

## Structure, Origin, Development and Senescence of Collecters in *Nerium indicum* Mill. (*N. odorum* Soland., Apocynaceae)

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### ABSTRACT

*Nerium indicum* has adaxial basal collectors on each petiole, bract, bracteole, calyx and corolla. The collectors are finger-shaped sometimes lobed structures with tall heads on short stalks. The petiolar collectors are more abundant than those on the bract, bracteole, calyx and corolla but their structure is similar. Meristematic activity in a small group of protodermal and hypodermal cells at the base of the developing leaf gives rise to the collector that further differentiates by frequent anticlinal and periclinal divisions. Many of the collectors are vascularized, sometimes a layer of radially elongated cells is present beneath the epithelial cells. Senescence of a collector begins when the leaves have either completed their growth or are still expanding. In senescent collectors, the central cell cytoplasm and nuclei are considerably reduced. Thin layer chromatography of the freshly harvested exudate of collector shows the presence of rhamnose, glucose and arabinose sugars.

### INTRODUCTION

Secretory structures present in angiosperm families such as the Rhizophoraceae, Rubiaceae, Apocynaceae and Asclepiadaceae have been variously termed as shaggy hairs (Solcreder, 1908; