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Morphological Study of Trichomes in *Indigofera wightii* Grah. ex Wigh & Arn., Indigo Dye Species, Traditionally Used by "Thaisongdam" Thailand

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***Abstract*—**The study aimed to collect morphological data of secretory structures that contribute to taxonomy of *Indigofera*. Detail features of trichomes occurrence in vegetative and reproductive organs of *Indigofera wightii* Grah. ex Wigh & Arn., a species traditionally used as source of indigo to dye "Thaisongdam" clothing were investigated. Examination through light microscopy and scanning electrom microscopy were done. Non secretory, T-shaped trichomes appeared throughout surfaces of stems, leaves, flowers and fruits. Secretory or glandular trichomes occurred in two types; one has big cylindrical head and short peduncle, distributed on adaxial surface of sepals and around the pedicel, whereas another possesses smaller cylindrical head but long peduncle. The latter was found on apical surface of immature pods. No phenolic and lipophilic compounds were detected from these glands.

***Keywords*—**Indigofera, Thailand, Thaisongdam, trichome.

I. Introduction

INDIGOFERA, the largest genus of the tribe Indigoferae, family Leguminosae subfamily Papilionoideae, has approximately 700 species distributed in the warm tropical and subtropical regions of the world [1] and 30 species (32 taxa) were found in Thailand [2]. Several species in this genus are useful to mankind ranging from feed for livestock, soil protection, green humus covering, folk medicine, ornamental plants and as dye source for commercial purposes [3], [4]. In Thailand, 15 species of *Indigofera* were enumerated as native plants in Thai Plant Names [5]. The commonly known a source of indigo dye*, I. tinctoria* L. is an exotic shrub here. This species is the most favourably used to dye cotton and silk in the Northeast provinces of the country. However some native species are also substituted source of this dye for local people. An ethnic group variously called "Thaisongdam", "Tai Song Dum", "Song" or "Black Tai" whom migrated from Dian bian foo, the North of Vietnam to establish in the central region of Thailand for more than 230 years ago, preferred wearing traditional black costumes. Therefore the old unique culture and knowledge including preparation and dyeing of their costumes to specify the typical "Thaisongdam identity," are always kept secret and passed on to the younger generation by the elderly [6]. *Indigofera wightii* Grah. ex Wigh & Arn. (synonyms: *I. hainanensis* H.T. Tsai & T.T. Yu, *I. inamoena* Thwaites, *I. pallid* Craib), or known in vernacular name as "Khrambailek" has long been used as source of indigo dye for black color for their clothes and costumes. This species is found in deciduous and dry dipterocarp forests from the altitudes almost at the sea level up to ca 2000 m in some provinces in Northern, North-Eastern, Eastern and South-Western of Thailand and also distribute in Sri Lanka, India, Myanmar, China, Cambodia and Vietnam [2]. Since, trichomes have long been reported to play a significant role not only in plant biology including defence, pollination, dispersal of seeds, fruits, and propagules [7]-[9], but also in taxonomy [10]. To specify the right *Indigofera* species as dying source for "Thaisongdam" clothes at the vegetative developing stage, the trichome data may help their descents easily accumulate this plant from natural areas. Taxonomists may also use the morphological details found from this structure as diagnostic characters to delimitate this species in such a way that they have been previously used within Neotropical *Indigofera* species [11], [12]. In addition ethnobotanists may understand interrelationship between plant andthis ethnic group on the basis of plant distribution and restriction rule on knowledge transfer.

This study reported the morphological characters of trichomes in *Indigofera wightii* Grah. ex Wigh&Arn., source of indigo dye, traditionally used by the ethnic group in Thailand, called "Thaisongdam", on the basis of examination via light and SEM microscopy.

II. Materials and Methods

A. Plant Material

Stems, leaves, flowers and pods of *Indigofera wightii* were obtained from well established 6-month-old plants grown from seeds collected from Ampoe Khao Yoi, Petchaburi province, Thailand in July 2010. These different plant parts were used for the study. The voucher specimen was also prepared and deposited at the Department of Biology, Silpakorn University, Nakhon Pathom after gross morphological examination.

