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Structure and Ontogeny of Stomata in Vegetative and Floral Organs of Thunbergioideae (Acanthaceae)

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Structure and ontogeny of stomata in vegetative and floral organs of six species of *Thunbergia* are described. Diacytic stomata on both vegetative as well as floral organs show syndetocheilic or mesogenous type of ontogeny. Based on the results, the sub-family status of Thunbergioideae within the family Acanthaceae is justified.

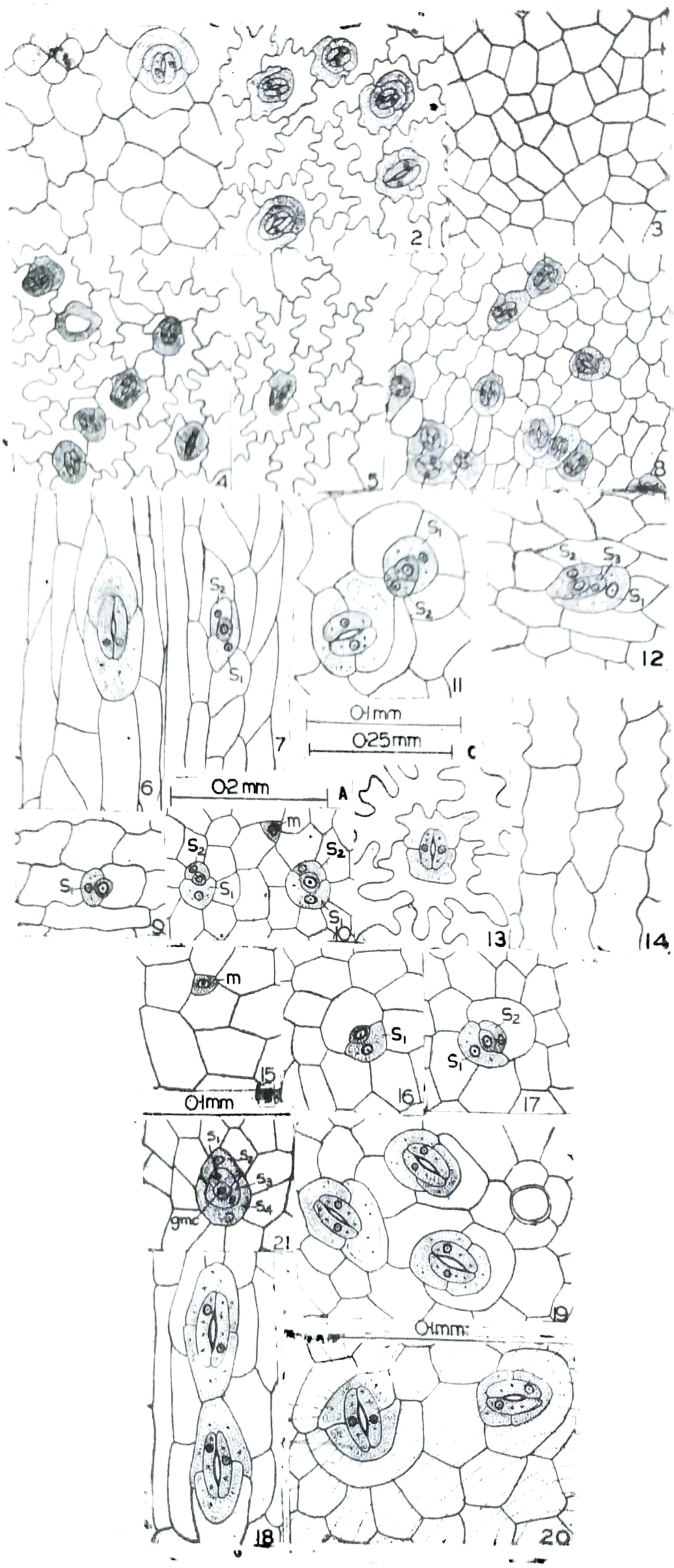
Key Words - Floral organ Ontogeny Sinnosity Stomata Wall

