

NOTES FROM THE ROYAL BOTANIC GARDEN  
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## STUDIES IN ACANTHACEAE TRIBE NELSONIEAE II: FOLIAR SCLEREIDS AND STOMATA

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**ABSTRACT.** The morphological nature, distribution and taxonomic value of sclereids in the tribe are discussed. Seven species of *Staurogyne* and one species of *Elytraria* are reported to have sclereids in their leaves; this may be the first record of foliar sclereids in the Acanthaceae. The development of the simple diacytic stoma of *Elytraria maritima*, and of the double diacytic stoma of *Staurogyne paludosa* is also described. The multiplication of subsidiary cells in Acanthaceae and other families is discussed.

### A. FOLIAR SCLEREIDS

There is plenty of literature on the occurrence of foliar sclereids, their distribution, and morphological nature in Angiosperms. But none of these has dealt with the members of the Nelsonieae. In an attempt to explore anatomical characters in the group, I became interested in the morphology of sclereids when I first discovered them in *Staurogyne merguensis* (T. Anders.) O. Kuntze during my investigation into epidermal peelings. Subsequent studies into all the genera and species of the Nelsonieae demonstrated that sclereids are also present in a few other species of the genus *Staurogyne* and in a single species of the genus *Elytraria*.

**METHOD.** Small pieces of dried leaves from herbarium sheets were first soaked overnight in 20% KOH solution (thick leaves required a higher concentration) with a few drops of 25% H<sub>2</sub>O<sub>2</sub>. The softened material, after thorough washing with water, was treated by the quick clearing technique used by Bokhari (1970). The morphological nature, orientation, and distribution of the sclereids were studied by both the clearing technique and hand-sectioning of the lamina and mid-rib at comparable levels.

The data were also supplemented by studies of macerated tissues following Jeffrey's method as outlined by Foster (1949). Staining and mounting were done according to Bokhari's modified technique (cf. Bokhari, p. 44).

**DISCUSSION.** Out of the sclereid-containing species of *Staurogyne* (*S. aristata* E. Hossain, *S. cremostachya* Brem., *S. griffithiana* (Nees) O. Kuntze, *S. kerrii* E. Hossain, *S. merguensis* (T. Anders.) O. Kuntze, *S. pedicellata* E. Hossain, and *S. tenuispica* Brem.), four (*cremostachya*, *griffithiana*, *merguensis* and *tenuispica*) possess diffuse astrosclereids. Regarding their taxonomic significance it is noted that *S. merguensis* and *S. tenuispica* belong to Subsect. *Microchlamydeae*, while the other two are in Subsect. *Macrosepalia*; all are in the same Sect. *Staurogyne*. Probably similar forms of sclereids have arisen independently in the two taxonomic groups.

Size differences of the sclereids can be a guide in separating *S. cremostachya* (smaller types, fig. 1b) from *S. griffithiana* (much longer sclereids, fig. 1a).

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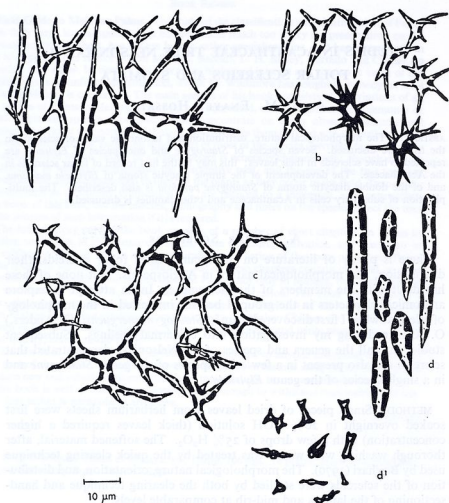


FIG. 1. Types of sclereids and fibres, macerated: a, foliar astrosclereids in *Staurogyne griffithiana*; b, foliar sclereids in *S. cremostachya*; c, foliar sclereids in *S. merguensis*; d, foliar sclereids in *Elytraria shaferi*, elongated macrosclereids along the veins and d', smaller polymorphic sclereids of the mesophyll.

The occurrence of similar sclereids in these two species, along with other morphological features, clearly indicate their close affinities. Therefore, Bremekamp's (1953) remark about the relationship of *S. cremostachya* as an isolated species in Series A (*Heterosepalae*) seems to be incorrect.

Size differences of sclereids between *S. merguensis* and *S. tenuispica* are not so great, but the former differs in having more complexly branched sclereids than the latter.

