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Full Length Research Paper

Studies of the leaf and floral anatomy of two species of *Ixora*

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Floral and epidermal characters of two species of *Ixora* were studied. The aim was to establish the useful diagnostic feature that may be employed in combination with other characters as intra and inter-specific or generic tools for their delimitation. The flowers of *Ixora coccinea* were hypostomatic while *Ixora finlaysoniana* flowers were hypoamphistomatic. The matured stomata types were anisocytic, anomocytic, staurocytic, paracytic, laterocytic, brachyparacytic and hemiparacytic stomata. *I. coccinea* leaf and flower are different from *I. finlaysoniana* in having hemiparacytic stomata in the abaxial surfaces of the leaf and flower. Parallel contiguous stomata occurred only in *I. finlaysoniana* leaf while it was absent in *I. coccinea.* Long unicellular trichome were distributed on adaxial surface of *I. coccinea.* The shape of the anticlinal cell wall, stomatal index, guard cell area can be used to distinguish the species.

Key words: Epidermal, *Ixora* species, hypostomatic, hypoamphistomatic, trichome, stomata, anticlinal cell wall.

INTRODUCTION

Ixora is a genus of flowering plant in the Rubiaceae family. There are about 500 species (Nayak et al., 1999). Rubiaceae are easily recognizable and characterized by opposite leaves that are simple and entire with interpetiolar stipules, tubular sympetallous corollas and an inferior ovary. Eventually there are some plants that have only one single leaf at each node, alternating from one side to the other. In these cases, the alternate leaf arrangement is produced through the suppression of one leaf at each node (Takhtajan, 2009). *Ixora finlaysoniana* and *Ixora coccinea* are shrubs or small trees. *Ixora i*s a genus of flowering plants in the Rubiaceae family which contains more than two species but two of them are considered in this research, and they are *I. finlaysoniana* L. and *I. coccinea* L.

I. coccinea beside its obvious value are used to make wreaths (Sofowora, 2008). The roots, leaves and flowers of the plant are ethnomedically very important. Leaves are useful to cure jaundice and the flowers are used to cure conjuctivities and asthma (Beg et al., 2005). They are one of the medicinal plants with therapeutic agent (Burkill, 2000).

The dilute tinctures of roots are used for mouth wash and gargles for sore throat (Burkill, 2000). *I. finlaysoniana* root are given with cow fresh milk in white leprosy. The decoration and ornament are used for hedges. In the Philippine, root decoction is used as sedative in the treatment of nausea, hiccups and loss of appetite, fever and gonorrhea (Yasmeen et al., 2010). In traditional medicine, the leaves and root are used to treat a wide variety of ailments like heap for protective, chemo protective, antimicrobial antioxidant, antinociceptive, anti-inflammatory, dysentery, ulcers and gonorrhea (Benkeblia, 2004).

Pharmacologically, the leaves are reported for their anti-microbial anti-diarrheal and antinociceptive activities (Annapurna et al., 2003). The flower possesses antioxidant, anti-inflammatory, wound healing activities (Nayak et al., 1999). Leaves are given in diarrhoea and also used in treatment of dysentery, leucorrhoea, dysmenorrhoea, hemoptysis and catarrhal bronchitis (Ghani, 2003). In the traditional medicine of India, infusions of the leaves and juice from the roots are used

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Table 1. Epidermal features of the leaves of *Ixora coccinea* and *Ixora finlaysonia*.

Species	Species stomatal size (µm)				Epidermal cells				Guard cell area (µm^2)		Stomatal index (%)		Stomatal distribution			Non-glandular trichome (µm)	
	Ad		Ab		Ad		Ab		Ad	Ab	Ad	Ab	Ad	Ab		Ad	Ab
	L	B	L	B	L	B	L	B									
Ixora coccinea	-	-	44	26	60	26	60	23	-	10	-	33	Hypostomatic	Slightly undulate	Slightly sinous anticlinal walls	-	-
Ixora finlaysonia	-	-	39	13	39	26	65	21	-	10	-	30	Hypostomatic	Straight to sinous anticlinal walls	Slightly sinous walls	-	-

to treat a wide variety of ailments including dysentery, ulcers and gonorrhoea (Parretta, 2001).

