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Leaf Epidermal Anatomy of Some *Crotalaria* L. (Fabaceae) Species in Thailand

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ABSTRACT. – The leaf epidermal anatomy of 23 *Crotalaria* species was studied. The leaf epidermis was prepared by peeling and clearing methods and observed by a light microscope. The results of the leaf epidermis showed that the shape of an epidermal cell is polygonal, irregular or jigsaw-like and the anticlinal wall is straight, curved or undulated. There are two stomatal types: anomocytic and anisocytic, while the trichome is uniseriate. The papillae are presented in *C. acicularis*. The leaf epidermal anatomical characteristics cannot be used for species identification, however the shape and anticlinal wall of the epidermal cell and the presence of trichomes can be used to determine species pairs.

KEYWORDS: Crotalarieae, Leguminosae, species pairs, taxonomy

INTRODUCTION

Crotalaria is the largest genus of the tribe Crotalarieae and belongs to the subfamily Papilionoideae of the family Fabaceae. The genus consists of approximately 700 species. It is mainly distributed in tropical and subtropical regions with around 500 species found in Africa and Madagascar (Adema, 2006; Mabberley, 2008; Subramaniam and Pandey, 2013). Several species are medicinal plants such as *C. alata*, *C. albida*, *C. bracteata*, *C. lejoloba*, *C. pallida*, *C. sessiliflora*, and *C. spectabilis* (Suwanchaikasem et al., 2013; Sutjaritjai et al., 2019), while *C. cunninghamii* and *C. juncea* are important crops in fibre production (Lewis et al., 2005; Clarke, 2008).

In recent years, there has been discrepancies in the number of species recognized by different authors. Both vegetative and reproductive parts have been reported to have interesting anatomical characteristics including the roots, stems, leaves, flowers, fruits and seeds. The anatomical characters are of great taxonomic value in the classification and identification of several families such as Arecaceae (Horn et al., 2009), Asteraceae (Mabel et al., 2013), Myrtaceae (Al-Edany and Al-Saadi, 2012), Solanaceae (Chiarini and Barboza, 2007) and Sapotaceae (Prasawang and Srinual, 2020). The anatomical features of the family Fabaceae provide useful taxonomic characters for the tribe Phaseoleae (Lackey, 1978), subtribe Cassiinae (Saheed and Illoh, 2010) and many genera such as *Astragalus* (Al-Joboury, 2016), *Butea* (Ninkaew and Chantaranothai, 2015), *Lotus* (Barykina and Kramina, 2006), *Phyllodium* (Saisorn and Chantaranothai, 2015), and *Vigna* (Mbagwu and Edeoga, 2006).

For the genus *Crotalaria*, Parveen et al. (2000) investigated 17 Indian species and found the leaf epidermal cells are taxonomic characters that can be used to construct the dichotomous key of these species. The anatomical characters of 16 Brazilian species belonging to four sections of Le Roux's system (Le Roux et al., 2013) including the sections Calycinae, Hedriocarpae, Incanae, and Stipulosae were examined by Devecchi et al. (2014), who investigated the venation, trichome and leaf blade. Their results indicated that only trichomes with a base composed of radially distributed cells are diagnostic characters in the section Calycinae. The other features have no taxonomic value at the sectional level. However, some anatomical characters are of diagnostic values for the classification and identification of species pairs. In Thailand, there are 38 *Crotalaria* species that can be classified and identified by both their vegetative and reproductive parts, especially flowers and fruits (Ninkaew et al., 2017). They are similar in their habits and leaf characters. Anatomical data was reported by Niyomdham (1978), who stated that the genus has non-glandular, uniseriate, and tricellular hairs. Stomata are either present on both surfaces of the leaf in *C. pallida*, *C. retusa*, *C. spectabilis*, *C. uncinella*, and *C. verrucosa* or found only on the adaxial surface in *C. cytisoides* and *C. incana*. Previous works have shown that anatomical characters are important taxonomic evidence. Thus, this study investigates the leaf epidermal anatomical characters of Thai *Crotalaria* in order to determine its implication for species identification and classification. This work expands our understanding of Thai *Crotalaria*.