Inamdar (1970), Ahmad (1974d) and Kumar & Paliwal, (1975) have studied the mature structure of foliar stomata in some species of *Thunbergia* and ontogeny of stomata of foliar epidermis of *T. erecta* (Paliwal, 1966). However, no attempt has been made earlier to study the structure and development of stomata from other vegetative and floral organs. It is of interest, to study the structure and development of stomata on the leaf, stem, bracteole, corolla, stamen and pericarp in 6 species of *Thunbergia*: *T. affinis* S. Moore, *T. alata* Boj. ex. Sims., *T. erecta* T. Anders, *T. fragrans* Roxb., *T. grandiflora* (Roxb. ex. Rottl.) Roxb. and *T. laurifolia* Lindl.

MATERIALS & METHODS Materials of 6 species were collected locally or procured from different places. The material was fixed in F.A.A. and stored in 70% ethanol. The epidermal peels were obtained mechanically or by following the technique of Boulos & Beakbane (1971). Peels for developmental studies were stained with Delafield's haematoxylin and mounted in glycerine.

OBSERVATIONS Mature epidermis **The leaves are hypostomatic except in *T. fragrans* and *T. grandiflora*. In amphistomatic leaves, the stomata are more on the abaxial than on the adaxial surface. Entire surface of stem is stomatiferous. Epidermis of corolla and the stamens is non-stomatiferous (Fig. 14). In dorsiventral organs such as leaves and bracteoles stomata are evenly distributed without any definite pattern of orientation though occasionally they are arranged in distant groups as in the abaxial bracteole epidermis of *T. grandiflora* (Fig. 8). In stem epidermis, stomata are arranged in the direction of the long axis of the organ (Figs. 6, 7). In the pericarp epidermis, stomata are randomly distributed (Figs. 19, 20) but in the beak portion of the fruit, stomata are arranged with their long axis oriented parallel to the radially elongated cells (Fig. 18).**

The foliar epidermal cells in *T. affinis* are polygonal with straight anticlinal walls on adaxial surface and sinuous anticlinal walls on abaxial surface (Figs. 3, 4). In other species, they are irregular with sinuous anticlinal walls on both the surfaces but sinuousities are more pronounced on the abaxial surface (Figs. 1, 2). Epidermal cells of stem are large, radially elongated with straight walls (Figs. 6, 7). Walls of epidermal cells in the bracteoles are slightly arched in *T. erecta* and *T. grandiflora* (Figs. 8-10), greatly sinuous in *T. alata* (Fig. 13) and straight in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T. fragrans (Figs. 11, 12). Epidermal cells of pericarp are small, polygonal with straight walls while epidermal cells in the beak portion of the fruit are radially elongated (Figs. 18-20). A thick cuticle with prominent striations is observed in the pericarb epidermis of *T. fragrans* (Fig .20).

The mature stomata are diacytic in the different vegetative as well as the floral organs. The stomata may be monocylic having two subsidiary cells with their common walls placed at right angles to the guard cells, or incompletely amphicyclic having a third subsidiary cell or amphicyclic with two rings of two subsidiary cells each (Figs. 1,2,4,6,8,13, 18-20). Although contiguous stomata are absent, groups of two or three stomata are commonly observed (Figs. 8. 18) Stoma with single guard cell with or without pore (Fig. 5), stoma with aborted guard cells (Fig. 4) and arrested development (Fig. 4) have been observed. Variations have been noted in the average number of stomata mm^{-2} and stomatal indices in the vegetative and floral organs. The size of stomata is generally greater than those occurring in floral organs (Table 1).

Ontogeny of Stomata : Developmental stages of stomata are found along with mature stomata on vegetative and floral organs. In all cases, meristemoid is cut off from a protodermal cell by the formation of straight or curved wall and is small, triangular or lenticular in shape with densely staining cytoplasm (Figs. 10, 15). The meristemoid divides transversely forming a larger and a smaller daughter cell. The former does not divide further becoming the first subsidiary cell while the latter divides unequally into two cells, the larger one becoming the second subsidiary cell (Figs. 7, 9-11, 16, 17) Two simitar divisions in the smaller cell after each division produce the third and fourth subsidiary cells resulting in two concentric rings of two subsidiary cells each (Fig. 21).