Patil and Patil (2011) reported on the investigations of foliar epidermis in some Rubiaceae. The foliar epidermal features of Rubiaceae have been documented by Metcalfe and Chalk (1979). There are further studies by different workers, Mathew and Sucarajan (1987), Viera et al. (2001), Rathmakumari et al. (2002) and Tarsila et al. (2009). The ethanolic extract of the plant wax proved to have estrogenic abortifacient and anti-implantatient effects (Singh et al., 1993).

The present study described the leaf and floral anatomy of two species of *Ixora*, with the aim of providing useful taxonomic data that would give further insight into proper and easy identification and an attempt to fill in the gap in our knowledge.

MATERIALS AND METHODS

The fresh leaves and flowers of *I. coccinea* and *I. finlaysoniana* were collected in October 2012 from a farmland in Uyo Local Government Area, Akwa Ibom State. The plant was authenticated by Dr. (Mrs.) U. A. Essiett in the Department of Botany and Ecological studies, Faculty of Science, University of Uyo. Anatomical studies were carried out using the method below:

Microscopic examination

For the purpose of anatomical studies, small sizeable portion was obtained from the standard median part of the mature leaves and flower. Epidermal peel of both abaxial and adaxial was surface taken from standard median portion of the leaves and flower on the clean glass slab.

The specimen surface to be studied was placed facing down, and was irrigated with water holding down the specimen from the end. The epidermis above the desired surface was scraped off carefully with a sharp razor blade. Loose cells were washed away from the epidermal peel with the soft carmel hair brush and watered until the desired epidermis below was reached. The epidermal peels were stained with aqueous 1% solution of Safranin for 4-8 min and rinsed carefully in water to remove excess stain and then mounted in 10% glycerol.

Guard cell area was calculated by Francos constant method (Guard cell area = length × width × 0.7854). The stomata index was determined according to Metcalfe and Chalk (1979), using the formula:

$$\frac{S}{E+S} \times \frac{100}{1} = \text{Stomata index (SI)}$$

Where: S = Number of stomata per unit area; E = number of epidermal cell in the same area.

All measurements were made with the aid of an ocular micrometer and finally converted by the ocular constant with respect to the power under which they were taken.

RESULTS

Epidermal cell

The epidermal cell shape seems to be determined by the pattern of its wall and the species vary remarkably in shape and size. The largest cells were observed in adaxial surface of the *I. coccinea* flower while *I.* finlaysoniana flower adaxial surface recorded the smallest cells (Table 2, (Plate 1G). The epidermal cells are slightly sinuous, undulated, while some are straight to sinuous (Table 1) in the two species investigated (Plate 1E and 2N).

Stomata

The distribution of stomata in the investigated taxa is hypostomatic (stomata absent on the adaxial and present on the abaxial) of the leaves of *I. coccinea* and *I.* finlaysoniana, while hypoamphistomatic (stomata abundant on abaxial and scanty on adaxial surfaces) of the flowers of *I.*

Table 2. Epidermal features of the flowers of *Ixora coccinea* and *Ixora finlaysonia*.

Species	Species stomatal size (µm)				Epidermal cells				Guard cell area (µm^2)		Stomatal index (%)		Stomatal distribution		Epidermal cell wall		Non-glandular trichome (µm)			
	Ad		Ab		Ad		Ab		Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad		Ab	
	L	B	L	B	L	B	L	B												
Ixora coccinea	29	21	31	23	80	29	42	26	15	19	16	27	Hypostomatic		Slightly sinous anticlinal walls	Slightly undulate anticlinal walls	50	11	-	-
Ixora finlaysonia	31	10	29	13	23	13	34	18	21	13	29	54	Hypoamphistomatic		Slightly undulate	Sinous anticlinal walls	-	-	-	-

Keys: Ad - Adaxial surface (upper); Ab - Abaxial surface (lower); L - Length; B - Breadth.

