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Comparative Micro-Morphology, Anatomy and Architecture of Leaf of *Physalis*

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***Abstract*—**Two species of *Physalis*, *P. angulata* L. and *P. peruviana* L. were used as models for comparative study to understand the values of micro-morphological, -anatomical and architectural characteristics of leaf for taxonomic purposes and possibly breeding and commercial applications. Both species possess amphistomatic leaves with 1-layer epidermis, 3-4-layer spongy mesophyll and bicollateral bundle midrib. Palisade parenchyma cells of *P. angulata* were almost twice longer (65-75µm) than the other one. Type of stomata was similar as anomocyticbut stomatal index (SI) at adaxial surface and abaxial surface of *P. angulata* were less than of *P. peruviana*as 3.57, 4.00 and 6.25, 6.66 respectively. Some leaf architectural characteristics such as leaf shape, order of venation also provided information of taxonomic significance

Keywords*—Physalis*, *Solanaceae*, micromorphology, anatomy, leaf architecture.

I. Introduction

PHYSALIS, a genus of the family *Solanaceae*, consists of 75 species, most of which are found in America with only a few species in Europe and Asia [1]. There are four species introduced to Thailand and half of them are weeds (*P. angulata* L. and *P. minima* L.) which usually distribute during rainy season whereas the rest are ornamental and fruit crops (Chinese lantern, *P. alkekengi* L. and Cape gooseberry, *P. peruviana* L.), cultivated in cool season. Some taxonomists tried to place this genus in the appropriate systematic position on the basis of comparatively data of pollen, seed, and folia epidermal features taken from herbarium dry specimens of hypothetic relative genera in the same family [1]. However to obviously determine the significances of the informative data of vegetative organ as leaves without environmental controversy, fresh specimens of species members in the problem genus are needed to repeatedly examination. Additionally, leaf architectural and anatomical characteristics which considered the important sources for taxonomic positioning of taxonomic unit [2]-[5] are deficits and needed especially in the family *Solanaceae* [6].

This study reports the micro-morphology, anatomy, and architecture of leaf of 2 *Physalis* species, *P. angulata* L. and *P. peruviana* L. as comparing models to allow an application of foliar data for taxonomy of the genus *Physalis*.

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II. Materials and Methods

A. Plant Material

Two species from the genus *Physalis*, *P. angulata* (Figs. 1 A and B) and *P. peruviana* (Figs. 1 C and D) collected from the fields were grown in pots, placed in glasshouse at Biology Department, Faculty of Science, Silpakorn University, Nakhon Pathom, Thailand. Plants were watered twice a day. The plant materials for study on morphology, anatomy, and architecture of leaf were taken when plants were in the flowering period.

B. Leaf Micromorphology and Architecture Investigation

Leaf blades of 2 *Physalis* species were prepared for micromorphological and architectural studies according to standard methods used in previous researches [7], [8]. Qualitative characteristics i.e. the sculpture of the adaxial and abaxial epidermal cells were examined on semipermanent slides and on cross section of leaf blades taking $1cm^2$ segments from the middle portion of leaves previously overnight fixed in FAA. Material was macerated in a sodium hypochlorite solution (5.25%) at room temperature (29-35ºC) for an hour, thrice washed with distilled water, stained with 0.5% aqueous toluidine blue and mounted in a 1:1 solution of water: glycerine for microscopic examination and photograph with an OLYMPUS CH30 RF 300 microscope and photographic camera (Olympus Optical, Tokyo, Japan).