TABLE 1. List of *Crotalaria* specimens examined for leaf anatomy.

Species	Voucher specimens	Locality
1. *C. acicularis* Buch.-Ham. ex Benth.	*Ninkaew* 324 (KKU)	Nan
	Rakarcha et al. 961 (QBG)	Chiang Mai
2. *C. alata* Buch.-Ham. ex D. Don	*Ninkaew* 284 (KKU)	Khon Kaen
3. *C. albida* Heyne ex Roth	*Ninkaew* 294 (KKU)	Sakon Nakhon
	Ninkaew 317 (KKU)	Phetchabun
	Thammarong et al. 2714 (QBG)	Chiang Mai
4. *C. assamica* Benth.	*Ninkaew* 312 (KKU)	Chiang Mai
	Ninkaew 321 (KKU)	Nan
	Thammarong et al. 2698 (QBG)	Chiang Mai
5. *C. bracteata* Roxb. ex DC.	*Ninkaew* 253 (KKU)	Nan
	Ninkaew 310 (KKU)	Chiang Mai
6. *C. calycina* Schrank	*Rakarcha et al.* 955 (QBG)	Chiang Mai
7. *C. chinensis* L.	*Ninkaew* 316 (KKU)	Phetchabun
	Rakarcha et al. 963 (QBG)	Chiang Mai
8. *C. dubia* Graham ex Benth.	*Ninkaew* 307 (KKU)	Chiang Mai
	Rakarcha et al. 988 (QBG)	Tak
9. *C. filiformis* Wall. ex Benth.	*Ninkaew* 286 (KKU)	Tak
10. *C. gorrensis* Guill. & Perr.	*Ninkaew* 398 (KKU)	Khon Kaen
11. *C. incana* L.	*Ninkaew* 390 (KKU)	Chumphon
12. *C. juncea* L.	*Ninkaew* 280 (KKU)	Nakhon Ratchasima
	Ninkaew 378 (KKU)	Nong Bua Lam Phu
13. *C. kurzii* Baker ex Kurz	*Rakarcha et al.* 977 (QBG)	Tak
14. *C. lejoloba* Bartl.	*Ninkaew* 309 (KKU)	Chiang Mai
	Ninkaew 400 (KKU)	Chiang Mai
	Thammarong et al. 2715 (QBG)	Chiang Mai
15. *C. medicaginea* Lam.	*Ninkaew* 281 (KKU)	Khon Kaen
	Ninkaew 320 (KKU)	Yasothon
16. *C. montana* Heyne ex Roth	*Ninkaew* 296 (KKU)	Phetchabun
	Ninkaew 384 (KKU)	Sakon Nakhon
	Rakarcha et al. 956 (QBG)	Chiang Mai
17. *C. neriifolia* Wall. ex Benth.	*Rakarcha et al.* 992 (QBG)	Tak
18. *C. pallida* Aiton	*Ninkaew* 399 (KKU)	Khon Kaen
19. *C. prostrata* Rottler ex Willd.	*Thammarong et al.* 2742 (QBG)	Chiang Mai
20. *C. sessiliflora* L.	*Rakarcha et al.* 953 (QBG)	Chiang Mai
21. *C. spectabilis* Roth	*Ninkaew* 313 (KKU)	Chiang Mai
	Rakarcha et al. 986 (QBG)	Chiang Mai
22. *C. tetragona* Roxb. ex Andrews	*Rakarcha et al.* 985 (QBG)	Tak
23. *C. verrucosa* L.	*Ninkaew* 322 (KKU)	Ratchaburi
	Rakarcha et al. 982 (QBG)	Chiang Mai

MATERIALS AND METHODS

Twenty-three species (39 taxa) of Thai *Crotalaria* were collected from various parts of Thailand (Table 1). Three to five mature leaves were collected from each species and preserved in 70% ethanol. All field collection specimens were deposited at Khon Kaen University herbarium (KKU) and Queen Sirikit Botanic Garden herbarium (QBG). The samples were anatomically interpreted. They were prepared by peeling and clearing methods which modified Itharat's protocol (2009). The size of the mature leaf blades, approximately 5 x 5 mm were mechanically scraped at the midway point between the base and apex before being stained with 1% safranin O in 70% alcohol for 20–30 min. The samples were rinsed in tap water to remove the excess staining solution. They were then dehydrated by washing with a series of increasing alcoholic content aqueous solutions (70%, 95% and 100% ethanol, respectively) for 15 min in each step and cleared with xylene (1:1 100% ethanol:100% xylene and 100% xylene, respectively) for 15 min in each step. Finally, the epidermal samples were permanently mounted on slides in the DePeX artificial mounting medium. All slides were examined by a light microscope (LM). Photographs were taken with the aid of a Nikon series E200 LM.

TABLE 2. Anatomical characters of the leaf surfaces by epidermal peeling.

Species	Cell shape		Anticlinal wall		Stomata type		Trichome		Trichome base with radial cells
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	
1. *C. acicularis*	Po + Pa	Po, Ir + Pa	St	St, Cu	Ano	Ano	+	+	+
2. *C. alata*	Ir, Po	Ir, Po	Cu, St	Cu, St	Ani	Ano	+	+	+
3. *C. albida*	Ji	Ji	Un,	Un	Ano	Ano	-	+	+
4. *C. assamica*	Ir, Po	Ir	Cu, St	Cu	Ano, Ani	Ano	-	+	+
5. *C. bracteata*	Ji	Ji	Un	Un	Ano	Ano	-	+	+
6. *C. calycina*	Ir, Po	Ir, Po	Cu, St	Cu, St	Ani	Ano, Ani	+	+	+
7. *C. chinensis*	Ir, Po	Ir, Po	Cu, St	Cu, St	Ani	Ano, Ani	+	+	+
8. *C. dubia*	Ji	Ji	Un	Un	Ani, Ano	Ani, Ano	+	+	+
9. *C. filiformis*	Ir, Po	Ir, Po	Cu, St	Cu, St	Ano	Ano	+	+	+
10. *C. gorrensis*	Ji	Ji	Un	Un	Ano	Ano	+	+	-
11. *C. incana*	Ji	Ji	Un	Un	Ano	Ano	+	+	-
12. *C. juncea*	Ji	Ji	Un	Un	Ano	Ano	+	+	-
13. *C. kurzii*	Ir	Ir	Cu	Cu	-	Ano	+	+	+
14. *C. lejoloba*	Ir, Po	Ji	Cu, St	Un	Ano	Ano	+	+	+
15. *C. medicaginea*	Ji	Ji	Un	Un	Ano	Ano	+	+	-
16. *C. montana*	Ir, Po	Ir	Cu, St	Cu	Ani	Ano	+	+	+
17. *C. neriifolia*	Ir	Ir	Cu	Cu	Ano, Ani	Ano	-	-	-
18. *C. pallida*	Ir	Ji	Cu	Un	Ano	Ano	-	+	-
19. *C. prostrata*	Po	Po	St	Cu	Ano	Ano	+	+	+
20. *C. sessiliflora*	Ir	Ir	Cu	Cu	Ano, Ani	Ano, Ani	+	+	+
21. *C. spectabilis*	Ir, Ji	Ir, Ji	Cu, Un	Cu, Un	Ani	Ano, Ani	-	+	+
22. *C. tetragona*	Ji	Ji	Un	Un	Ano	Ano	+	+	-
23. *C. verrucosa*	Ir, Ji	Ir	Cu, Un	Cu	Ano, Ani	Ano, Ani	+	+	+

Notes: Ani = anisocytic; Ano = anomocytic; Cu = curved; Ir = irregular; Ji = jigsaw-like; Pa = papillae; Po = polygonal; St = straight; Un = undulated; + = present; - = absent

RESULTS

In this study, the anatomical characters of the leaf surfaces were present in Table 2. The figures illustrate the epidermal cells of 12 representative species *viz. Crotalaria acicularis*, *C. assamica*, *C. calycina*, *C. chinensis* (Fig. 1), *C. dubia*, *C. kurzii*, *C. neriifolia*, *C. prostrata* (Fig. 2), *C. sessiliflora*, *C. spectabilis*, *C. tetragona* and *C. verrucosa* (Fig. 3). It was found that epidermal cells in the genus *Crotalaria* are polygonal (Fig. 1A–B, Fig. 2G–H), irregular (Fig. 2C–D, Fig. 3A–B) and jigsaw-like shape (Fig. 2A–B, Fig. 3E–F). Anticlinal walls were straight, curved or undulated. There are nine species that have a jigsaw-like shape and 11 species have irregular shape on both surfaces. There were two types of stomata *viz*. anomocytic and anisocytic (Fig. 1E–F, Fig. 3G–H). Seventeen species were found anomocytic on both surfaces. The papillae were found only in *C. acicularis.* Trichomes were uniseriate and present in all the studied species, except *C. neriifolia*. The trichomes of *C. assamica*, *C. bracteata*, *C. pallida*, and *C. spectabilis* appeared only on the abaxial surface, while the others were present on both surfaces. The base of the trichome was radially distributed cells except for *C. gorrensis*, *C. incana*, *C. juncea*, *C. medicaginea*, *C. pallida*, and *C. tetragona*.

DISCUSSION

The epidermal cells of many species were jigsaw-like with an undulated anticlinal wall. Most Thai *Crotalaria* species have anomocytic stomata. The results are congruent with Niyomdham (1978), who reported that stomata are found on both surfaces of the leaf in *C. pallida*, *C. spectabilis*, and *C. verrucosa*. The only difference was that for *C. incana*, Niyomdham (1978) reported stomata are only on the adaxial surface, but in this study anomocytic stoma were found on both surfaces. This genus has non-glandular and uniseriate trichomes, which is agree with previous reports (Niyomdham, 1978; Parveen et al., 2000; Devecchi et al., 2014). Moreover, cells at the base of trichomes were observed and found to have the same characteristics as reported by Devecchi et al. (2014).

The anatomical characters can be used for characterizing some species. The presence of papillae is a diagnostic feature of *C. acicularis* (Fig. 1A–B).

