Adaptation and taxonomic value of leaf anatomical characteristics of selected *Ipomoea* L. species

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Abstract

Aim: To investigate the taxonomic value of leaf anatomical characteristics and to determine the anatomical features that enabled the species to adapt to different environmental conditions.

Methodology: Three methods were applied for leaf anatomy observation: sectioning using a sliding microtome, leaf clearing and epidermal peels.

Results: The findings showed some common anatomical characteristics shared in all species studied, such as incomplete leaf venation, the presence of trichome and schlerenchyma cells, and the presence of mucilaginous idioblast cells in the petiole and midrib. Straight-to-sinuuous anticlinal cell walls, amphistomatic leaves, and three types of stomata: parasitic, anomocytic and staurocytic were on the abaxial side of leaf lamina in this study. Peltate and capitate glandular trichome, as well as simple trichome, were all present in the petiole, midrib and lamina. However, peltate glandular trichome were only found in the root of *I. triloba*. Druses were observed in all species, except *I. batatas*.

Interaction: Leaf anatomical characteristics possess taxonomic value, especially in species differentiation and identification, that can be subsequently useful in explaining plant's adaptation to their environment.

Key Words: Convolvulaceae, *Ipomoea*, Leaf adaptation, Leaf anatomy

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Introduction

There are more than 650 species that can be found in the world (Jayeola and Oladunjoye, 2012; Judd *et al.*, 2002; Miller *et al.*, 2004), with 40 species in Malaysia (Khadijah *et al.*, 2016). The species distribution is widely in tropical and subtropical countries (Austin and Huaman, 1996; Meira *et al.*, 2012), but some can be found in temperate countries (Cao *et al.*, 2005; Meira *et al.*, 2012).

Ipomoea L. belongs to family Convolvulaceae has the largest number of species and is an ornamental plant because of their flowers (Jayeola and Oladunjoye, 2012). This genus consists of woody and herbaceous lianas, scandents, shrubs or small trees. The flower is campanulate, bisexual and actinomorphic, with variations in petal colour from purple, red, pink, orange-reddish or white (Folorunso *et al.*, 2013; Olorode 1984). Meanwhile, the fruit is oblong or globose in shape, capsulated with four to six seeds.

According to Noraini and Cutler (2007) and Ruzi *et al.* (2009), anatomical characteristics are alternative identification method beside morphological characteristics for many plant species. The presence of latex and parallel vascular bundles are the diagnostic characteristics of this genus (Folorunso *et al.*, 2013; Shukla and Mistra 1979). The presence of resin glycoside is a chemotaxonomic bioindicator for *Ipomoea* within family Convolvulaceae (Meira *et al.*, 2012; Wagner, 1973). Folorunso (2013) showed *Ipomoea* species can be divided into two groups on the types of stomata. The presence of brachyparacytic stomata is diagnostic in *I. batatas*, *I. carnea*, *I. intrapilosa*, *I. asarifolia*, *I. nil*, *I. mauritiana*, *I. aquatica*, *I. triloba*, *I. alba* and *I. involucreta*, the remaining species have paracytic stomata.

There is no previous study that relate *Ipomoea* leaf anatomical characteristics to the environment and on its taxonomic significance to this genus. In view of the above, this study was conducted to investigate the leaf anatomical characteristics in *Ipomoea* species that were systematically significant and helpful in evolving anatomical adaptations to their environments.

