COMPARATIVE LEAF SURFACES OF ORCHIDACEAE SPECIES FROM THAILAND

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Received: March 18, 2019; Revised: September 24, 2019; Accepted: November 04, 2019

Abstract

Leaf surfaces anatomy was investigated in thirteen species, seven genera of Ochidaceae from Thailand. Leaf blade anatomy characteristics were studied using epidermal peeled slides before being observed under light microscope. The results showed the comparable data of leaf anatomical characters among thirteen species of the orchids. Distinct variation in the anatomical characters of the orchids including stomata anomocytic, cyclocytic, paracytic, pentacytic and tetracytic; the presence or absence of secretory cell, grandular trichomes, crystal (druses, long tube, raphides, rectangle and square), shape ofepidermal cell, length of guard cells and density of trichome on adaxial surfaces were presented. Detailed leaf anatomical characters have been used successfully to clarify taxonomic status and important to support species identification. The key to species of Ochidaceae based on leaf surfaces anatomy characters is constructed.

Keywords: **Leaf surfaces, Orchidaceae, anatomy**

Introduction

Orchidaceae or the orchid family is one of the species richest families of seed plants. It is composed of five subfamilies, 880 genera and more than 25,000 species in the world (Dressler, 2005) with highly various morphological and anatomical characters (Dressler, 1993). Chantanaorrapint and Thaithong (2005) reported 177 genera and 1,333 species of Orchidaceae from Thailand. Orchidaceae is widely distributed in the tropics and subtropics with different life forms (Cribb *et al*., 2003). Recent studies suggested that distribution of orchids were limited by the joint effect of habitat availability and pollination limitation (McCormick and Jacquemyn, 2014). Anatomical characters of orchids have been widely studied in a wide range of species within tribes, subtribes and genera levels, such as tribe *Calypsoeae* (Stern

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and Carlsward, 2008); subtribes *Laeliinae* (Stern and Carlsward, 2009); *Aeridinae, Angraecinae* and *Aerangidinae* (Carlsward *et al*., 2006); *Stanhopeinae* (Stern and Whitten, 1999), *Orchidinae* (Stern, 1997); *Habenariinae* (Stern, 1997), *Oncidiinae* (Stern and Carlsward, 2006); and genera *Caladenia* (Pridgeon, 1993), *Dendrobium* and *Rhizobium* (Carlsward *et al*., 1997), *Ophrys, Orchis* and *Dactylorhiza* (Aybeke *et al*., 2010).

Anatomical characters are important to supported identification and classification in Orchidaceae (Pridgeon, 1982; Aybeke *et al*., 2010; Fan *et al*., 2014). Many botanists confirmed for this report. Pridgeon (1982) and Pridgeon and Norris (1979) showed diagnostic characters to distinguish genera within subtribe *Pleurothallidinae* (*Acostaea, Barbosella, Brachinionidium, Cryptophoranthus, Dracula, Dresslerella, Dryadella, Lepanthes, Masdevallia*, and other genera within the subtribe) using anatomical characters of leaf such as cuticle, epidermis, hypodermis, spiral thickenings, and vascular bundle number. Kaushik (1983) showed the taxonomic significance from stomata features and identified the orchids into four sub-families, namely anomocyticeae, diacyticeae, cyclocyticeae and paracyticeae. Leaf anatomical structures are the foundations of leaf physiological functions and consequentially any 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 Mc Elwain, 2008). In Orchidaceae, some recognized vegetative anatomy is similar to other ordinary monocots (Dressler, 1993). Four types of stomata have been reported in leaves of Orchidaceae; paracytic, diacytic, tetracytic and anomocytic stomata (Dressler, 1993).