Qualitative features on leaf epidermis considered in this study were epidermal cell shape and cell wall sinuousity from a front paradermal view; type, location and distribution of stomata; trichometype and distribution, whereas the quantitative features were concentrated on stomatal length, stomatal width, stomatal index (SI). Stomatal index was calculated using the following equation [9],

$$\text{Stomatal Index (SI)} = S/\ E+S \times 100$$

S = Stomatal number per leaf area unit

E = Epidermal cell number in the same leaf unit

The venation was study using leaf skeletons prepared according to adapted clearing method [10]. Complete fresh leaves were macerated in 2.5% NaOH at room temperature (29-35ºC) for 2 days, thrice washed with distilled water, decolorized with 50% ethanol, cleared with 70% lactic acid, rehydrated with 50% ethanol, stained with 1% Safranin in 50% ethanol, dehydrated with 95% ethanol prior mounted in glycerol. The terminology of leaf architecture follows [11].Observations and photographs were performed using

stereomicroscope OLYMPUS SZ40and OLYMPUS CH30 RF 300 microscope

C. Leaf Tissue Anatomy

Leaf samples were cut into segments transversally to the midrib, 5mm long, and overnight fixed in FAA. The materials were dehydrated through a graded ethanol series, cleared by incubation in xylene, and embedded in paraffin wax. Transverse sections of 12µm thickness were prepared using rotary microtome. All sections were made from the midrib and the margin of the leaf blades. The sections were double stained with 1% Safran in and 0.2% Fastgreen, mounted in DPX, examined and photographed using an OLYMPUS CH30 RF 300 microscope (Olympus Optical, Tokyo, Japan).

III. Results

A. Micromorphological Characteristics of Leaf Epidermis

The micromorphological features of 2 *Physalis* species due to microscopic examination on leaf epidermis revealed mostly similar data (Table I). The anomocytic stomata surrounded by 3-5 epidermal cells were observed on both leaf surfaces (Fig. 1 E-H). However, slightly higher stomata length and stomatal index were obtained from *P. peruviana* (Table I). In addition to epidermal cell outline taken from a front paradermal view, *P. angulata* possessed irregular cell shape with strongly angular sinuous polygonal cell wall distributed on abaxial surface (Fig. 1 F). The sinus herein was almost twice deeper compared to that on another side of the same species (Fig. 1 E). In contrast *P. peruviana* possessed similar irregular cell shape, lining with rounded sinuous polygonal cell wall which almost equal length of sinus of approximately a half of cell width and presented on the both surfaces (Figs. 1 G and H; Table I).

TABLE I
Leaf Epidermal Morphological Features of 2 *Physalis* Species

Features \ Species	*Physalisangulata*		*Physalisperuviana*	
	Adaxial surface	Abaxial surface	Adaxial surface	Abaxial surface
Stomata width (µm)	22.07	18.25	22.82	17.05
Stomata length (µm)	32.67	28.42	32.69	32.69
Stomatal index(SI)	3.57	4.00	6.25	6.66
Epidermal cell shape	irregular	irregular	irregular	irregular
Pattern of anticlinal walls	sinuous	sinuous	sinuous	sinuous
Stomata type	anomocytic	anomocytic	anomocytic	anomocytic
Guard cell shape	elliptic (sausage)shape	elliptic (sausage)shape	eliptic (sausage)shape	eliptic (sausage)shape
Guard cell width-length ratio at opening stage	1:1.5-1:2 (1-1.5:1.5-2)	1:1.3-1:2 (1.5-2:2-3)	1:1.3-1:2.2 (3-5 : 4-6.5)	1:2-1:3.6 (2.5-6.5 : 5-9)

