The Thailand Natural History Museum Journal 12(1): 1-8, June 2018
©2018 by National Science Museum, Thailand

Original article

Differences in Leaf Sizes and Stomatal Distribution along the Leaf Spiral of Benguet Lily (*Lilium philippinense* Baker)

Jones t. Napaldet*

PhD Botany Candidate, University of the Philippines Los Baños (UPLB)
Instructor, Biology Department, Benguet State University
Km. 6, La Trinidad, Benguet

ABSTRACT: Though leaf spiral phyllotaxy has been subjected to most modeling approaches, its implication to leaf characteristics has not been given much emphasis in literatures. Thus this study attempted to document the differences in leaf sizes and stomatal distribution along the leaf spiral of Benguet lily (*Lilium philippinense* Baker). Basal leaves are the widest while middle leaves are the longest. The mesophyll generally is thickest at the base of the lamina then narrows apically. Between leaf groups, the middle leaves generally have thicker lamina than apical leaves. The stomata of Benguet lily are of anomocytic type. Stomatal density in mature leaves ranges from 31 to 124 stomata/ mm^2 with higher values in apical leaves than lower ones. Much higher stomatal density is observed in developing leaves ranging from 132 to 242. Between parts of lamina in mature leaves, the stomatal density is lowest in the base, highest in the middle then decreases apically. However, different trend is observed in young leaves wherein stomata in the base are almost twice denser than in the apex. These results show that leaf sizes and stomata density could vary significantly among leaves of the same plant even if they are just few cm apart. Stomata were also observed in adaxial epidermis but only sporadically in the basal and middle leaves. This contradicts previous report that the adaxial epidermis of Liliaceae has higher or equal stomatal density with the abaxial epidermis.

KEY WORDS: Benguet lily (*Lilium philippinense* Baker), leaf spiral, stomatal density

INTRODUCTION

The leaf of plants, though sharing many features in common, is highly diverse in gross morphology and to a lesser extent in internal anatomy. This diversity is related not only to leaf function, but also to the environment in which leaves have evolved as well as to that in which they develop (Beck, 2010). Even in the same individual, the morphology of leaves may vary depending on environmental factors such as spectral quality and light intensity. Experiments by Buisson and Lee (1993) on the effects of simulated canopy shade demonstrated that leaves of *Carica papaya* (papaya) grown under reduced irradiance were significantly thinner, with lower specific weight, had fewer stomata, produced more chlorophyll per unit area, and were characterized by a larger volume of air space in the mesophyll than leaves growing under conditions of high light intensity.

*Corresponding author.
E-mail: jones_napaldet@yahoo.com

Stomata density and distribution in the leaf are also highly affected by environmental factors. According to Brownlee (2001), low stomatal density is often attributed to low light condition, high atmospheric carbon dioxide concentrations and dry environment. Stomata are also more common on the abaxial surface; but are usually sparse or absent on the adaxial surface. However, Zarinkamar (2006) found that in some monocot families (Poaceae, Liliaceae, Iridaceae and Orchidaceae), the adaxial stomatal density is equal or even higher than the abaxial.

Another factor that could possibly influence leaf structures such as stomatal density is its ontogeny or developmental pattern pathways, though this was not addressed in literatures. For example, spiral phyllotaxy pattern, wherein leaves are formed 137.5° apart, had been subjected to extensive studies due to its predominance in nature (Williams, 1975) and the focus of several modeling approaches (Scwabe, 1984). However, its implication to leaf sizes and stomatal features has not been investigated. Thus, there is a need to investigate if ontogeny, spiral phyllotaxy pattern in this case, would dictate the features of the leaf it give rise to. This is the main aim of the study– to determine the differences of leaf structure and stomatal distribution as influence by their position in leaf spiral using Benguet lily (*Lilium philippinense* Baker) as the test plant. The study also aims to compare the stomata of Benguet lily with common stomata theories like those of Earnes'. According to Earnes *et al.* (1925), stomata are arranged apparently without regularity in majority of leaves. Salisbury (1975), on the other hand, found that frequencies are greater at the leaf margins than near the midrib in broad-leaved plants while in monocot, there is an increasing frequency from the base, reaching maximum at the middle before decreasing toward the apex.