coccinea and *I. finlaysoniana* respectively (Table 2). The mature stomatal types were anisocytic, anomocytic, staurocytic, paracytic, laterocytic, hemiparacytic and brachyparacytic (Plate 1A, C, D, E and F) and (Plate 2B, C). *I. coccinea* are distinguished from *I. finlaysoniana* in having hemiparacytic stomata in the abaxial surfaces of the leaf and flower (Plate 2A). Stomatal index varied between the two species on the abaxial surfaces of the leaves and the both surfaces of the flowers (Table 1). The highest stomatal index was found on the abaxial surface of *I. finlaysoniana* flower (54%) and the lowest stomata index were recorded on the adaxial surface of the *I. coccinea* flower (16%). There are various abnormal stomata structures observed which include unopened stomata pore (Plate 1B), one guard cell (Plate 2D), two stomata sharing one subsidiary cell (Plate 2M) and parallel contiguous stomata (Plate 1G). Parallel contiguous stomata were present only on the abaxial surface of *I. finlaysoniana* leaf, one guard cells are found on the surface of *I. finlaysoniana* leaf and abaxial surface of *I. coccinea* respectively, while two stomata sharing one subsidiary cell are found only on abaxial surface of *I. coccinea* leaf (Plate 2M).

Hairs

Non glandular were present in only abaxial surface of *I. coccinea* flower (Table 1, Plate 4K), but absent in other surfaces.

DISCUSSION

The taxonomic value of epidermal feature has been documented in literature where they have been used to delimit a number of plant taxa (Kadiri and Ayodele, 2003; Kotresha and Seetharam, 2000; Ogundipe and Wujek, 2004).
The epidermal cell size varies significantly and can be fairly used for the separation of the two species studied. *I. coccinea* flower has the largest epidermal cell size (Table 2). The presence of abnormal stomata with unopened stomata pore (Plate 4H), one guard cell (Plate 2F) and two stomata sharing one subsidiary cell (Plate 2M) observed in this study can be used to distinguish the two species. The present of parallel contiguous stomata on the leaf of *I. finlaysoniana* distinguished it from the other species and flower. There is no evidence to suggest that such stomata abnormalities are the result of disease factor. Dehnel (1960) has induced stomatal degeneration by wounding in *Begonia* species but *Ixora* species extensively showed single guard cell and degenerated stomatal cells in their leaves and flowers which have been found to be quite normal and healthy. The cause of these abnormalities in nature as in many Rubiaceae family is yet to be determined (Patil and Patil, 2011). The mature stomatal types and epidermal cell structure appeared to be in different forms in the investigated members of the genus *I. coccinea* and *I. finlaysoniana* flowers which are hypoamphistomatic and which could be one of the adaptive features that partly account for its wide distribution of wild species (Essiett and Akpabio, 2003). The function of stomata is associated with various physiological processes and with the success of plant individuals (Essiett, 2004). It is expected that stomatal structure will be under strong and highly integrated genetic control and that modification of stomata during evolution would reflect both general relationship and evolutionary trend in the two species studied, different in each other in having hemiparacytic stomata on abaxial surface of the leaves (Plate 2A, K) and flower of *I. coccinea* with abundance paracytic stomata present (Plate 4D). But in addition, staurocytic, brachyparacytic,laterocytic, anisocytic and anomocytic are present as shown by Patel and Inamdar (1971). In some *Polemoniales*, Essiett and Akpabio (2009), on the species of *Talinum*, showed that it is possible for most species to have more than one type of stomata. In this study, stomatal index (SI) is highly constant for any given species (Olatunji, 1983,

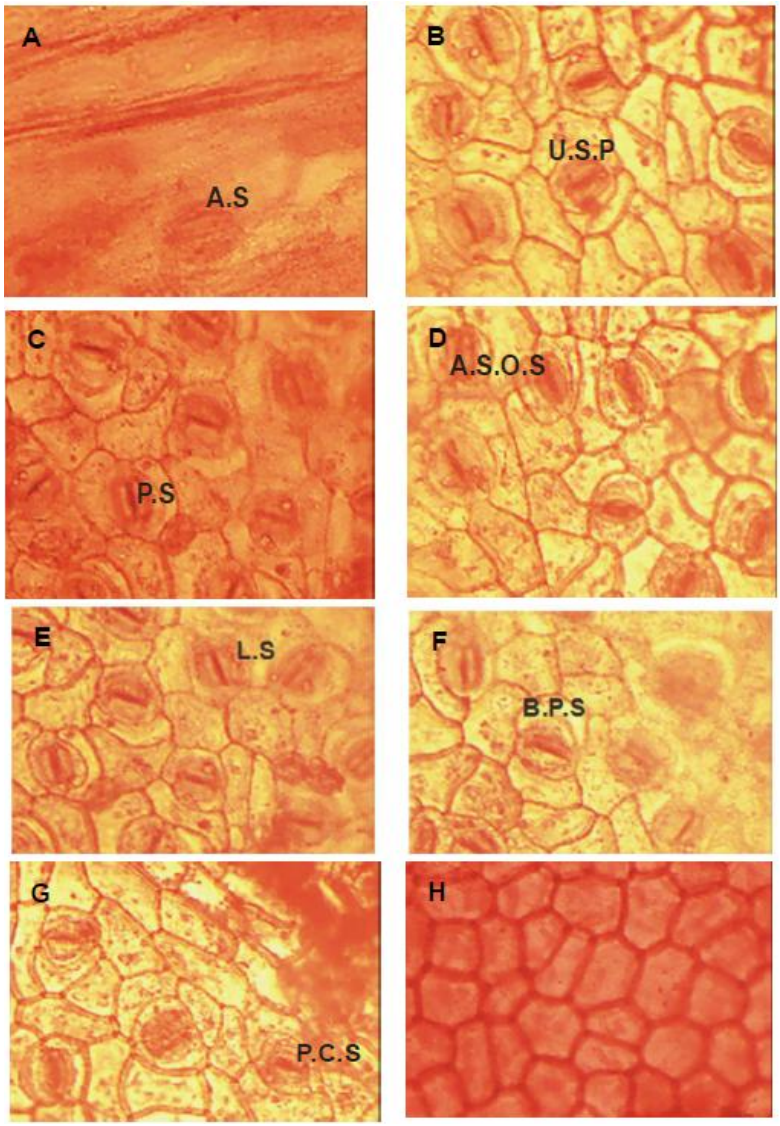

Plate 1A. (A.S): Anisocytic stomata of *I. finlaysoniana* (abaxial surface) × 400; **1B:** (U.S.P): Unopened stomatal pore of *I. finlaysoniana* leaf (abaxial surface) × 400; **1C:** (P.S): Paracytic stomata of *I. finlaysoniana* leaf (abaxial surface) × 400; **1D:** (A.S.O.S): Anomocytic stomata of *I. finlaysoniana* leaf (abaxial surface) × 400; **1E:** (L.S): Laterocytic stomata of *I. finlaysoniana* leaf (abaxial surface) × 400; **1F:** (D.P.S): Brachyparacytic stomata of *I. finlaysoniana* leaf (abaxial surface) × 400; **1G:** (P.C.S): Parallel contiguous stomata of *I. finlaysoniana* leaf (abaxial surface) × 400; **1H:** Epidermal cell of *I. finlaysoniana* leaf (abaxial surface) × 400.

Adedeji; Jewoola, 2008). Stomatal index vary between the two species on the abaxial surface of the leaves and on both adaxial and abaxial surface of the flowers. The higher stomatal index was found in the flower of *I. finlaysoniana* (54.0) while the lowest was found on the flower of *I. coccinea* (16.0). It is useful for the separation of *I. coccinea* and *I. finlaysoniana* (Table 2). In line with the findings of Abdulrahaman and Oladele (2003) and Isawumi (1986), the guard cell area provides values that would save as parameters for comparison among taxa. The unicellular hairs in them help to reduce the rate of transpiration in the plant. The importance of trichome in taxonomy has been highlighted in the family Combretaceae. The non-glandular trichomes on adaxial surface of *I. coccinea* (red flower) have distinguished the two species studied.