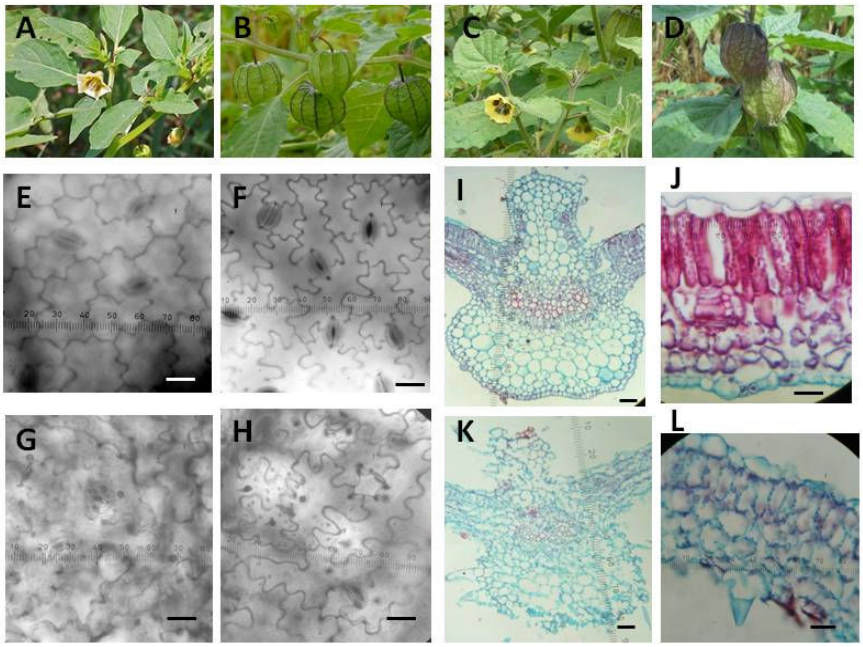

Fig. 1 Comparative characteristics of 2 *Physalis* species, *P. angulata* L.(A-B, E-F, I-J), *P. peruviana* L.(C-D, G-H, K-L): Flowering shoots (A, C); Fructescence shoots (B ,D); Light micrographs of leaf epidermis on the adaxial surface (E,G), on the abaxial surface (F, H); Cross sections of leaf at midrib position (I,K), at lateral part of blade (J, L). Bars = 25 µm

B. Leaf Architectural Characteristics

On the basis of 28 characteristic data obtained (Fig. 2; Table II), 7 characters (i.e. petiole length, leaf base shape, 1^0 vein category, 2^0 vein category, number of highest vein order, 5^0vein category and Freely ending ultimate vein or F.E.V.S.) could be used to distinguish this 2 species (Table II). There were slightly variations appeared in laminar shape, apex shape and base angle of leaf blade which were mostly broader and more degree of angle (or curve) presented in *P. peruviana* (Table II).

C. Leaf Anatomical Characteristics

Observation of leaf segments provided qualitative and quantitative features of leaf tissues in the studied species (Figs. 1 I-L; Table III). Both *Physalis* species showed similar structure and tissue components regarding to examination on transverse sections taken from the same compared positions either at the midrib (Figs. 1 I and K) or the blade margin (Figs. 1 J and L). They possess 1-layered epidermis, scarcely covered with finger hairs along the leaf margin (Figs. 2 D, E, I, and J), and 1-layered mesophyll (Table III). Type of vascular bundle in the midrib was bicollateral bundle (Figs. 1 I and K). There were clear differences between two types of mesophyll occurred in *P. angulata* (Fig. 1 J). Palisade cells in *P. angulata* were slightly narrower but almost twice longer than that of *P. peruviana* (Table III) leading to be a columnar shaped appearance in the former species (Fig. 1 J), whereas being a rectangular shaped in the later (Fig 1 L). In contrast, spongy mesophyll cells of *P. peruviana* were bigger (12.50-47.50µm wide by 11.25-40.00µm long) than that of another species (Table III).

TABLE II
LEAF ARCHITECTURE IN 2 *PHYSALIS* SPECIES

Features \ Species	*Physalis angulata* L.	*Physalis peruviana* L.
Leaf attachment	spiral	spiral
Petiole outline	reniform	reniform
Petiole length(cm)	1-11	2-5
No. of lateral veins/side	5-7	5-7
Laminar shape	elliptic, ovate, oblong	broad ovate
Laminar symmetry	symmetrical to base asymetrical	symmetrical
Apex angle	acute	acute
Apex shape	acute, long acuminate	short acute
Base angle	acute, obtuse	obtuse, wide obtuse
Base shape	concavo-convex	lobate
Position of petiolar attachment	marginal	marginal
Margin type	entire, dentate	entire, dentate
Highest vein order	5	4
1 ˚vein category	pinnate	suprabasal
2 ˚vein category	semicraspedodromus	semicraspedodromus
2 ˚veinspacing	decreasing toward base	decreasing toward base
2 ˚veinangle	smoothly increasing toward base	abruptly increasing toward base
3 ˚vein category	alternate percurrent	alternate percurrent
3 ˚vein course	sinuous	sinuous
4 ˚vein	regular polygonal reticulate	regular polygonal reticulate
5 ˚vein	dichotomizing	-
Areolation	moderately developed	moderately developed
Freely ending ultimate veins(F.E.V.S.)	unbranched	absent
Marginal ultimate	looped	looped
Teeth order	1	1
Tooth spacing	irregular	irregular
Tooth shape(apical side/basal side)	concave/concave	concave/concave
Sinus shape	rounded	rounded