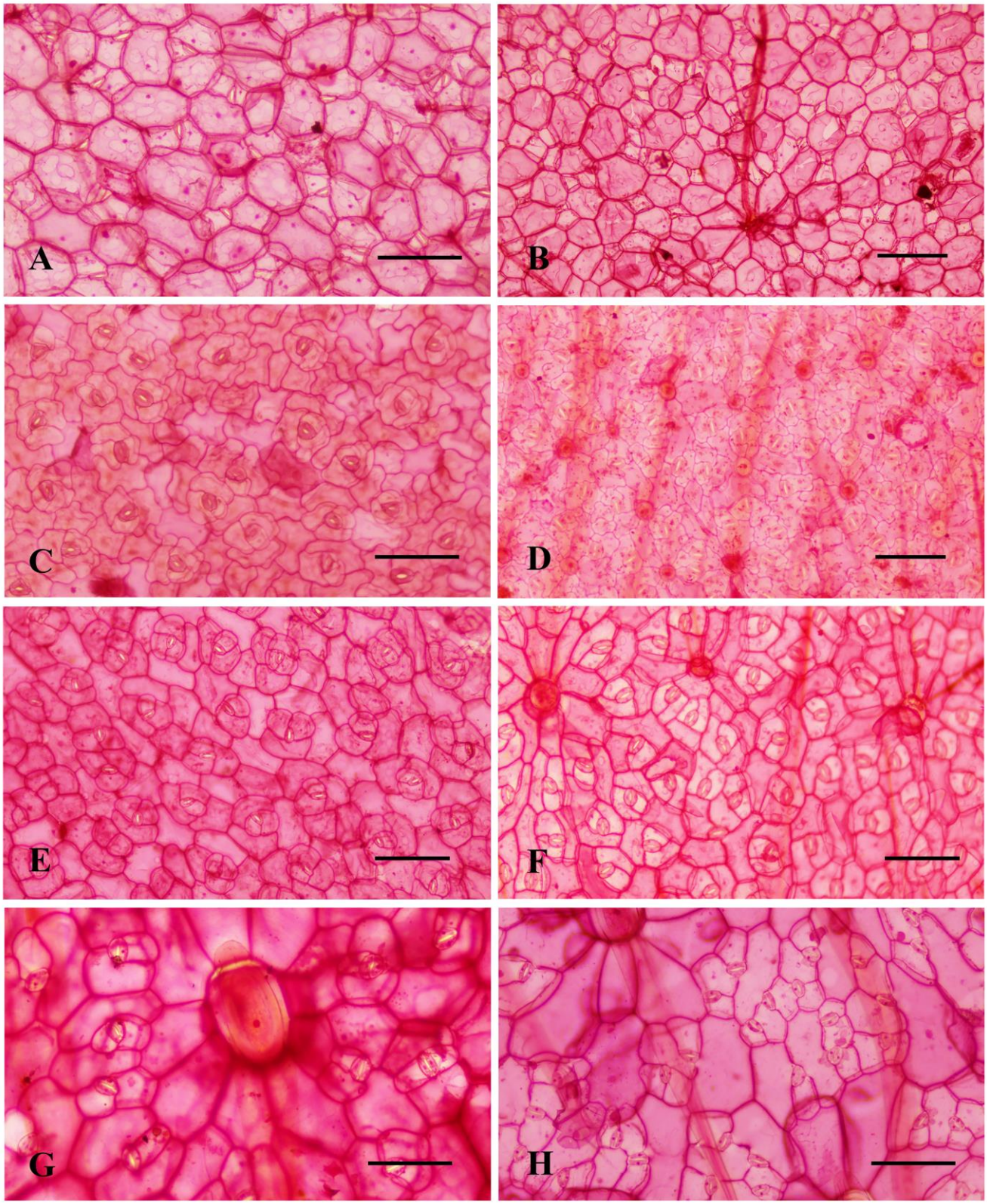

FIGURE 1. The epidermal cells on adaxial surface (A, C, E, G) and abaxial leaf surface (B, D, F, H) of *Crotalaria*: A–B) *C. acicularis*; C–D) *C. assamica*; E–F) *C. calycina*; G–H) *C. chinensis*; Scale bars: 50 µm.

Crotalaria neriifolia is the only species that does not have trichomes on either surface (Fig. 2E–F). The results also indicated that the anatomical properties of this genus cannot be used for the delimitation of all species, but these characters do provide important taxonomic characters for the classification and

