stomatal index, stomatal length and breadth with the guard cell were noted down. Four different types of stomata were observed: anomocytic (*A. praemorsa, B. affine, D. aphyllum* and *D. lindleyi*) tetracytic (*C. graminifolia*), diacytic (*R. retusa*) and paracytic (*D. transparens*). The stomatal index ranged from 14.89±0.62 (*D. lindleyi*) to 32.27±1.28 (*R. retusa*). The maximum and minimum stomatal length with guard cell was noted in *R. retusa* (52.34±0.24) and minimum *C. graminifolia* (26.46±0.36), respectively. The maximum and minimum stomatal width with guard cell was noted in *R. retusa* (28.69±0.29) and minimum *C. graminifolia* (11.28±0.21), respectively. According to previous studies (23), all of the selected epiphytic orchids exhibit amphistomatic behaviour. (41) Amphistomatic conditions are often found in environment with high light intensity and/or dry, reflecting orchids adaptation to their environment. The presence of an amphistomatic condition also favours improving CO_2 conductivity (42).

Conclusion

The examined orchids exhibit epiphytic life patterns dependent on mycorrhiza, despite having variations in agro climatic attributes such as temperature, habitat and the availability of the compatible host plants. Anatomical traits that identify the distinctive features of their vegetative

forms are also explored in present investigation. Additionally, stomatal characteristics contribute to their natural habitats and are linked with the improved photosynthetic efficiency. This study opens a new avenue for expanding research on the mycological aspect of epiphytic orchid plants from Tripura, particularly focusing on their mycorrhizal relationships. Notably, this research is the first to document the relationship of mycorrhiza and orchids in Tripura. While the current data clearly show that mycorrhizae are common in epiphytic orchids of Tripura, furthermore research on the characterization of mycobionts may provide better understanding on their pivotal role. The study's finding on the leaf, root, stomatal morphology and anatomy along with mycorrhizal characteristics strongly support the epiphytic adaptability of orchids, highlighting the evolutionary mechanism behind their survival.

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Authors' contributions

KC and TC conducted the work. PD and KC analyzed the data. KC and PD designed the work. KC, AKS and PD wrote the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: There are no conflicts of interest.

Ethical issues: None

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