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B. Light Microscopy

The morphological features, types, natural coloration and primary distributions of trichomes were studied using a stereoscopic microscope Olympus SMZ (Japan). To detect trichome properties on possessions of phenolic compounds and lipophilic substances, free hand sections from fresh materials (sepal and pod) were stained with ferric chloride [13] and Sudan III [14] respectively. The unstained sections were also prepared for comparisons. Sections were processed for temporary slides by mounting in glycerine jelly prior to be examined and photographed using an OLYMPUS CH30 RF 300 microscope and photographic camera (Olympus Optical, Tokyo, Japan).

To observe the origin of trichomes, the transverse sections of stem and leaf were done. The FAA fixed materials were processed through TBA series and embedded in paraffin wax, prior the transversely cut sections of 12 µm thickness were stained with 1% Safranin and 0.2% Fast green combination [13]. Subsequently sections were mounted in DPX mounting media and examined under OLYMPUS CH30 RF 300 microscope and photographic camera (Olympus Optical, Tokyo, Japan).

C. Scanning Electron Microscope

Three pieces of 3 x 5 mm^2 plant materials were cut from each organ and fixed in FAA (Formalin: Acetic acid: 70% Ethyl alcohol= 8:1:1 v/v) for 3 days, then dehydrated in an ethyl alcohol series followed by trice soak in absolute ethanol. Each specimenwas mounted on an aluminum stub with double side sticky tape and coated with gold in a Cressington sputtercoater-180. The samples were observed and photographed with a CAMSCAN MX-2000 scanning electron microscope at 10 kV.

III. Results

A. Plant Morphology

Indigo ferawightii is shrub of 1-1.5 m high. Leaves are oddpinnate and alternate phyllotaxy. Petioles are 0.4-1 cm long. Stipules are triangular in shape, hairy outside but glabrous inside. Rachis is 3-6 cm long. Leaflet number is 11-13, with opposite or subopposite arrangement and scabrous. Each leaflet has petiole (petolule) of 1-2 mm long, elliptic or obovate lamina of 1-2 cm by 0.6-1 cm with emarginated, (rarely rounded), apex and cuneate base. Lateral veins are indistinct. Both sides of leaf surfaces are covered with grayish, symmetric T-shaped hairs, but denser on abaxial. Flowers are in raceme of 1-4 cm long. Bracts are ovate in shape, ca 1 mm long. Pedicels are 1.5-2 mm long and hairy. Calyx fuses at base into cup-shaped, of 1-3 mm long and apically separates into 5 triangular lobes of 0.5-1 by 0.5 mm with acute apex and hairy margin. Corolla is pinkish color with broadly ovate shaped standard of 3-5 by ca 4 mm. It has acute apex, rounded base, entire margin and been covered densely with hairs outside but glabrous inside. Wings are of 4-6 by 1-2 mm with acute apex, entire margin, glabrous both outside and inside and possessing claw of ca 0.5 mm long. Keel is of 4-5 by 1-2 mm, hairy outside and glabrous inside. Stamen number is 10 and diadelphous with glabrous staminal tube of 4-5 mm long and filament of ca1 mm long. Anthers possess an apiculate connective. Ovary is superior and covered densely with hairs. The ovary has 10-12 ovules. Style is of ca 2 mm long. Pods are cylindrical shape, straight, of 2-3 cm long, hairy and dehiscent. Seed number per pod is 8-12.Each seed is cylindrical shape with blunt ends, of ca 1.5 mm long and ca 1 mm in diameter and glabrous.