Conclusion

The study has attempted to highlight anatomical features

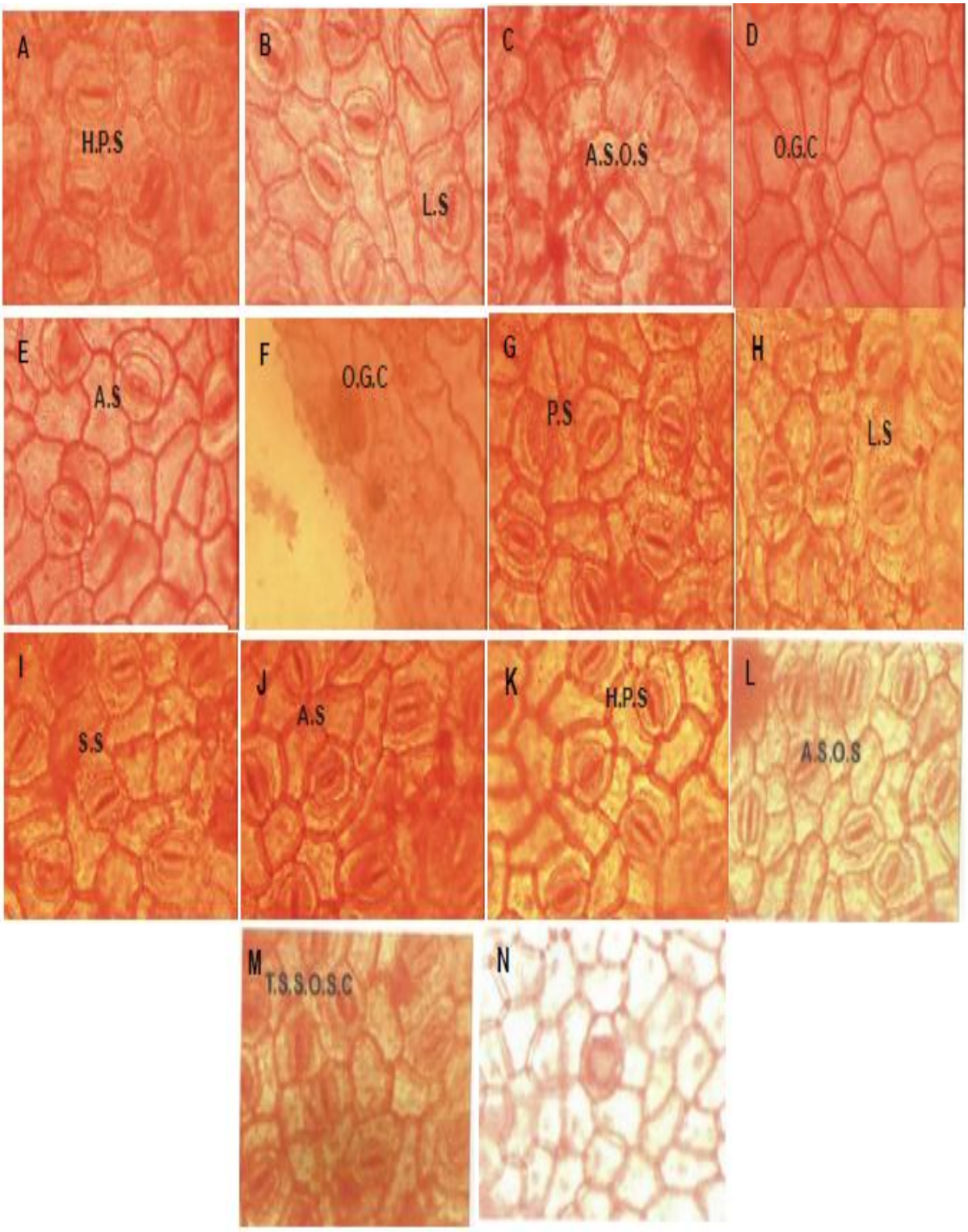

Plate 2A. (H.P.S): Hemiparacytic stomata of *I. coccinea* leaf (abaxial surface) × 400; **2B:** (L.S): Laterocytic stomata of *I. coccinea* leaf (abaxial surface) × 400; **2C:** (A.S.O.S): Anomocytic stomata of *I. coccinea* leaf (abaxial surface) × 400; **2D:** (O.G.C): One guard cell of *I. coccinea* leaf (abaxial surface) × 400; **2E:** (A.S): Anisocytic stomata of *I. coccinea* leaf (abaxial surface) × 400; **2F:** (O.G.C): One guard cell of *I. coccinea* leaf (abaxial surface) × 400; **2G:** (P.S): Paracytic stomata of *I. coccinea* leaf (abaxial surface) × 400; **2H:** (L.S): Laterocytic stomata of *I. coccinea* leaf (abaxial surface) × 400; **2I:** (S.S): Starocytic stomata of *I. coccinea* leaf (abaxial surface) × 400; **2J:** (A.S): Anisocytic stomata of *I. coccinea* leaf (abaxial surface) × 400; **2K:** (H.P.S): Hemiparacytic stomata of *I. coccinea* leaf (abaxial surface) × 400; **2L:** (A.S.O.S): Anomocytic stomata of *I. coccinea* leaf (abaxial surface) × 400; **2M:** (T.S.S.O.S.C): Two stomata shared one subsidiary of *I. coccinea* leaf (abaxial surface) × 400; **2N:** Epidermal cell of *I. coccinea* leaf (abaxial surface) × 400.