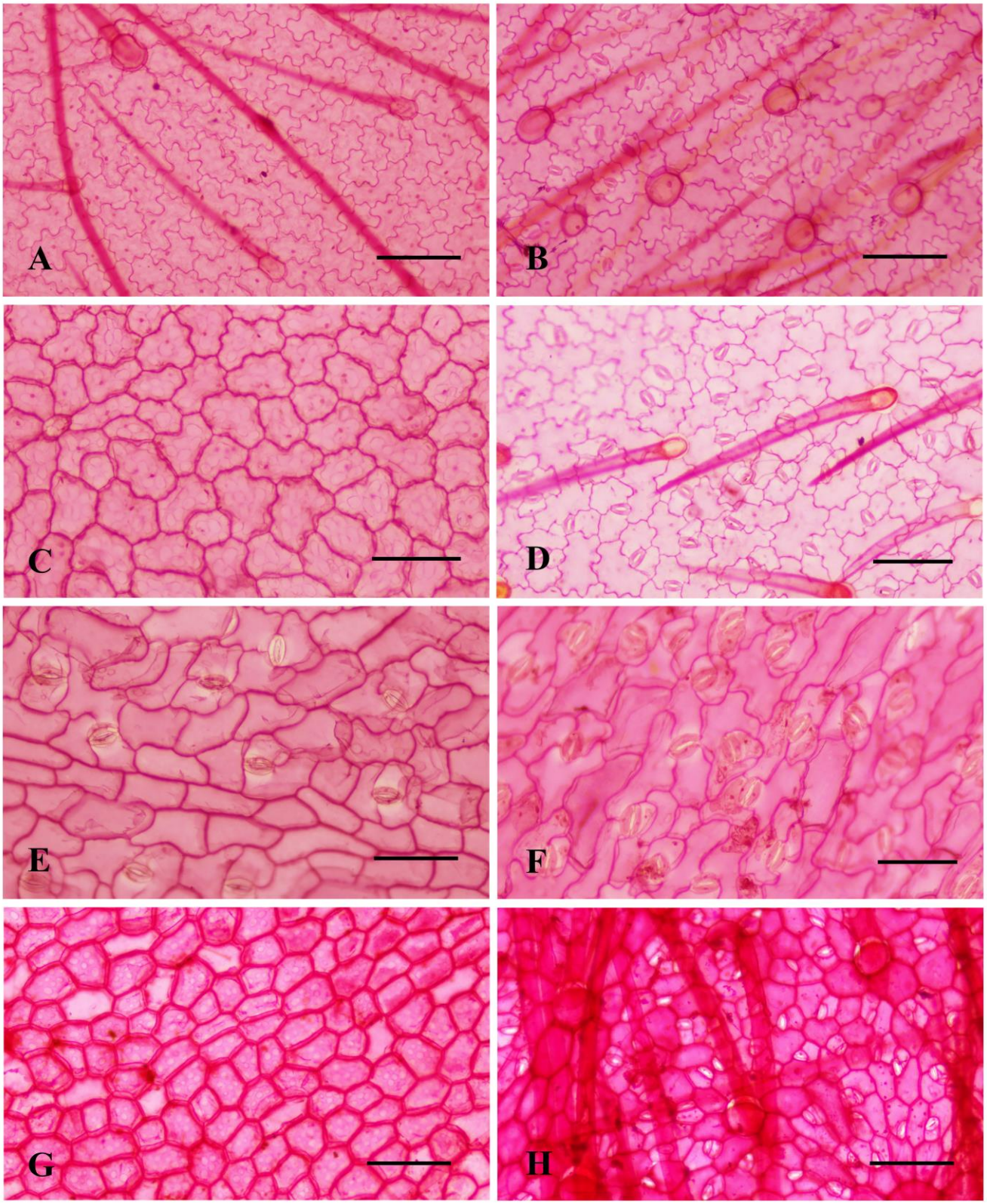

FIGURE 2. The epidermal cells on adaxial surface (A, C, E, G) and abaxial leaf surface (B, D, F, H) of *Crotalaria*: A–B) *C. dubia*; C–D) *C. kurzii*; E–F) *C. neriifolia*; G–H) *C. prostrata*; Scale bars: 50 µm.

identification of some species pairs. For example, *C. albida* and *C. montana* that are erect herbs with oblong leaves and sharing similar floral traits. Both species can be separated by the epidermal cell shape and the position of the trichome. The epidermal cells of *C. albida* are a jigsaw-like shape, and the trichomes are