B. Trichome Morphological Characteristics

Under the light microscope and positions where the trichomes belonged, 2 groups of trichomes were classified (Table I; Figs. 1-3). Firstly, the non secretory (non glandular), uniseriate, T-shape trichome was recognized. This trichome type appeared all over surface of the vegetative organs of *Indigofera wightii*. Each trichome composed of one cell peduncle or foot with thickened wall (Fig. 1, Figs. 3 (b) and (g)); surrounded by ordinary epidermal cells (Fig. 1). Head was unicellular with symmetric two arms as T-shaped and ends pointed. Ornamentation on trichome wall surface was smooth on peduncle but pitted on head (Fig. 1 (c)). Secondly, 2 types of the secretory trichomes were observed. Type I was reddish colored trichome. Each trichome possesses short peduncle with cylindrical massive head (15-30 µm dia.) and presented in cluster on raceme and pedicel axis, bract and sepal margin (Figs. 3 (c) and (d); Table I). Type II was greenish colored trichome with long biseriate peduncle and cylindrical massive head (30-45 µm dia.). This was found along the pod tip (Figs. 3 (e) and (f); Table I). These secretory trichomes possessed no phenolic and lipophilic compounds as the result of no differences occurred after being stained with ferric chloride and Sudan III (Fig. 2).

IV. Discussions

The 6-month-old *Indigofera wightii* plants grown from seeds showed similar morphological characteristics of the species as being recognized from parental plants. The quantitative characters such as number and size of leaflets number of seeds per pod slightly varied but were still in the range that had been described for this species [2].

The microscopy of *Indigofera wightii* studied here revealed valuable basic information for plant taxonomy and ethnobotanical application. Regarding to morpho-anatiomical data, the non secretory, uniseriate, symmetric T- shape trichomes appeared all over surface of every organ of *Indigofera wightii* except on adaxial surface of stipule and corolla segments. The studied results herein corresponded to the previous descriptive report [2]. Although this trichome type was commonly present on many organs such as leaves and flowers of *Indigofera* spp. [15], the ornamentation on wall surface was found useful to distinguishing at species level of the genus. For examples, the non secretory medifixed trichome with papillate wall was reported in *I. trita* L. whereas trichome with warty wall was present in *I. enneaphylla* L. and *I. tinctoria* L. [16]. Ornamentation character of trichome wall surface of *I. wightii* was firstly reported herein as smooth on peduncle but pitted on head. This data clearly supports an idea

of useful application of trichomes for distinguishing *Indigofera* species.

The secretory trichomes Type I (short peduncle with cylindrical massive head) and Type II (long biseriate peduncle and cylindrical massive head) resulting from this study were previously observed in Neotropical species of *Indigofera* and recognized as Type 5 and Type 7 respectively [12]. However the natural color of Type I secretory trichome herein was reddish not greenish as of Type 5.

According to distribution of secretory trichomes, Type I occurred in cluster on parts of reproductive organs, such as inflorescence, pedicel axis, bract and sepal margin whereas Type II was found along the pod tip, the defensive function against herbivores and the attraction of pollinators might possibly relate [17]. In addition, an appearance of secretory trichomes in cluster form in nodal regions and axillae of organs which protect structures during initial developmental states, may allow these trichomes easily to secreting lubrication substances, i.e. mucilaginous. However, after the preliminary histochemical tests, no evidences (no color changes from ferric chloride or Sudan III stained secretory trichome Type I and Type II) were found for the secretions either phenolic compounds or lipophilic substances from Type I and Type II trichomes. In comparison to important indigo dye source, *I. tinctoria* L. these secretory trichomes were absent [16]. Therefore, it would be interesting to investigate in detail, the chemical composition elucidated from these trichomes and the relative function in different organs.

TABLE I
TRICHOME CHARACTORS OF *INDIGOFERA WIGHTII* GRAH EX.WIGH & ARN. FOUND IN THAILAND

Trichome type	Natural color	Size (µm)		Morphological description		Distribution			
		Trichome length	Head length or diameter	Peduncle	Head	Stem	Leaflet	Flower	Fruit
Non secretory									
T- shape	green	20-50	300-400	Very short cylindrical	Long cylindrical with tapering towards ends	present	present	present	present
Secretory									
Type I	reddish	80-140	15-30	Short cylindrical	Solid cylindrical massive	absent	absent	Bract, Calyx, Pedicel	absent
Type II	greenish	45-60	30-45	Long cylindrical, biseriate	Cylindrical massive	absent	absent	absent	Pod tip