Species like *S. aristata* of Subsect. *Macrosepale* and *S. pedicellata* and *S. kerrii* of Subsect. *Michrochlamydeae* show a peculiar arrangement of sclereids which cannot be accommodated into a strait-jacket of morphological terms depending on their topography. Most of these sclereids are

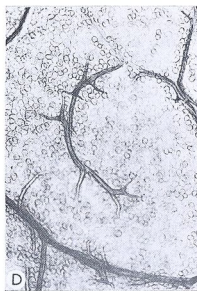
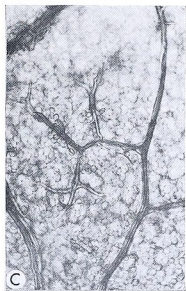
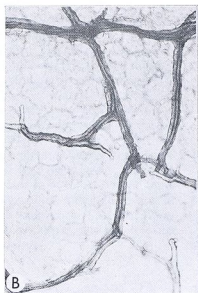
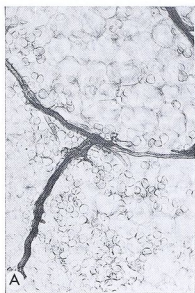


PLATE 3. Pseudoterminial sclereids in *Staurogyne*; A, *S. aristata*; B, *S. pedicellata*; C, *S. kerrii* (16307); D, *S. kerrii* (15257).

strikingly elongated, less branched than the astrosclereids and occur both at the extremities of the veinlets and also run parallel with the veins (Pl. 3 A-D). These are termed here as pseudoterminal sclereids.

By far the most remarkable arrangement of sclereids has been encountered in *Elytraria shaveri* (P. Wilson) Leonard. This species contains two types of sclereids in the same leaf (diffuse and parallel types). The diffuse sclereids are very small, diversely shaped, and often looking like small dots (fig. 1 d, d<sub>1</sub>), while the parallel type embraces unbranched, elongated, and more or less bluntly pointed macrosclereids (fig. 1d). The dorsiventral leaves of the *Nelsonieae* as a rule show 2-3 layers of palisade parenchyma below the upper epidermis and 2-4 layers of spongy parenchyma above the lower epidermis. The sclereids are usually formed in the mesophyll (palisade + spongy tissues), often well extended into the spongy tissue, but sometimes found to protrude into the palisade tissue. In the pseudoterminal type the sclereids are often seen just below the veins in transverse section. It is to be noted that all these sclereid-containing species also possess acicular fibres along the veins in addition to their sclereids in the mesophyll. The occurrence of acicular fibres in the phloem tissue of the vascular bundles in roots, stems, and leaves is one of the constant anatomical features of the *Nelsonieae*.

#### B. STOMATAL DEVELOPMENT

During these investigations on *Nelsonieae* living material of two species was available for the study of stomatal development. Fortunately one, *Elytraria maritima* J. K. Morton, showed simple diacytic stomata, while the other, *Staurogyne paludosa* (Mangenot & Aké Assi) Heine showed the double diacytic type. Young leaves of these plants were fixed for 10-12 hours in 3:1 absolute alcohol:acetic acid and then teased out in 1% acetocarmine, the slide being warmed to intensify staining.

**OBSERVATIONS.** The stomatal meristemoids are easily recognizable by their smaller size, denser cytoplasm, prominent nuclei, and spheroidal or semilunar shape (fig. 2, a, i). The first division of the meristemoid cell in each case (i.e. either in *E. maritima* or *S. paludosa* (fig. 2b, j), produces two unequal cells with two unequal nuclei. The cell containing the smaller nucleus is the  $S_1$  (first subsidiary cell) which later on enlarges quite considerably along with the meristemoid. The next division of the cell containing the larger nucleus again produces two unequal cells of which the middle one is lenticular in shape (the guard mother cell, GMC) and the other cell ( $S_2$  = second subsidiary cell) remains exactly opposite to the  $S_1$  (fig. 2e, f, m). Up to this point, the stages are the same in both the species investigated. But in *S. paludosa*, the stomatal meristemoid divides twice more to form two other subsidiary cells ( $S_3$  &  $S_4$ ) (fig. 2, o) in a similar way as to the formation of  $S_1$  and  $S_2$  before the guard mother cell finally divides to form two crescentic guard cells in each case (fig. 2g, h, q, r). A small pore develops gradually at the middle region of the common wall and ultimately the mature guard cells become full of chloroplasts. In both cases, the subsidiary cells (either 2 or 4) remain exactly opposite to one another. Their common walls are always at right angles to the longitudinal axis of the guard cells (fig. 2g, h, q, r). As the

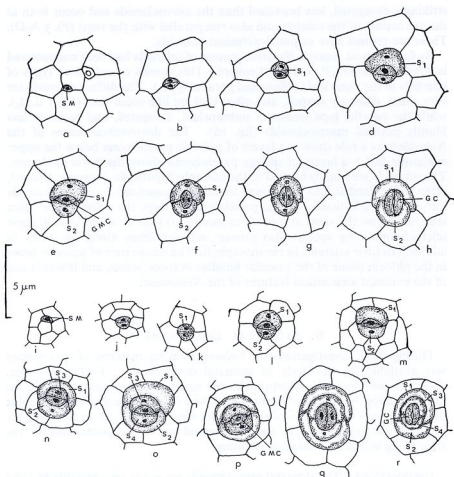


FIG. 2. Stomatal development in Nelsonieae: a-h, development of simple diacytic stoma, *Elytraria maritima*; i-r, development of double diacytic stoma, *Staurogyne paludosa*. S<sub>1</sub>, 1st subsidiary cell; S<sub>2</sub>, 2nd subsidiary cell; S<sub>3</sub>, 3rd subsidiary cell; S<sub>4</sub>, 4th subsidiary cell; SM, stomatal meristemoid; GMC, guard mother cell; GC, guard cell.