Materials and Methods

Fresh leaf samples of *Ipomoea* were collected from different localities in Selangor, Malaysia (Table 1). The samples were fixed in ethanol and acetic acid in ratio 1:3). For leaf venation study, the leaf lamina was cleared using basic fuchsin solution and kept at 60°C in an oven for 1-2 days, depending on the leaf specimen. Clear leaves were then dehydrated in alcohol series, soaked in xylene and mounted on slides using Canada balsam (R&M Chemical, Essex, UK). The samples were then placed in an oven at 60°C for nearly 2 week. The slides were photographed with a digital camera (Olympus BX43F, Tokyo) mounted on Olympus microscope (Olympus Soft Imaging Solutions GmbH, Münster, Germany). The leaf venation patterns were observed with Cell^B Software (Olympus Soft Imaging Solutions GmbH, Münster, Germany) under 10×, 20×, 40× and 100× magnifications. Standard botanical microtechniques of Johansen (1940) and Sass (1958), were followed with slight modifications to mould the material. Leaf sectioning was conducted using a sliding microtome (thickness of 10-15 µm). These sections were stained in safranin and alcian blue and then dehydrated in an alcohol series of 50%, 70%, 95% and 100%. The specimens were then mounted on slides using euparal, followed by photography using an Olympus Diaphlan microscope. The images were processed using Analysis Docu Software.

Results and Discussion

All species studied had complete marginal venation, swollen tracheids, trichome at leaf margins and sclerenchyma cells in the petiole and midrib vascular bundles. The pattern of anticlinal cell walls observed in this study were straight-to-curve, curve-to-wavy, wavy-to-sinuuous and sinuuous (Table 2). According to Metcalfe and Chalk (1950), Convolvulaceae has straight-to-sinuuous anticlinal cell walls, while Folorunso (2013) stated that *Ipomoea* has sinuuous anticlinal cell walls. Our study discovered other types of anticlinal cell walls. Based on Hong-zhi (2001), the pattern of anticlinal cells wall is rigid and genetically controlled. Ecological and biological specializations which have arisen in response to peculiarities of environment are of more limited use.

Table 1 : List of *Ipomoea* species studied

Species	Code specimen	Locality
<i>I. batatas</i> (L.) Lam.	FND01	Rumah Tumbuhan, UKM, Bangi
	FND02	Port Dickson, Negeri Sembilan
<i>I. aquatica</i> Forssk.	FND04	Tasik FKAB, UKM, Bangi
	FND05	Kuala Selangor, Selangor
<i>I. cairica</i> (L.) Sweet	FND06	Rumah Tumbuhan, UKM, Bangi
<i>I. pes-caprae</i> (L.) R. Br.	FND07	Port Dickson, Negeri Sembilan
	FND08	Kuala Selangor, Selangor
<i>I. purpurea</i> (L.) Roth.	FND09	Tanah Rata, Cameron Highland, Pahang
	FND10	Port Dickson, Negeri Sembilan
<i>I. triloba</i> L.	FND11	Rumah Tumbuhan, UKM, Bangi

However, Noraini et al. (2019) and Gunasegaran et al. (2021) reported that in the experience of systematic anatomists, although the structure of a species can and does vary with the habitat, anatomical variation in response to ecological change is much less marked than is commonly believed and is more often quantitative than qualitative.

Wilkinson (1979) stated that in dry and hot weather, plant species have straight anticlinal cell wall, especially on adaxial side whereas understorey plant is expected to have sinuous anticlinal cells wall on both adaxial and abaxial side (Mussury et al., 2012). Based on this study, *I. pes-caprae* was the most tolerable species in the hot and dry environment compared to others.

According to Metcalfe and Chalk (1979), stomata in Convolvulaceae is usually present on both abaxial and adaxial leaf epidermis or simply known as amphistomatic. This observation is supported by this research findings and corresponds with the study on *I. cairica* by Mandal et al. (2015). Three types of stomata were present in *Ipomea* species studied; staurocytic, paracytic and anomocytic. Some species were homostomatic (where only one type of stomata was present), as present in *I. batatas*, *I. pes-caprae*, *I. purpurea*, *Ipomea triloba* (all species had anomocytic stomata). Some species were heterostomatic (more than one type of stomata were present), as in *I. aquatica* (paracytic, anomocytic) and *I. cairica* (paracytic, anomocytic, staurocytic). Table 3 shows the stomata anatomical characteristics in *Ipomea* species studied.