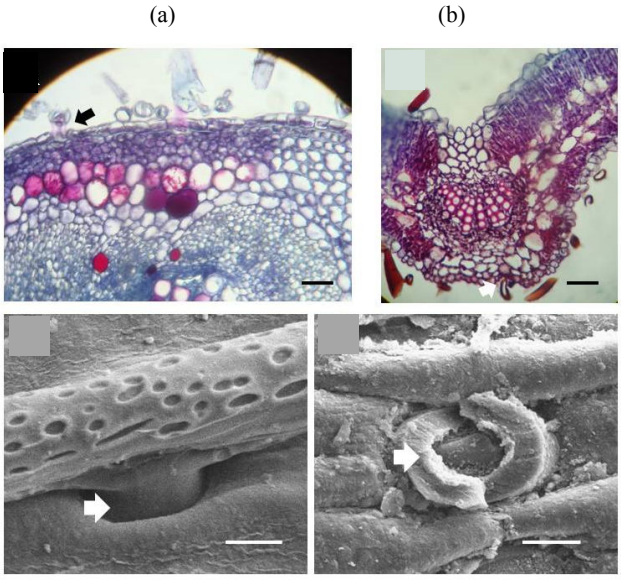

Fig. 1 Non secretory trichomes in *Indigofera wightii* Grah. ex Wigh & Arn.. Photomicrographs of cross section of stem (a) and leaf (b) showing peduncle or foot of trichomes (arrow). Electron micrographs (SEM) of wall ornamentation of peduncle and head of trichome (c) and thickness of peduncle wall after trichome head detaching (d). Scales bars (a, b) = 25 µm; (c, d) = 10 µm.

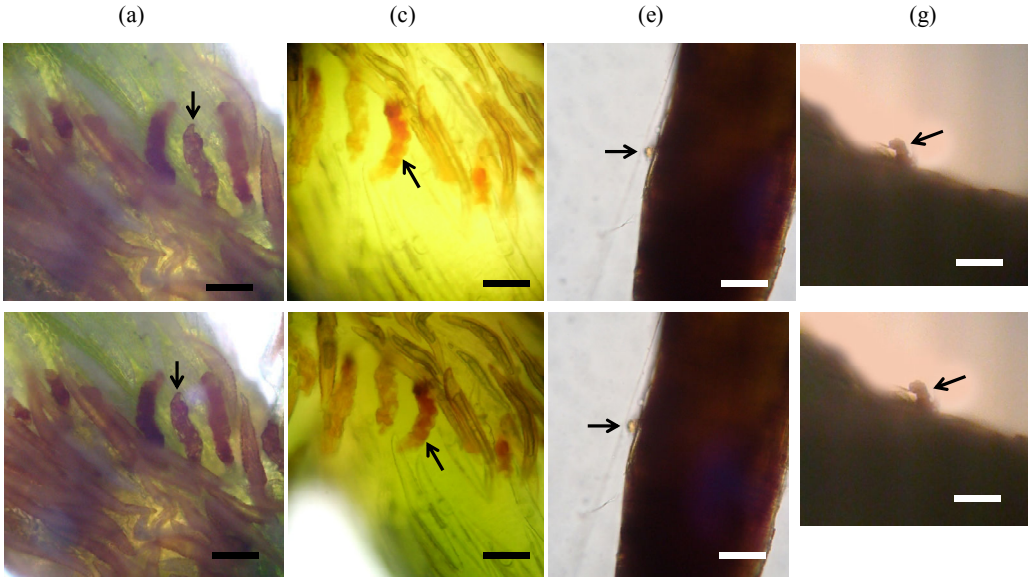

Fig. 2 Comparative characters of 2 secretory or glandular trichome types in *Indigofera wightii* Grah. ex Wigh & Arn. under unstained and stained conditions. Type I (a-d) on pedicel, Type II (e-h) on pod tip. Unstained secretory trichomes (a, c, e, g); stained with ferric chloride (b, f); stained with Sudan III (d, h). Scales bars = 50 µm.