subsidiary cells (2 or 4) in both cases, are derived from the same stomatal initial (meristemoid cell) proper, the stomatal complexes are mesogenous in origin.

DISCUSSION. Pant & Mehra (1963) stated that the stomatal meristemoid in *Asteracantha longifolia* Nees (= *Hygrophila auriculata* (Schumacher) Heine) divides five times successively to form two encircling cells (outer ring), two subsidiary cells (inner ring), and two guard cells of the stomatal complex. They recognised the stomatal meristemoids as special, densely staining cells of the protoderm. Paliwal (1966) contradicted their contentions. In his opinion, Acanthaceous stomata are always formed by three (instead of five) successive mitotic divisions of the meristemoid cell. Further he contends that the stomatal meristemoid is always cut off from a protoderm cell.

Inamdar (1970) has supported the earlier observations of Pant & Mehra (1963). He has shown the development of four subsidiary cells encircling a stoma in *Justicia gendarussa* L. (cf. Pant & Mehra, fig. 76-79, p. 268). By the survey of Indian Acanthaceae he concludes that the diacytic stomata in Acanthaceae can arise through either three, four or five successive mitotic divisions of the stomatal meristemoid.

The present observations on the stomatal development of *Elytraria maritima* and *Staurogyne paludosa* fully support the findings of Pant & Mehra (1963) and Inamdar (1970). A stomatal complex with four subsidiary cells whose common walls are at right angles to the longitudinal axis of the guard cells is described here as double diacytic as against the usual type with only two subsidiary cells (simple diacytic). Abnormalities in the number of subsidiary cells are also met with in the same leaf: stomata with one or sometimes no subsidiary cells (possibly due to arrested development) are also recorded.

Occasionally simple diacytic stomata may contain one or more enlarged encircling cells, but these are not actually subsidiary cells. Their occurrence is not constant and their outer walls show undulations similar to those characteristic of other epidermal cells. They become specialized (enlarged) only to accommodate the enlarging subsidiary cells of the stomatal complex and are therefore an example of variation in the epidermal cells proper.

The phenomenon of multiplication in the number of subsidiary cells in a basic stomatal type occurs widely in various plant groups—both in dicots and monocots. Payne (1970) has introduced new terms to describe such stomatal variation. He refers to paracytic and diacytic stomata having more than two subsidiary cells as 'parallelcytic' and 'diallelocytic' types respectively. On the other hand, his 'helicytic' type conforms to the anisocytic stoma having more than three subsidiary cells in the complex. As all these types are nothing but minor variations of widely occurring basic stomatal types in angiosperms, there is little justification for further complicated terms.

The mature stomatal complexes having more subsidiary cells than those of the basic stomatal types can be easily described in explanatory words. Thus diacytic stomata with 4 subsidiary cells can be described as double diacytic, and likewise double paracytic, double anisocytic (with 6 subsidiary cells) and so on. Further, from the developmental point of view, Payne's 'helicytic' and 'allelocytic' patterns can be elucidated by Pant's (1965) useful ontogenetic terminologies (mesogenous, meso-perigenous and perigenous). As a result, Payne's terms seem to be superfluous.

The multiplication of subsidiary cells can however be explained by the fact that in these cases either the stomatal meristemoid retains its meristematic capacity for a prolonged period or the encircling epidermal cells become meristematic to give rise to additional subsidiary cells or a combination of both processes gives rise to the mature stomatal complex. Thus the mature stomatal complexes may vary in their ontogenetic history.

So far as the development of double diacytic and simple diacytic stomata in *Nelsonieae* and other Acanthaceae is concerned, it is always mesogenous.

It would be interesting to know whether there is any physiological or ecological relationship in the formation of double diacytic stomata. In *Nelsonieae*, they occur only in a few forest shade-loving plants—never in an open sun-loving plant. It is also observed that in transverse section of these

leaves the stomata remain slightly above the level of other epidermal cells. Thus the stomata look like turrets. Their substomatal chambers are also much wider than those of simple diacytic stomata. This turret-formation by the stomata is also quite frequent in the leaves of many Gesneriaceous genera, e.g. *Cyrtandra* (Bokhari & Burt, 1970, p.13), *Didymocarpus* (personal observations) and others.

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