Van Cotthem (1970) stated that stomata not only have diagnostic value but can also be related to natural surrounding. Devi et al. (2013) also agreed that variation and stomata type can be used in biological, ecological, and environmental studies of plant species. Conclusively, our study corresponds with the findings by Folorunso et al. (2013) as *Ipomea* has paracytic, anisocytic, and anomocytic stomata on both adaxial and abaxial leaf surface.

The outlines of leaf margin for *I. purpurea*, *I. batatas*, *I. aquatica*, *I. cairica*, *I. purpurea* and *I. triloba* were all round and recurved 10°-30° to abaxial side. Conversely, *I. pes-caprae* had acute outline. Therefore, this characteristic can be used to differentiate species in *Ipomea*. Table 4 shows variation in the outline of leaf margin in *Ipomea* species studied.

The presence of vascular bundles in the leaf margin was only observed in *I. pes-caprae*, which can be used as a diagnostic character for this species. Nevertheless, the vascular bundles were also observed in the leaf lamina, in the middle of leaf lamina in *I. aquatica*, *I. pes-caprae*, *I. cairica* and *I. triloba*, near to adaxial epidermis in *I. batatas*, and near to abaxial epidermis in *I. pupurea*. This character is useful for species differentiation in *Ipomea*.

The number of palisade cells layer does not have systematic significant but this character is relatively related to the environment (Noraini, 2012). The number of palisade layers change according to light intensity (Ashton and Berlyn, 1992). Findings of this study discovered that all species studied had one layer of mesophyll palisade, except *I. aquatica* with 2-4 layers. Most species had one palisade layer cell representing $\frac{1}{2}$ thickness of leaf lamina, except for 1-3 layers in *I. pes-caprae* and 3-4 layers in *I. purpurea*. For spongy mesophyll, *I. purpurea* had three layers representing $\frac{1}{4}$ of leaf lamina thickness, 3-4 layers in *I. cairica* and *I. triloba*, 4-5 layers in *I. batatas*, *I. aquatica* and *I. pes-caprae*.

According to Esau (1977), aerenchyma cells refer to plant tissue with intercellular or airy spaces. Aerenchyma cells were only present in the leaf lamina of *I. pes-caprae*, *I. purpurea* and *I. triloba* (Fig. 1D). The presence of intercellular or airy spaces in these three species have shown the leaf anatomical adaptation to aquatic habitat. Table 5 shows variation in the presence of aerenchyma, spongy mesophyll, and palisade layers in all species studied.

Metcalfe and Chalk (1950) stated that the common trichome present in family Convolvulaceae is glandular capitate trichomes, whilst simple and capitate glandular trichomes are found in the genus *Ipomea*. In this study, trichome was found in the leaf margin, lamina and midrib. The type of trichomes found in this study were simple, peltate and capitate glandular trichomes (Fig. 1B). Variation in trichome types in all *Ipomea* species studied is presented in Table 6.

Previous studies have shown that *I. triloba* and *I. pes-caprae* have simple and glandular trichome (Ahmad et al., 2016). Folorunso et al. (2013) also reported that *I. batatas* and *I. aquatica* have glandular trichome but simple trichome is found in *I. triloba*. Our results support the findings of previous studies that the present and type of trichomes are proven can be used to differentiate *Ipomea* species and this character definitely has taxonomic significant.

Calcium oxalate crystals can be found in many plant species (Doege, 2003). Classification at species and genus level can be done on the presence of crystals (Franceschi and Horner, 1980; Genua and Hillson, 1985; Hsieh and Huang, 1974; Noraini et al., 2016; Prychid and Rudall, 1999; Solederer, 1908). Sahreen et al. (2010) stated that cystals can be found in the paerenchyma cell wall and intercellular spaces in the form of solitary crystals or clusters. Metcalfe and Chalk (1950) reported that in *Ipomea*, normally crystals can be found in clusters form (Fig. 1E). The availability of calcium oxalate crystals in all *Ipomea* species studied is given in Table 7.