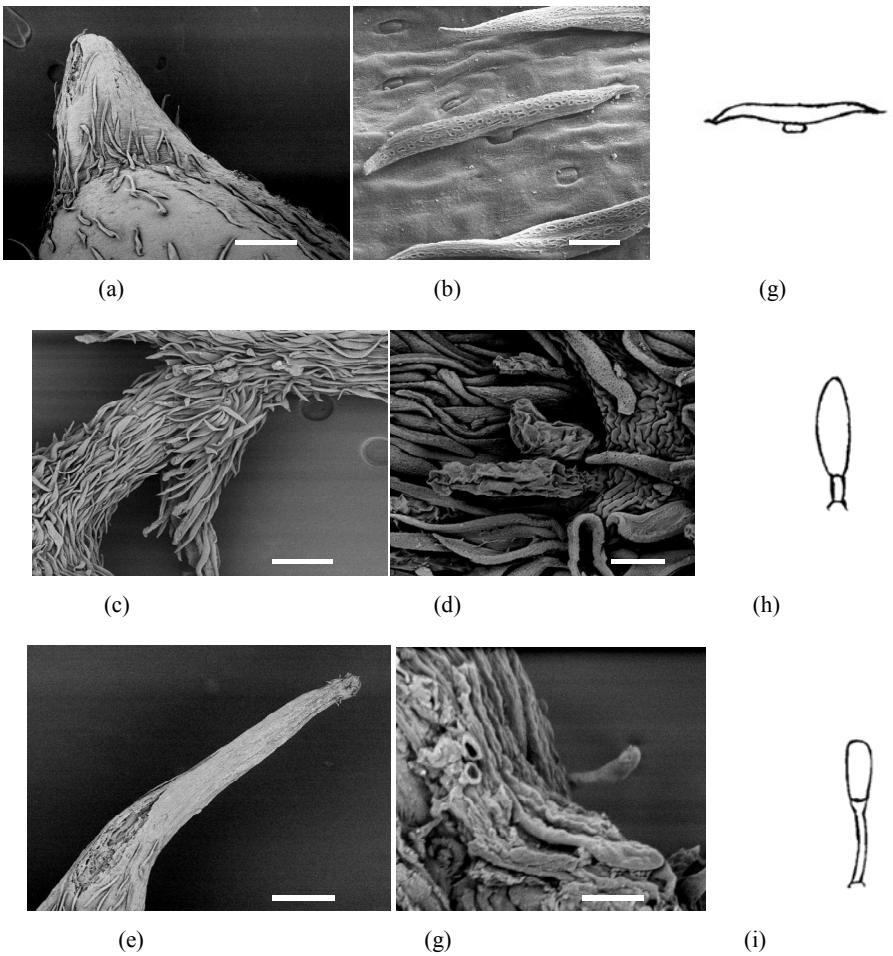

Fig. 3 Electron micrographs (SEM) (A-F) and schematic drawings (G-I) of 3 trichome types in *Indigofera wightii* Grah. ex Wigh & Arn.. Non secretory trichome (a-b, g) on pod; Secretory trichome Type I: short peduncle with cylindrical head (c-d, h) on pedicel axis; Type II: long peduncle with cylindrical head (e-f, i) on pod tip. Scales bars (a, c, e) = 150 µm; (b, d, f-i) = 50 µm.

V. Conclusion

Microscopy characteristics of trichomes provided useful data to distinguish *Indigofera wightii* Grah. ex Wigh & Arn. found in Thailand. Non secretory, T-shaped trichomes occurred almost throughout surfaces in both vegetative and reproductive organs. Secretory or glandular trichomes were presence in 2 types; Type I had big cylindrical head and short peduncle, clustery distributed on adaxial surface in axil of sepals and around the pedicel, whereas Type II with smaller cylindrical head but longer peduncle were found on apical surface of immature pods. No phenolic and lipophilic compounds were detected from these glands.

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