Previously study, several botanists from Indonesia have involved the anatomical characters of orchids in Indonesia, namely Nurfadilah *et al*. (2016) studied roots of epiphytic orchids from Sempu Island, Betty (2011) reported leaf anatomy of nine species of *Bulbophyllum*, Metusala *et al*. (2017) recognized comparative leaf and root anatomy of two species of *Dendrobium*. Krüger (1883) studied the leaf anatomy of *Cymbidium ensifolium* and *C. aloifolium* (L.) Sw. and reported that stomata occurred only on the abaxial surface with covered by a thick cuticle and cells of the leaf tissue were characterized by raphide idioblasts, while the epidermis possessed a thick adaxial cuticle. Tominski (1905) examined two species of *Cymbidium*, namely *C. bicolor* and *C. ensifolium*. The leaf cuticle in both the species appeared one third as thick as the epidermal cells. Stomata in both the species were occurred superficial on the abaxial leaf surface. Trichomes in both the species were absent. Kaushik (1983) studied the anatomy of *C. cyperifolium* Wall., *C. eburneum*, and *C. lancifolium* Hook. and found that paracytic stomata occurred on the abaxial leaf surface only, trichome was absent, a thick cuticle covered both surfaces. Abraham *et al.* (2016) studied the stomata from leaves of epiphytic orchids, namely *Aerides ringens, Bulbophyllum sterile, Dendrobium aphyllum* and *Oberonia brachyphylla*, which were examined as anomocytic stomata. Mulgaonkar (2006) studied on dermal anatomy of four species of *Dendrobium* Sw., namely *D. microbulbon* A. Rich., *D. mabalae* Gammine., *D. ovatum* (Willd) Kranzl., and *D. barbatulum* Lindl. and observed that stomata are presented as anomocytic stomata and flushed with the epidermis as it is true majority of the orchids. Trichomes were presented on abaxial side in *D. microbulbon* and absent in other three species. The present study aimed to investigate the anatomical characters, to describe the comparative anatomical characters and to provide useful characteristics in relation to taxonomy of thirteen species of Orchidaceae from Thailand.

Materials and Methods

Living the orchid species were collected from natural areas under Plant Genetic Conservation Project under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn in Kut Bak District, Sakon Nakon Province, Thailand. The specimens were analyzed by epidermal peeling method according to

Thammathaworn (1995). Epidermal peeling of specimens was prepared by mechanical scraping between midrib and margin of the lamina. The adaxial and abaxial epidermis of middle leaf parts of mature leaves were peeled from fresh leaves. After that, the specimens were dehydrated through an alcohol series; 30%, 50%, and 70% respectively, and stained in safranin. Then the specimens were washed in 70% alcohol twice and dehydrated through 80%, 95%, 100%, mixture of absolute alcohol and xyline (1:1) and xyline and mounted in DePeX and photographed at 100× and 400× magnification under the light microscope ZEISS Axio Lab. A1. The analyze specimens were examined from more than 15 images per leaf of each species and characters of epidermal cell types, shapes and numbers of subsidiary cells surrounding pairs of guard cells and stomatal numbers per leaf area were observed. All specimens examined and slide collections are kept at the Mahasarakham University Herbarium.

Results and Discussion

The results of the surface leaf anatomy showed some variations and similarities in the leaf anatomical characteristics of the orchid species studied. A summary of the leaf anatomical characteristics observed in this study is presented in Tables 1 and 2.

Shape of Epidermal Cell

Most of the shape of epidermal cells on both the surfaces were square, rectangle to polygonal with 4-6 sides (Tables 1 and 2) and straight-sided anticlinal walls except the shape of epidermal cells of *C. bicolor*, *D. puchellum*, *D. lindleyi*, *Eulophia macrobulbon*, *Geodorum attenuatum*, *R. retusa* which were square,

Table 1. Comparison of characteristics of leaf surface on adaxial side in Orchid

Species	Shape of epidermal cell	Type of stomata	Length of guard cells (µm)	Length of subsidiary cells (µm)	Density of stomata (number/ leaf area) (mm^2)	Granular trichome (µm)	Density of granular trichome (number/ leaf area) (mm^2)	Type of crystal	Secretory cell
Aerides falcata Lindl. & Paxton. (Saensouk 3000)	5-7	-	-	-	-	-	-	-	-
Cymbidium aloifolium (L.) Sw.EO. (Saensouk 3001)	4-6	-	-	-	-	11.71±1.44	92±1	-	-
C. bicolor Lindl. (Saensouk 3002)	4-7	T	35.15±2.77	37.89±3.05	32±2	-	-	Square	+
Dendrobium aphyllum (Roxb.) C.E.C.Fisch. (Saensouk 3003)	4-6	C, P	61.58±7.48	75.66±8.11	9±1	-	-	-	-
D. delacourii Guillaumin (Saensouk 3004)	4-6	A	41.65±2.78	58.98±3.67	21±2	-	-	-	-
D. lindleyi Steudel. (Saensouk 3005)	4-8	-	-	-	-	-	-	Druses	+
D. puchellum Roxb. ex Lindl. (Saensouk 3006)	4-7	-	-	-	-	-	-	-	+
Eulophia macrobulbon Par. & Reichb.f. (Saensouk 3007)	4-8	T, Pen	43.00±3.17	38.33±4.38	8±1	-	-	Long tube	-
Geodorum attenuatum Griff. (Saensouk 3008)	4-7	-	-	-	-	-	-	Long tube	-
Grammatophyllum speciosum Blume (Saensouk 3009)	4-6	-	-	-	-	10.53±1.34	120±2	-	-
Rhynchostylis coelestis (Rchb.f.) Rchb.f. ex H.J. Veitch (Saensouk 3010)	4-6	P	43.77±3.27	51.27±5.05	24±2	-	-	Raphides	-
R. gigantea (Lindl.) Ridl. (Saensouk 3011)	4-6	P	63.48±5.10	88.75±6.68	9±1	-	-	-	-
R. retusa (L.) Blume. (Saensouk 3012)	4-7	P	48.58±5.59	57.71±3.67	8±1	-	-	-	-