Ipomea is a group of plant species that has specific anatomical characteristics in adapting extreme environment and

Table 2 : Pattern of anticlinal cell wall of *Ipomoea* species studied

Species	Pattern of anticlinal cells wall	
	Adaxial epidermis	Abaxial epidermis
<i>I. batatas</i>	Curve to sinuous	Sinuous
<i>I. aquatica</i>	Wavy to sinuous	Curve to sinuous
<i>I. pes-caprae</i>	Straight to curve	Straight to curve
<i>I. cairica</i>	Straight to curve	Sinuous
<i>I. purpurea</i>	Wavy to sinuous	Wavy to sinuous
<i>I. batatas</i>	Curve to sinuous	Sinuous

Table 3 : Stomata anatomical characteristics in *Ipomoea* species studied

Species	Presence	Types
<i>batatas</i>	Amphistomatic and homostomatic	Anomocytic
<i>aquatica</i>	Amphistomatic and heterostomatic	Paracytic, Anomocytic
<i>I. pes-caprae</i>	Amphistomatic and homostomatic	Anomocytic
<i>I. cairica</i>	Amphistomatic and homostomatic	Anomocytic, staurocytic, paracytic
<i>I. purpurea</i>	Amphistomatic and homostomatic	Anomocytic
<i>I. triloba</i>	Amphistomatic and homostomatic	Anomocytic

Table 4 : Variation in the outline of leaf margin in *Ipomoea* species studied

Species	Description
<i>I. batatas</i>	Rounded, recurved 10° to abaxial side
<i>I. aquatica</i>	Rounded, recurved 10° to abaxial side
<i>I. pes-caprae</i>	Acute, recurved 10° to abaxial side
<i>I. cairica</i>	Rounded, recurved 10° to abaxial side
<i>I. purpurea</i>	Rounded, recurved 30° to abaxial side
<i>I. triloba</i>	Rounded, recurved 10° to abaxial side

Table 5 : Variation in the presence of aerenchyma cells, number of spongy mesophyll and palisade cell layers in *Ipomoea* species studied

Species	Number of palisade cell layers	Number of spongy mesophyll cell layers	Aerenchyma cells
<i>I. batatas</i>	1	3-4	Absent
<i>I. aquatica</i>	1	2-4	Absent
<i>I. pes-caprae</i>	4-5	1	Present
<i>I. cairica</i>	4-5	3-4	Absent
<i>I. purpurea</i>	3	3	Present
<i>I. triloba</i>	3-4	3-4	Present

Table 6 : Location and variation in trichome types in *Ipomoea* species studied

Species	Leaf margin	Lamina	Petiole	Midrib
<i>I. batatas</i>	Capitate glandular, simple	Peltate glandular	Capitate glandular	Capitate glandular
<i>I. aquatica</i>	Peltate glandular	Capitate glandular	Capitate glandular	Capitate glandular
<i>I. pes-caprae</i>	Absent	Peltate glandular	Absent	Peltate glandular
<i>I. cairica</i>	Peltate glandular	Peltate glandular	Absent	Peltate glandular
<i>I. purpurea</i>	Peltate glandular	Simple	Simple, Peltate glandular	Simple, peltate glandular
<i>I. triloba</i>	Capitate glandular	Peltate glandular	Capitate glandular	Capitate glandular

Table 7 : Location and variation in the presence of calcium oxalate crystals

Species	Lamina	Petiole	Midrib
<i>I. batatas</i>	Absent	Absent	Absent
<i>I. aquatica</i>	Absent	Absent	Druses
<i>I. pes-caprae</i>	Absent	Druses	Druses
<i>I. cairica</i>	Absent	Druses, solitary	Absent
<i>I. purpurea</i>	Druses	Druses	Druses
<i>I. triloba</i>	Druses	Druses	Absent

Table 8 : Diagnostic leaf anatomical characteristics in *Ipomoea* species studied

Species	Diagnostic characteristics
<i>I. batatas</i>	Anticlinal cells wall curve or wavy.
<i>I. aquatica</i>	Anticlinal cells wall curve to sinuous on abaxial epidermis. Air spaces present in petiole pith.
<i>I. pes-caprae</i>	Epicuticular waxes present on leaf epidermis. Druses present in leaf lamina. Outline of leaf margin acute.
<i>I. purpurea</i>	Anticlinal cells wall wavy to sinuous on abaxial epidermis. Simple trichome present in the petiole. Simple trichome on abaxial epidermis.