Lilium philippinense (Liliaceae) is endemic to the Philippines (Elwes, 1880) and occurs in the southern part of the Cordillera Central Range (CCR). Its fragrant, white, trumpet-shaped flowers sometimes have a reddish tinge at the corolla base, and annual mass flowering events are spectacular. In the Benguet and Mountain Province, this lily is often a favorite adornment for wedding ceremonies and special occasions because of its delicate white flowers and fragrance. However, there is scanty research on Benguet lily particularly on its morphology and anatomy. It is hope by the author that this endeavour may contribute to better understanding this plants and help, in a way, in its conservation.

MATERIALS AND METHODS

The Benguet lily used in the study were randomly collected along the summit and slope of Mt. Jambo, La Trinidad, Benguet, Philippines. The area is now being offered as an ecotourism destination as it offers a scenic 360^0 view of Baguio City, La Trinidad and the surrounding mountain ranges. Thus, there is a need to document the economically and ecologically important flora in the area as part of conservation efforts and impact assessment.

The plant has a 1 continuous spiral of 20–50 leaves (Fig 1). Three leaves each from the base, middle and apical portion of the spiral were collected from each plant. From the apical portion, only mature leaves were considered. Mature leaves are characterized by dark-green coloration. These basal, middle and apical mature leaves served as the leaf groups which were compared in the study. The distance between the leaf groups ranges from 5 to 8 cm apart. The leaves were first measured in terms of length and width. After that, transverse sections were derived from the base, middle and apical portion of



Figure 1. The plant habit, from vegetative to reproductive stage.

the lamina for documentation and measurement under light miscrocopy. Epidermal sections and stomatal imprints of the abaxial epidermis were also derived from the base, middle and apex of each leaf. Since it was reported in literatures (Beck, 2010; Zarinkamar, 2006) that stomata also occur in upper epidermis of monocot, stomatal imprints were also derived from the adaxial base, middle and apex of the lamina. The leaf sizes and stomatal density were computed and compared among these leaf groups. Also, the stomatal distribution in young developing leaves, characterized by pale green coloration and shorter length, were also documented and compared with the mature ones. However, the young leaves are significantly shorter thus only the base and apex of the lamina could be objectively sampled for stomatal imprints.

Stomatal density was computed by counting the number of stomata per square millimeter. The area was determined through ImageJ. Supposedly, stomatal index would be computed using the same program but the epidermal cells cannot be accurately counted from the imprints. Also, the stomatal size (guard cell plus stomatal pore) was documented in terms of length and width

Data gathered on leaf sizes, stomatal density and sizes were compared among the basal, middle, apical and young leaves using Analysis of Variance (ANOVA). Duncan's Multiple Range Test (DMRT) as post hoc analysis was also employed to determine which specific means are different statistically.

RESULTS

Leaf size. Majority of the leaves in the leaf spiral of Benguet lily stays trough-out the plant's life cycle until the seeds matured. The sizes of these leaves are presented in Table 1 in terms of length and width of the lamina.

Table 1. Leaf sizes along the leaf spiral of Benguet lily.

Leaf Groups	Length (cm)		Width (cm)	
	Mean	Standard Deviation	Mean	Standard Deviation
Young Leaves	7.800[a]	±1.91	0.333a	±0.06
Apical Mature Leaves	16.167[c]	±0.67	0.667 b	±0.06
Middle Leaves	18.700[d]	±0.26	0.833c	±0.06
Basal Leaves	12.600[b]	±0.87	1.033d	±0.06

Note: Means with the same letter along column are not statistically significant at 0.05 DMRT

The basal leaves are the widest at 1.033 cm but also the shortest. On the other hand, middle leaves are the longest followed by the apical leaves at 18.7 and 16.17 cm, respectively.

Thickness of the lamina was also documented thru measurement of transverse sections in the different parts of the lamina (Table 2). However during sectioning, the basal leaves had already wilted and turned yellow so no representative sections were gathered. Among the available leaves, the mesophyll generally is thickest at the base particularly at the midrib portion then narrows apically. The midrib also narrows apically with the lamina. Between leaf groups, the middle leaves generally have thicker lamina. It is only in the mid-portion of the lamina that the apical mature leaf is thicker.

Stomata density. The stomata of Benguet lily are of anomocytic type (Fig 2a–d). The stomata are mostly concentrated in the abaxial epidermis but few are noted sporadically in adaxial epidermis in the base of middle and basal leaves. Stomatal density of Benguet lily ranges from 31 to 242 stomata/mm^2 (Table 3, Fig 2a–d). The density varies depending on the leaf groups and part of the lamina. Among the mature leaves, stomatal density ranges from 31 to 124 with higher values in the apical leaves consistently in all parts of the lamina. The basal leaves, on the other hand, have the lowest stomatal densities. On the other hand, much higher stomatal density is observed in the young developing leaves ranging from 132 to 242.

Between parts of the lamina, the stomatal density is significantly lowest in the base of lamina among all mature leaf groups. It is highest in the middle part of the lamina followed by apex. However, different trend is observed in young developing leaves. The base contains the highest stomatal density of 242 stomata/mm^2 which is almost twice that of the apex.

Lastly, the sizes of stomata in mature leaves are generally not significantly different regardless of location in the lamina and leaf groups (Table 4). Stomatal length ranges from 58 to 72 μm among the mature leaves while width ranges from 20 to 29 μm. Stomatal length is higher among basal and middle leaves. Between parts of lamina, stomatal length is highest at the base then decreases apically which is inversely proportional with stomatal density. Developing leaves,

Table 2. Cross-section thickness of Benguet lily leaf.

Leaf Groups	Part of the Lamina					
	Base (mm)		Middle (mm)		Apex (mm)	
	Lamina	Midrib	Lamina	Midrib	Lamina	Midrib
Young Leaves	0.208	0.733	0.230	0.401	0.210	0.267
Apical Mature Leaves	0.417	1.270	0.403	0.756	0.239	0.383
Middle Leaves	0.431	1.711	0.375	0.916	0.346	0.451

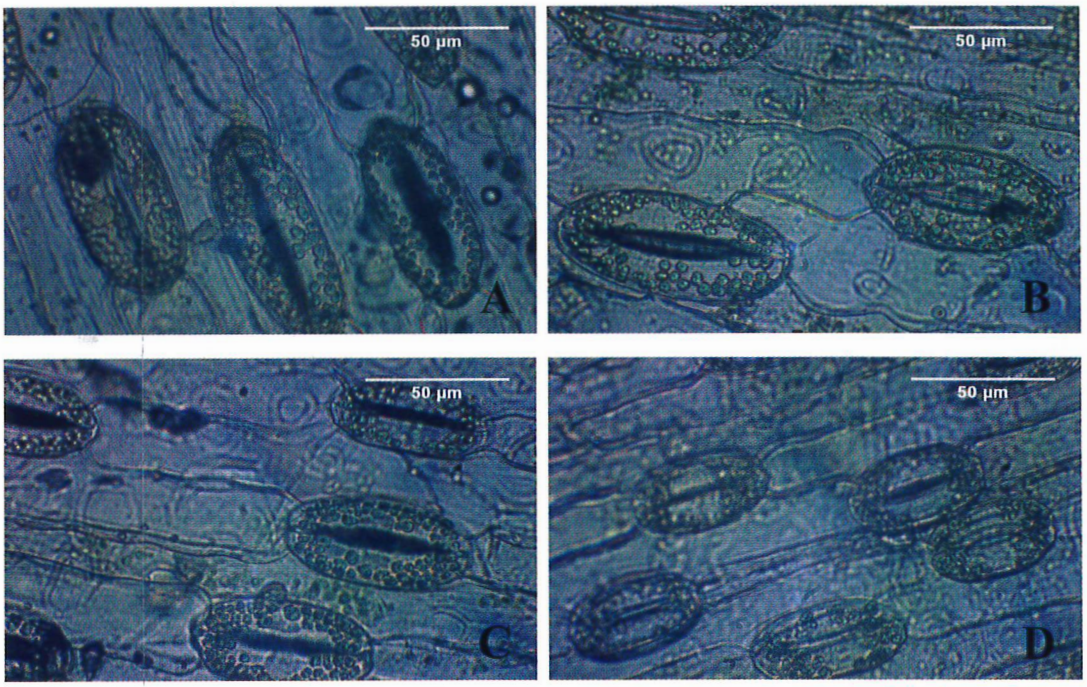


Figure 2. a – d. Epidermal sections at HPO showing stomates in abaxial epidermis of the different leaf groups (a, apical leaves; b, middle leaves; c, basal leaves; d, young developing leaves)

Table 3. Abaxial Stomatal density (ni/mm^2) in the leaf spiral of Benguet lily.