IV. DISCUSSIONS

The results from this study indicate that the two species of *Physalis* are similar in most characteristics of leaf epidermal morphology and leaf anatomy which those characteristic evidences seem to be commonly found in the genus *Physalis* [1]. However there are comparatively consistent in the shapes of epidermal cells on both surfaces. The irregular shaped epidermal cells with strongly sinuous anticlinal wall occurred similarly on both sides of the leaf in *P. peruviana* (Figs. 1 G-H), but only found on abaxial side in *P. angulata* (Fig. 1 F). On adaxial suface, these cells depicted shallower lobes (Fig. 1 E) than those on the adaxial surface (Fig. 1 F).The uniformity and sinuousity patterns on anticlinal wall of each epidermal cell were hypothesized affected by the variation in number of contacting cells in small area between epidermis and mesophyll and needed to have the sinuous growth pattern to provide secure attachment between these tissues [12]. Korn [13] suggested that by regional expansion of basal periclinal

walls in accommodation to lobe growth, more contact space with mesophyll cells is possible than by uniform growth alone and may cause more uniform spacing between mesophyll cells. On the basis of mesophyll cell shape and size and its distribution data obtained from *P. angulata* in this study, by narrower but longer palisade cells as columnar shape (Fig. 1 J) and more compacted arrangement in association with more cell number in an area of contact to each epidermal cell, possibly involve causing angular sinuous feature than round sinuous in this species (Fig. 1 E; Table III). However, to assure that this feature is not affected by the surrounding environment in their habitat; more evidences on leaf anatomy and ontogeny of this species from different climatic and geographic regions are needed.

TABLE III
MICROSCOPIC LEAF ANATOMICAL FEATURES OF 2 *PHYSALIS* SPECIES

Features \ Species	*Physalis angulata*	*Physalis peruviana*
Epidermis	1 layer	1 layer
Trichome	finger hair	finger hair
Mesophyll structure	dorsiventral	dorsiventral
Palisade mesophyll	1 layer	1 layer
Palisade cell shape	columnar	rectangular
Palisade cell width(µm)	5.00-17.50	15.00-20.00
Palisade cell length(µm)	65.00-75.00	37.50-45.00
Palisade cell arrangement	perpendicular to epidermis	perpendicular to epidermis
Spongy mesophyll	3-4 layers	3-4 layers
Spongy cell shape	irregular	irregular
Spongy cellwidth (µm)	7.50-22.50	12.50-47.50
Spongy cell length(µm)	7.50-27.50	11.25-40.00
Spongy cell arrangement	irregular	irregular
Vascular bundle type in midrib	bicollateral bundle	bicollateral bundle