Fig. 1 : (A) Sinuous anticlinal cell wall in *I. batatas*; (B) Simple trichome in *I. aquatica*; (C) Air spaces in petiole pith of *I. aquatica*; (D) Aerenchyma in *I. pes-caprae* and (E) Druses in *I. pes-caprae*.

surrounding. *Ipomoea pes-caprae* for example, is one of the species that has capability to adapt in sandy and salty area such as beach. This type of plant species is known as halophyte plant species. The presence of epicuticular wax is one of the mechanisms for coping extreme environment. This is similar to *I. aquatica* that adapts in aquatic area. This plant species is known as hydrophyte plant. The presence of aerenchyma cells help the hydrophyte plant to emerge above water.

Diagnostic characteristics are prominent in only one species, but this character has systematic significant that can be useful for identification of species (Noraini *et al.*, 2017). Diagnostic leaf anatomical characteristics found in this study are tabulated in Table 8.

An identification key for *Ipomoea* species studied was constructed based on the leaf anatomical characteristics:

Key to *Ipomoea* species studied based on leaf anatomical characteristics:

Vascular bundles present in the middle of leaf lamina, glandular trichome present in the lamina and midrib. Vascular bundles adjacent to leaf epidermis, non glandular trichome present in the lamina and midrib *Ipomoea purpurea*; Outline of leaf margin rounded and recurved 10° to 30° to leaf abaxial side. Outline of leaf margin acute and recurved 10° to leaf abaxial side, epicuticular waxes present on the leaf epidermis of *I. pes-caprae*; Aerenchyma cells absent in the leaf lamina, curve to sinuous, wavy to sinuous, or straight to curve anticlinal cell wall present on leaf epidermis adaxial side. Aerenchyma cells present in the leaf lamina, sinuous anticlinal cells wall present on leaf epidermis adaxial and abaxial side of *I. triloba*; Air spaces absent in the petiole pith, sinuous anticlinal cells wall present only on leaf epidermis abaxial side. Air spaces present in the petiole pith, druses present in the midrib of *I. aquatica*; Trichome present in the petiole, homostomatic, only anomocytic stomata present, druses and solitary crystals present in the petiole of *I. batatas*; Trichomes absent in the petiole, heterostomatic, anomocytic, staurocytic and paracytic stomata present, druses and solitary crystals present in the petiole of *I. cairica*.

The findings of this study definitely have shown that leaf anatomical characteristics in the studied genus has taxonomic value for species identification and classification, especially in the type of leaf venation, presence of trichomes and sclerenchyma cells, in the presence of mucilaginous idioblast cells in petiole and midrib, presence and types of stomata, presence and type of trichomes and presence of druses. Some anatomical adaptations to the environment were found in this study such as presence of aerenchyma cells in *I. pes-caprae*, *I. purpurea* and *I. triloba*, presence of epicuticular cuticles in *I. pes-caprae* and presence of air spaces in petiole and stem of *I. aquatica* signified its floating ability.

In conclusion, leaf anatomical characteristics have taxonomic value for species differentiation and identification, and also show leaf anatomical adaptation towards extreme environment.

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Add-on Information

Authors' contribution : T. Noraini: Analysis of anatomical characteristics; A.J. Amirul-Aiman: Processing data on anatomy and habitat; A.R. Ruzi: Taxonomic area on *Ipomoea*; H. Bunawan: Using some facilities and techniques on equipment in INBIOSIS; S.F. Nurdiana: M. Sc. student working on this project.

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