Leaf Groups	Part of the Lamina		
	Base	Middle	Apex
Young Leaves	241.687$^{b\ II}$	n/a	132.200$^{a\ I}$
Apical (Mature) Leaves	76.237$^{a\ I}$	124.087$^{b\ II}$	107.870$^{a\ II}$
Middle Leaves	41.363$^{a\ I}$	94.077$^{a\ II}$	88.400$^{a\ II}$
Basal Leaves	30.820$^{a\ I}$	90.833$^{a\ II}$	79.483$^{a\ II}$
Basal Leaves	30.820$^{a\ I}$	90.833$^{a\ II}$	79.483$^{a\ II}$

Note: Means with the same letter along column are not statistically significant at 0.05 DMRT
Means with the same Roman numeral along rows are not statistically significant at 0.05 DMRT

Table 4. Stomatal size in Benguet lily.

Leaf Groups	Average Stomatal Size (µm)					
	Base		Middle		Apex	
	L	W	L	W	L	W
Abaxial (lower) Epidermis						
Young Leaves	33.97$^{a\ I}$	20.33$^{a\ I}$	n/a	n/a	50.41$^{a\ II}$	28.52$^{a\ I}$
Apical Leaves	67.36$^{b\ I}$	24.37$^{a\ I}$	62.34$^{a\ I}$	24.32$^{a\ I}$	58.42$^{a,b\ I}$	22.85$^{a\ I}$
Middle Leaves	71.74$^{b\ I}$	25.68$^{a\ I}$	68.70$^{a,b\ I}$	24.22$^{a\ I}$	68.60$^{b\ I}$	22.63$^{a\ I}$
Basal Leaves	70.44$^{b\ I}$	25.68$^{a\ I,II}$	70.30$^{b\ I}$	28.91$^{a\ II}$	68.88$^{b\ I}$	19.58$^{a\ I}$
Adaxial (upper) Epidermis						
Middle Leaves	69.21$^{b\ I}$	28.31$^{a\ I}$	n/a	n/a	n/a	n/a
Basal Leaves	74.72$^{b\ I}$	22.83$^{a\ I}$	n/a	n/a	n/a	n/a

Note: Means with the same letter along column are not statistically significant at 0.05 DMRT
Means with the same Roman numeral along rows are not statistically significant at 0.05 DMRT

on the other hand, have significantly shorter stomata with values ranging from 34 to 50 µm. Also, the apical stomata in young leaves are significantly longer than basal ones which could be attributed to the acropetal development of the leaf.

DISCUSSION

Leaf size. Basal leaves are the most shaded leaves in the leaf spiral of Benguet lily so it is expected that they are the widest. Shaded leaves, according to literatures (Buisson and Lee, 1993; Brownlee, 2001; Beck, 2010) are generally broader than upper exposed leaves. It could also be readily gleaned from the leaf width that the higher the leaf in the spiral, the narrower the lamina. This trend is not just statistically significant in the means but morphologically obvious. Mostly likely, this

trend is for more efficient exposure of the leaves to sunlight by minimizing shading of lower leaves.

The middle leaves being thicker than apical exposed leaves in Benguet lily is contradictory with findings of other authors (Buisson and Lee, 1993; Brownlee, 2001) wherein directly exposed leaves are thicker than shaded leaves even within the same plant, in response to sunlight exposure. Most likely, the apical leaves are still developing thus did not yet attain their full thickness. These results shows that leaf sizes could vary significantly even if there just few centimeters apart.

Stomata density. Anomocytic stomata were also discovered in other species of Liliaceae (Zarinkamar, 2006). In anomocytic type, as defined by Metcalfe and Chalk (1950), the stomata remains surrounded by limited number of subsidiary cells which are quite alike the remaining cells.

The low stomatal density of basal leaves could be attributed to the low light condition and high atmospheric carbon dioxide concentrations from soil respiration, two of three (3) factors that contribute to less stomata according to Beck (2010), the other one being dry environment. Higher stomatal density on apical leaves of Benguet lily is consistent with the findings of Salisbury (1927), Poole *et al.* (1996), Kürschner (1997) and Wagner (1998) which found that upper exposed leaves or sun leaves have higher stomatal density. Also, the trend of stomatal density between parts of lamina is consistent with the findings of Salisbury (1975) wherein monocot leaves, stomatal density increases from the base, reaching maximum at the middle before decreasing toward the apex. On the other hand, it contradicts Earnes' theory that stomata are arranged apparently without regularity.

However, there is much higher stomatal density in the base of young developing leaves. This could be due to acropetal development of the leaf which is common among monocot. This means that the base is younger than apex. Upon maturity, the base now would become the middle part of lamina and the cells would elongate resulting to reduced density. Also, the base is white in color while the apex is starting to turn green. These young leaves are relatively erect thus the basal portion is also exposed to sunlight.