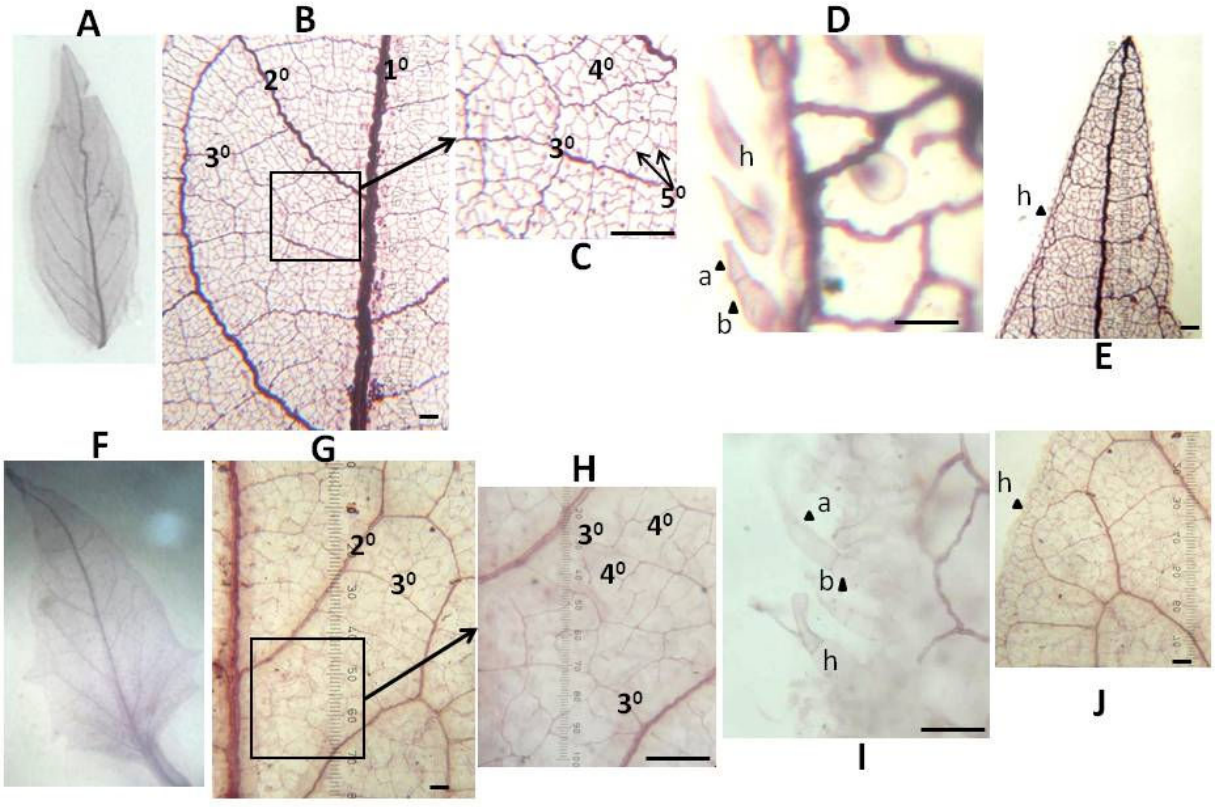

Fig. 2 Comparative leaf architectural features of 2 *Physalis* species, *P. angulata* L. (A-E), *P. peruviana* L. (F-J): (1^0=1^0 vein category, 2^0 = 2^0 vein category, 3^0=3^0 vein category, 4^0 = 4^0 vein category, 5^0 = 5^0 vein category; a=apical hair cell, b=basal hair cell, h= finger hair) Bars = 25µm

Leaves of both species involved in amphistomatic or dorsiventral type which palisade and spongy mesophyll tissues locating at different sides were agreed with previously reported [14]. The type of stomatal apparatus occurred in both species was anomocytic and existing on both surfaces. Zhang and Lu [1] reported that anomocytic stomata were restricted only on abaxial surface of leaf in *P. angulata* that was different from the observed data herein. Sandhya et al. [15] reported that stomatal apparatus observed in *P. angulata* was anisocytic type which was different from that of this.

The leaf architectural characteristics data in 2 *Physalis* species obtained from this study (Fig. 2; Table II) were firstly reported. The seven characteristics including petiole length, leaf base shape, 1^0 vein category, 2^0 vein category, number of highest vein order, 5^0vein category and freely ending ultimate vein or F.E.V.S. were constant within species, and thus could provide taxonomic values for generic classification and usage to distinguish this 2 species.

V. Conclusion

Some structure and micromorphology of leaf epidermis in association with relative anatomical features and leaf architectural characteristics reported herein, provided taxonomic significances at generic and specific ranks due to similarity and dissimilarity existing in the compared features, between 2 species of *Physalis*.

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