The inner ring of subsidiary cells surrounds a central much smaller cell which now acts as the guard cell mother cell (Fig. 21). The guard cell mother cell divides vertically to form the two guard cells of equal size. Pant & Mehra (1963) term the outer ring of subsidiary cells as encircling subsidiary cells in *Asteracantha longifolia*. Thus in this sequence of development, it is always the smaller cell which retains the meristematic activity and goes on dividing repeatedly. Suppression of one or two divisions before the formation of guard cells results in the formation of stomata with three or two subsidiary cells, respectively. As both guard cells and the subsidiary cells, originate from the same meristemoid, the diacytic stomata are mesogenous (Pant, 1965)

Fig. 1-14. 1,2. Portions of adaxial and abaxial intercostal leaf epidermides of *Thunbergia fragrans*. 3 & 4. Portions of adaxial and abaxial intercostal leaf epidermides of *T. affinis*. Note stomata with aborted guard cells and with arrested development in Fig. 4.5. A portion of abaxial intercostal leaf epidermis of *T. erecta* showing a stoma with single guard cell. 6 & 7. Portions of stem epidermides of *T. alata*. Note developmental stage of stomata in Fig. 7.8. A portion of abaxial bracteole epidermis of *T. grandiflora* 9 & 10. Portions of abaxial bracteole epidermides showing developmental stages of stomata in *T. erecta*. 11 & 12. Portions of abaxial bracteole epidermides of *T. fragrans*. showing developmental stages of stomata. 13. A portion of abaxial bracteole epidermis *T. alata*. 14. A portion of corolla abaxial epidermis of *T. alata*. 15-17, 21. Portions of pericarp epidermides of *T. grandiflora* showing developmental stages of stomata. 18 & 19. Portions of pericarp epidermides *T. alata* from beak and body portions of the fruit respectively. 20. A portion of pericarp epidermis of *T. fragrans* showing prominent cuticular striations.

(m = meristemoid; S_1 to S_4 -subsidiary cells; gmc - guard cell mother cell) Scale A for Fig. 1-5; Scale B for Fig. 6,7, 9-14, Scale C for Fig. 8. Scale A for Fig. 15-17, 21; Scale B for Fig. 18-20.

Table 1 Epidermal Characteristics of *Thunbergia*

Species	Organ	Epidermal Cells, shape and size (μ)	:Stomata size(μ)	;Stomata frequency per mm²	:Stomatal Index.
Thunbergia affinis	Leaf	Ad Polygonal, walls straight; 73x31	— —		
		Ab Irregular, walls sinuous; 79x46	28x18	135	31.0
	Bracteole	Ab Irregular, walls very sinuous; 82x54	31x20	60	20.4
T. alata	Leaf	Ad Irregular, walls sinuous; 73x44	— —		
		Ab Irregular, walls very sinuous; 88x40	25x17	135	33.0
	Stem	Elongated, walls straight; 63x14	26x17	65	6.4
	Bracteole	Ab Irregular, walls very sinuous; 37x27	23x17	25	5.2
	Pericarp (outer)	Polygonal, walls straight; 86x17	32x21	110	26.4
	Pericarp (Beka, outer)	Elongated, walls straight; 99x20	49x20	75	22.6
T. erecta	Leaf	Ad Irregular, walls sinuous; 86x69	— —		
		Ab Irregular, walls very sinuous; 121x75	25x17	50	19.0
	Stem	Elongated, walls straight; 72x17	31x20	50	18.8
	Bracteole	Ab Elongated, walls sinuous; 75x20	38x24	60	20.8
T. fragrans	Leaf	Ad Irregular, walls sinuous; 93x58	32x21	65	24.7
		Ab Irregular, walls very sinuous; 109x55	36x23	65	24.0
	Bracteole	Ab Irregular, walls slightly sinuous; 92x27	34x24	65	7.2
	Pericarp (outer)	Polygonal, walls straight; 55x38	41x24	50	6.4
	Pericarp (Beak, outer)	Elongated, walls straight; 103x24	37x20	65	5.8
T. grandiflora	Leaf	Ad Irregular, walls sinuous; 69x58	22x40	25	6.3
		Ab Irregular, walls very sinuous; 71x43	26x16	235	43.0
	Bracteole	Ab Irregular, walls sinuous; 79x55	41x24	75	21.2
	Pericarp (outer)	Polygonal, walls straight; 31x20	34x20	65	6.3
T. laurifolia	Leaf	Ad Irregular, walls sinuous; 61x54	— —		
		Ab Irregular, walls very sinuous; 79x44	23x15	225	40.0