*A = anomocytic, C = cyclocytic, P = paracytic, Pen = pentacytic, T = tetracytic, + = present, - = absent, ± = standard error (SE).

rectangle to polygonal with 4-7 side, while the shape of epidermal cells of *Aerides falcate* was found 5-7 side (Tables 1 and 2). According to Aybeke *et al*. (2010), there are various shapes of leaf epidermis of orchids such as polygonal, isodiametric, rectangular, and elongated. The epidermis of *D. subulatum* had the elongated epidermal cells (Rindyastuti *et al*., 2018).

Stomata

Stomata are small pores on leaves surfaces and have role of facilitating the gaseous movement in and out of leaves and, thus, the gas exchange in plants as a whole. Stomata have significant importance in the plant physiology, evolution, and ecology (Hetherington and Woodward, 2003). Stomata configuration of plants varied, such as anomocytic, tetracytic, cyclocytic, paracytic and pentacytic. The stomata configuration of thirteen orchids were found variously stomata which were cyclocytic in *Dendrobium aphyllum* (Figure 1(a)), anomocytic in *D. Delacourii* (Figure 1(b)), pentacytic in *Eulophia macrobulbon* (Figure 1(c)), tetracytic in *Cymbidium bicolor* (Figure 1(d)) and paracytic in *Rhynchostylis retusa* (Figure 1(e)). The length of guard and subsidiary cells on both surfaces of the orchids is significantly different among species (Tables 1 and 2). According to Williams (1979), 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. The stomatal numbers per leaf area were different in each species, on the adaxial surface ranging from 8±1 per mm^2 in *E. macrobulbon* to 32±2 per mm^2 in *C. bicolor*, while the density of stomata on the abaxial surface varies from 14±2 per mm^2 in *D. aphyllum* to 116±4 per mm^2 in *Geodorum attenuatum*. According to Abraham *et al.* (2016) were recorded anomocytic stomata in *Aerides ringens*, *Bulbophyllum sterile,*

Table 2. Comparison of characteristics of leaf surface on abaxial side in Orchid

Species	Shape of epidermal cell	Type of stomata	Length of guard cells (µm)	Length of subsidiary cells (µm)	Density of stomata (number/ leaf area) (mm^2)	Grandular trichome (µm)	Density of grandular trichome (number/ leaf area) (mm^2)	Type of crystal	Secretory cell
Aerides falcata Lindl. & Paxton. (Saensouk 3000)	5-7	P	52.86±1.98	81.32±9.76	83±6	-	-	Raphides	-
Cymbidium aloifolium(L.) Sw. EO. (Saensouk 3001)	4-6	P	33.03±2.77	48.08±6.16	79±4	11.01±1.73	102±3	-	-
C. bicolor Lindl. (Saensouk 3002)	4-7	T	35.75±3.68	41.79±6.76	64±5	-	-	-	+
Dendrobium aphyllum(Roxb.) C.E.C.Fisch. (Saensouk 3003)	4-6	P	59.98±3.35	78.35±8.11	14±2	-	-	-	-
D. delacourii Guillaumin (Saensouk 3004)	4-6	P	37.85±2.28	48.58±2.82	56±3	-	-	-	-
D. lindleyi Steudel. (Saensouk 3005)	4-8	P	29.15±2.54	34.50±5.73	20±2	-	-	Druses, Raphides	+
D. puchellum Roxb. ex Lindl. (Saensouk 3006)	4-7	P, T	42.29±2.98	50.10±5.84	92±5	-	-	Rectangle	+
Eulophia macrobulbon Par. & Reichb.f. (Saensouk 3007)	4-8	T	44.34±3.43	39.07±3.20	66±3	-	-	-	-
Geodorum attenuatum Griff. (Saensouk 3008)	4-7	P	27.47±1.80	35.47±5.30	116±4	-	-	-	-
Grammatophyllum speciosum Blume (Saensouk 3009)	4-6	P	28.49±3.30	41.24±8.29	113±3	8.81±1.21	124±3	-	-
Rhynchostylis coelestis (Rchb.f.) Rchb.f. ex H.J. Veitch (Saensouk 3010)	4-6	P	41.76±3.12	53.86±4.21	35±2	-	-	Raphides	-
R. gigantea (Lindl.) Ridl. (Saensouk 3011)	4-6	P	71.08±3.32	95.29±15.35	15±3	-	-	-	-
R. retusa (L.) Blume. (Saensouk 3012)	4-7	P	47.84±3.06	71.52±4.69	24±1	-	-	-	-