These results shows that stomatal density could vary significantly along the leaf spiral of Benguet Lily even if they are just few centimeters apart. However, the proximity of the observed leaf groups may suggests that the difference in stomatal density could not be totally due to environmental factors, namely lower irradiance and higher carbon dioxide concentrations in basal leaves while higher irradiance in apical leaves. There could be other factors and the data in this study suggest that ontogeny, spiral phyllotaxy in this case, also contribute to difference in stomatal density. However, more study in other plants with similar phyllotaxy is needed to support and establish this theory.

Stomata were also observed sporadically in the adaxial epidermis of basal and middle leaves but none in the apical and young leaves of Benguet lily. This finding is contradictory to the generalization that monocot have uniform distribution of stomata in the abaxial and adaxial epidermis. This also contradicts the generalization of Zarinkamar (2006) that family Liliaceae have equal or higher adaxial than abaxial stomatal density. However, the plant species used by Zarinkamar (2006) to represent Liliaceae are Allium atroviolaceum, Allium rubellum, Asparagus officinalis, Asphodeline dendroides and others, that were traditionally under this family sensu lato but were now circumscribed and transferred

into other related families and order (namely, *Allium* to family Amaryllidaceae, *Asparagus* to Asparagaceae and *Asphodeline* to Xanthorrhoeaceae of order Asparagales) under Angiosperm Phylogeny Group (APG) III (2009). The result of this study showed that generalization on stomatal density of family Liliaceae needs to be verified and checked, this time using species under this family sensu stricto such as *Lilium*.

Lastly, the stomatal lengths in Benguet lily are much higher than those reported by Zarinkamar (2006) for related species of Liliaceae. *Allium atroviolaceum*, *Allium rubellum*, *Asparagus officinalis* and *Asphodeline dendroides* have guard cell length ranging only from 23.64 to 39.48 μm. This large difference in stomata sizes with *Lilium philippinense*, as representative of family Liliaceae sensu stricto, may serves as an additional character supporting the circumscription of these related species used by Zarinkamar (2006) into separate families and order under APG III (2009).

ACKNOWLEDGEMENTS

REFERENCES

APG, I.I.I. 2009. "An Update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III". *Botanical Journal of the Linnean Society* 161(2): 105–121. doi: 10.1111/j.1095-8339.2009. 00996.x. Retrieved 3 January 2014.

Beck, C.B. 2010. *An Introduction to Plant Structure and Development*. New York, USA: Cambridge University Press.

Brownlee, C. 2001. The long and the short of stomatal density signals. *Trends in Plant Science* 6(10): 441-442.

Buisson D. and D.W. Lee. 1993. The developmental responses of papaya leaves to simulated canopy shade. *American Journal of Botany* 80: 947–952.

Elwes, J.H. 1880. *A Monograph of the Genus Lilium. London*: Taylor and Francis.

Earnes, A.J., H. Laurence and M. Daniels. 1925. *An Introduction to Plant Anatomy*. 1st ed. New York: McGraw-Hill Book Co. pp. 364.

Kürschner, W.M. 1996. Leaf stomata as biosensors of paeoatmospheric CO_2 levels. LPP Contrib. Series 5: 1-153.

Metcalf, C.R. and L. Chalk. 1950. Anatomy of Dicotyledons.https://archive.org/details/anatomyofthedico033552mbp. [Accessed on 17 May 2017]

Poole, I, J.D. Meyers, T. Lawson, Raven J.A. 1996. Variations in stomatal density and index: implications for paleoclimatic reconstructions. *Plant, Cell Environ.* 19: 705-712.

Salisbury, E.J. 1927. On the causes and ecological significance of stomatal frequencies, with special reference to the woodland flora. Philos. Tans. R. Soc. London B 216: 1-65.

Salisbury, E.J. 1975. Frequency, size and distribution of stomata in triticale leaves. *Crop Science* 10: 575-578.

Schwabe, W.W. 1984. Phyllotaxis. In: Barlow PW, Carr DJ, editors. Positional Controls in *Plant Development*. London: Cambridge University Press, pp. 403-440.

Wagner, F. 1998. The influence of environment of the stomatal frequency in Betula. *LPP Contrib* 9: 1-102.

Williams, R.F. 1975. *The Shoot Apex and Leaf Growth*. London: Cambridge University Press.

Zarinkamar, F. 2006. Density, Size and Distribution of Stomata in Different Monocotyledons. *Pakistan Journal of Biological Sciences* 9 (9): 1650-1659.

Received: 2 February 2018
Accepted: 1 April 2018