— Absent, Ab — Abaxial surface, Ad — Adaxial surface
Numerical values represent average of 5 readings.

or syndetocheilic (Florin, 1931, 1933) in their development.

DISCUSSION The family Acanthaceae, is uniformly characterized by diacytic stomata on the leaves (Metcalfe & Chalk, 1950; Pant & Mehra, 1963; Paliwal, 1966; Inamdar, 1970; Ahmad, 1974a, b,c,d, 1975, 1976). Murty & Varma (1976) and Varma (1978), however, reported occasional occurrence of anomocytic, paracytic, haplocytic, helicocytic and transitional types along with predominantly occurring diacytic type in some Acanthaceae. The present study on 6 species of *Thunbergia* has revealed that stomata occurring on both vegetative as well floral organs are of the diacytic type.

Pant & Mehra (1963) observed that the caryophyllaceous stomata in *Asteracantha longifolia*, having two subsidiary cells in the inner ring and two encircling subsidiary cells in the outer ring, are formed by five successive mitotic divisions of the meristemoid following syndetocheilic mode of development (Florin, 1931, 1933). They further added that the incompletely amphicyclic or monocyclic stomata are formed as a result of suppression of one or two divisions before the formation of the guard cells. Paliwal (1966), however, opined that diacytic stomata develop through three successive mitotic divisions of the meristemoid and the encircling cells regarded as subsidiary cells by Pant & Mehra (1963) may perhaps represent the ordinary epidermal cells which have changed their form due to the growth of stomatal apparatus in their vicinity. Inamdar (1970) stated that the epidermal cells are distinct from the subsidiary and encircling subsidiary cells and differ in the density of their cytoplasm. The present study has recorded the syndetocheilic or mesogenous type of ontogeny for diacytic stomata and these being formed by three, four or five successive mitotic divisions of the meristemoid, thus supporting the views of Pant & Mehra (1963) and Inamdar (1970).

Fryns-Claessens & Van Cotthem (1973) introduced a new classification of the ontogenetic types of stomata. Following their classification, the diacytic stomata observed by us with two subsidiary cells can be regarded as diamesogenous and those with three or four subsidiary cells as subtype diallelocytic of the allelomesogenous type.

Tognini (1897) pointed out that "Various modes of stomatal development exist in different organs of the same plant" while Stebbins & Khush (1961) considered that the "stomatal developmental modes are constant, even as to minute details, from organ to organ within the same plant." Examples in support of both the views are available in literature for angiosperms. The present investigation corraborates the views of Stebbins & Khush (1961).

Mohan Ram & Wadhi (1965) supported the proposal of Mauritzon (1934) of raising the subfamily Thunbergioideae of Lindau (1895) to the family level on certain morphological and embryological grounds. Their contention has also been supported by Sahi & Dixit (1969). The 6 species of *Thunbergia* examined resemble those of the other Acanthaceae as they show the presence of diacytic stomata. Thus as far as the structure and development of stomata is concerned, Thunbergioideae does not differ from other members of Acanthaceae and hence its sub-family status within the family Acanthaceae is justified.

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