* P = paracytic, T = tetracytic, + = present, - = absent, ± = standard error (SE).

Dendrobium aphyllum and *Oberonia brachyphylla*. From this study, we found cyclocytic and paracytic stomata in *Dendrobium aphyllum*, which is different from the study of Abraham *et al.* (2016). Rindyastuti *et al.* (2018) described *Dendrobium subulatum* anatomical characters showed that stomata configuration was cyclocytic including stomata width 36.50±0.72 µm and stomata length 37.40±2.07 µm.

Trichome

On both the surfaces of *Cymbidium aloifolium* (L.) Sw. EO. and *Grammatophyllum speciosum* Blume were observed glandular trichome (Figure 1(f)). However, the figure represent trichrome only on the adaxial surface. Density of trichome on adaxial side was more than abaxial side. The largest trichome was found in *G. speciosum* Blume (124±3 µm and 120±2 µm), while the smallest was occurred in *C. aloifolium* (L.) Sw. EO. (102±3 µm and 92±1 µm). Secretory cells are occasionally seen in a few species, such as in both surface of *Cymbidium bicolor Dendrobium lindleyi* Steudel. Lindl., and *D. puchellum* Roxb. ex Lindl (Figure 1(g)). The observations

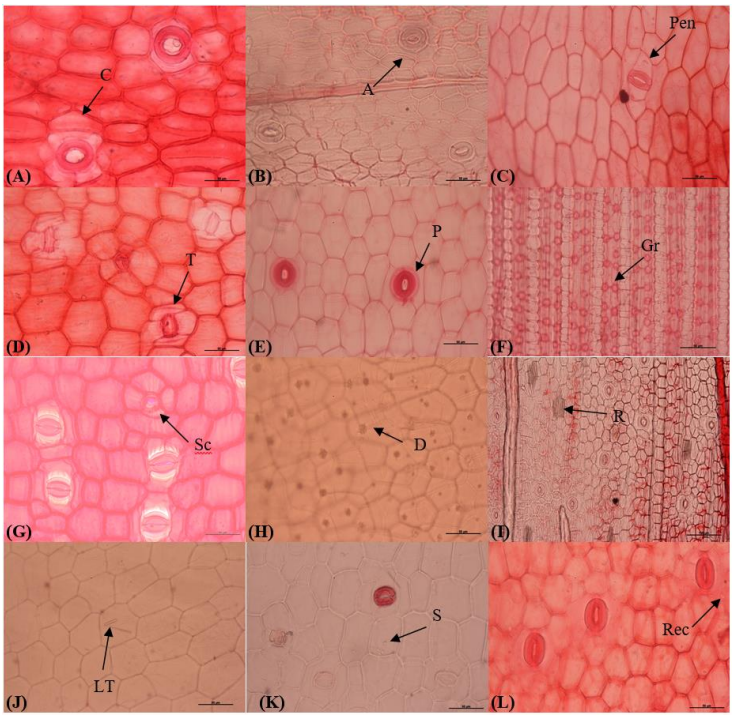

Figure 1. Leaf epidermal anatomy of the orchid species. (A) Cyclocytic stomata on adaxial surface of *Dendrobium aphyllum*. (B) Anomocytic stomata on adaxial surface of *D. delacourii*. (C) Pentacytic stomata on adaxial surface of *Eulophia macrobulbon*. (D) Tetracytic stomata on abaxial surface of *Cymbidium bicolor*. (E) Paracytic stomata on abaxial surface of *Rhynchostylis retusa*. (F) Grandular trichomes on adaxial surface of *Grammatophyllum speciosum*. (G) Secretory cell on abaxial surface of *D. puchellum*. (H) Druses crystal on adaxial surface of *D. lindleyi*. (I) Raphides crystal on abaxial surface of *R. coelestis*. (J) Long tube crystal on adaxial surface of *Geodorum attenuatum*. (K) Square crystal on adaxial surface of *C. bicolor* and (L) Rectangle crystal on abaxial surface of *D. pucellum*. scale bars = 50 µm. A = anomocytic, C = cyclocytic, P = paracytic, Pen = pentacytic, T = tetracytic, Gr = Grandular trichome, Sc = Secretory cell, D = Druses crystal, LT = Long