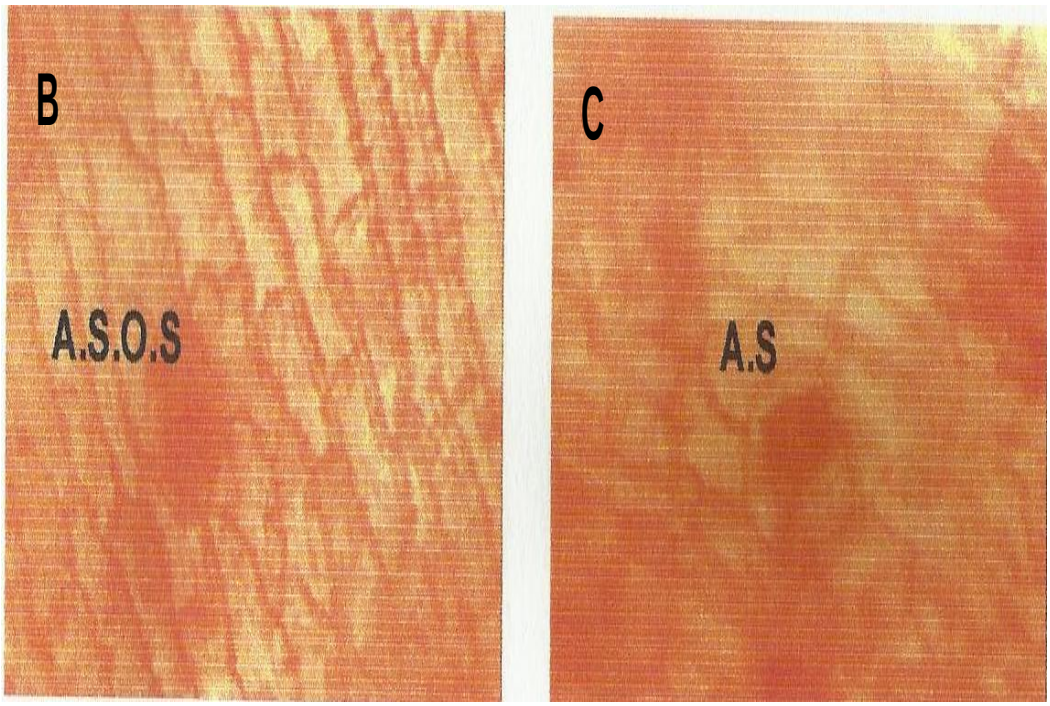

Plate 3A. (S.S): Staurocytic stomata of *I. finlaysoniana* flower (abaxial surface) × 400; **3B:** (A.S.O.S): Anomocytic stomata of *I. finlaysoniana* flower (abaxial surface) × 400; **3C:** (A.S): Anisocytic stomata of *I. finlaysoniana* flower (abaxial surface) × 400.