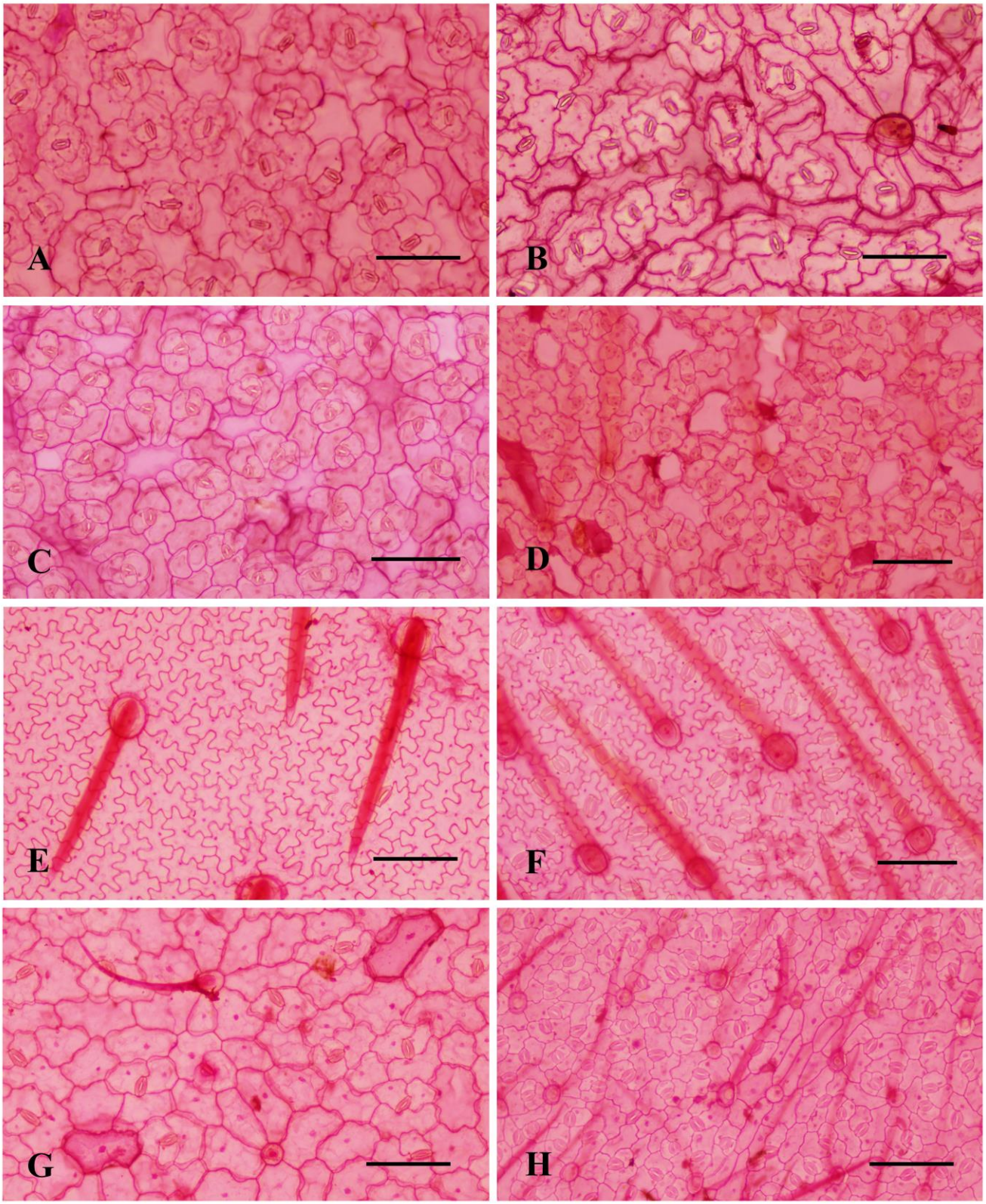

FIGURE 3. The epidermal cells on adaxial surface (A, C, E, G) and abaxial leaf surface (B, D, F, H) of *Crotalaria*: A–B) *C. sessiliflora*; C–D) *C. spectabilis*; E–F) *C. tetragona*; G–H) *C. verrucosa*; Scale bars: 50 µm.

present on the abaxial surface only. On the other hand, the epidermal cells of *C. montana* are irregular and polygonal, and trichomes are present on both surfaces. For *C. gorrensis* and *C. pallida*, both species have quite the same size of trifoliolate leaves, inflorescences and flowers. The jigsaw-like shape epidermal cell and

trichome are present on the adaxial surface of *C. gorrensis*, while *C. pallida* has an irregular shape epidermal cell and no trichome. For *C. assamica* and *C. spectabilis*, their leaves, flowers, and pods are similar but these species can be differentiated by their epidermal cells; *C. assamica* has a polygonal to slightly irregular shape on the adaxial surface and an irregular shape on the abaxial surface (Fig. 1C–D), while *C. spectabilis* has an irregular to jigsaw-like shape on both surfaces (Fig. 3C–D). The final species pair is *C. chinensis* and *C. lejoloba*. Both species are similar in habits, leaves, and flowers. They can be separated by the epidermal cells. The epidermal cells of both species are irregularly shaped on the adaxial surface, but *C. chinensis* has an irregular and polygonal shape on the abaxial surface (Fig. 1G–H), while *C. lejoloba* has a jigsaw-like shape.

In conclusion, anatomical characteristics cannot be used for species identification, however the shape and anticlinal wall of the epidermal cell and the position of the trichome can be used to determine some species pairs.

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