of the present study contradicts with the results of Kaushik (1983) where trichomes were found to be absent in *C. cyperifolium* Wall., *C. eburneum* Lindl. and *C. lancifolium* Hook.

Type of Crystal

Druses and Raphides crystal were found on both the leaf surfaces in *Dendrobium lindleyi* Steudel. and *Rhynchostylis coelestis* (Rchb.f.) Rchb.f. ex H.J. Veitch, while in *Aerides falcata* Lindl. & Paxton. with Raphides crystal on the abaxial surface (Figures 1(h) and 1(i)). For the adaxial surface square and long tube crystal are presented in *Cymbidium bicolor* Lindl. and *Eulophia macrobulbon* Par. & Reichb.f., *Geodorum attenuatum* Griff. respectively (Figures 1(j) and 1(k)). Rectangle crystal is found only on the abaxial surface in *Dendrobium puchellum* Roxb. ex Lindl. (Figure 1(l)). The observations of the present study are agreed with Krüger (1883) who also reported the presence of raphides in cells of leaf tissue of *C. ensifolium* and *C. aloifolium*.

Conclusions

From the results show that leaf surfaces anatomical features such as stomata presence or absence on adaxial surfaces, secretory cell presenceor absence on adaxial surfaces, grandular trichomes presence or absence on adaxial surfaces, density of stomata on the abaxial surface, shape of epidermal cell, types of stomata, length of guard cell on adaxial surface can be used for classification. Leaf anatomical studies have been used successfully to clarify taxonomic status. Therefore, the key to the species based on leaf surfaces anatomy is constructed below:

Key To Species Of Ochidaceae From Thailand Based On Leaf Surfaces Anatomy Features

1. Stomata absent on adaxial surface
    2. Secretory cell present on adaxial surface
        3. Druses crystal present on adaxial surface *Dendrobium lindleyi*
        3. Druses crystal absent on adaxial surface *Dendrobium puchellum*
    2. Secretory cell absent on adaxial surface
        4. Long tube crystal present on adaxial surface *Geodorum attenuatum*
        4. Long tube crystal absent on adaxial surface
            5. Grandular trichomes absent on adaxial surface *Aerides falcata*
            5. Grandular trichomes present on adaxial surface
                6. Density of trichome on adaxial surface >100 per mm^2 *Grammatophyllum speciosum*
                6. Density of trichome on adaxial surface <100 per mm^2 *Cymbidium aloifolium*
1. Stomata present on adaxial surface
    7. Density of stomata on adaxial surface >15 per mm^2
        8. Shape of epidermal cell on adaxial surface has a 4-7 side *Cymbidium bicolor*
        8. Shape of epidermal cell on adaxial surface has a 4-6 side
            9. Raphides crystal present on adaxial surface *Rhynchostylis coelestis*
            9. Raphides crystal absent on adaxial surface *Dendrobium delacourii*
    7. Density of stomata on adaxial surfaces <15 per mm^2
        10. Long tube crystal present on adaxial surface *Eulophia macrobulbon*
        10. Long tube crystal absent on adaxial surface
            11. Paracytic and cyclocytic stomata present on adaxial surface *Dendrobium aphyllum*
            11. Paracytic stomata present only on adaxial surface
                12. Length of guard cells on adaxial surface >55 μm *Rhynchostylis gigantea*
                12. Length of guard cells on adaxial surface <55 μm *Rhynchostylis retusa*

Acknowledgements

This research was financially supported by National Research Council of Thailand and Mahasarakham University. The authors thank the Walai Rukhavej Botanical Research Institute, Mahasarakham University and Kut Bak District, Sakon Nakhon Province for their facilities during the study. We would like to thank Mr. Phattaravee Prommanut who is specialist on family Ochidaceae for his identify correctly sciencetific name of the Ochidaceae. We wish to thank Dr. Paul Alexander Dulfer for language editing and suggestions to improve an earlier version of the manuscript.

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