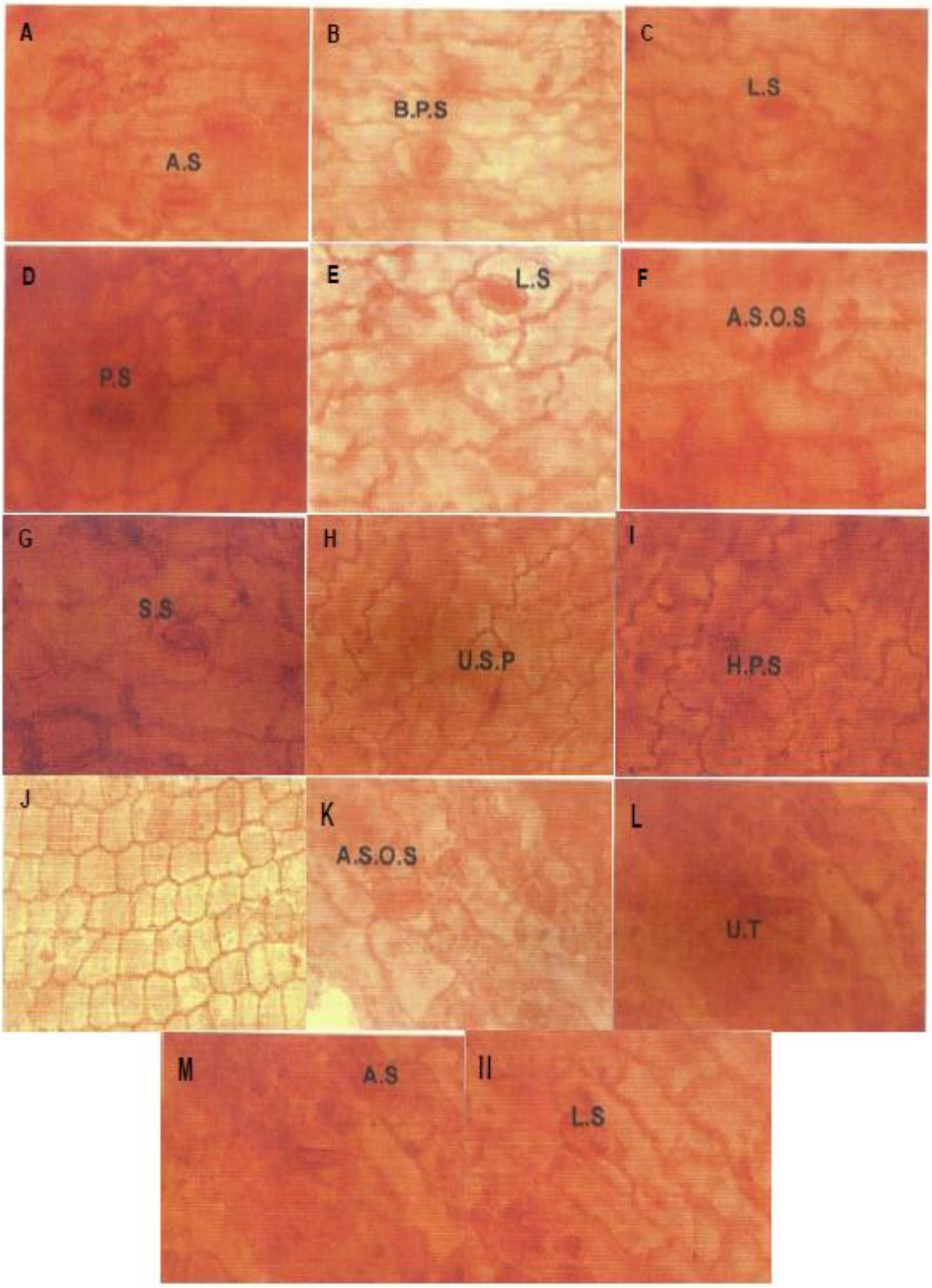

Plate 4A. (A.S): Anisocytic stomata of *I. coccinea* flower (abaxial surface) × 400; **4B:** (B.P.S): Brachyparacytic stomata of *I. coccinea* flower (abaxial surface) × 400; **4C:** (L.S): Laterocytic stomata of *I. coccinea* flower (abaxial surface) × 400; **4D:** (P.S): Paracytic stomata of *I. coccinea* flower (abaxial surface) × 400; **4E:** (L.S): Laterocytic stomata of *I. coccinea* flower (abaxial surface) × 400; **4F:** (A.S.O.S): Anomocytic stomata of *I. coccinea* flower (abaxial surface) × 400; **4G:** (S.S): Starocytic stomata of *I. coccinea* flower (abaxial surface) × 400; **4H:** (U.S.P): Unopened stomata pore of *I. coccinea* flower (abaxial surface) × 400; **4I:** (H.P.S): Hemiparacytic stomata of *I. coccinea* flower (abaxial surface) × 400; **4J:** Epidermal cell of *I. coccinea* flower leaf (abaxial surface) × 400; **4K:** (A.S.O.S): Anomocytic stomata of *I. coccinea* flower (abaxial surface) × 400; **4L:** (U.T): Unicellular trichomes of *I. coccinea* flower (abaxial surface) × 400; **4M:** (A.S): Anisocytic stomata of *I. coccinea* flower (abaxial surface) × 400; **4N:** (L.S): Laterocytic stomata of *I. coccinea* flower (abaxial surface) × 400.

in the two species. The *I. coccinea* and *I. finlaysoniana* of the leaves and flowers have been distinguished on the basis of the presence of epidermal cell size, stomatal index and guard cell area and be used for identification and collection of these species.

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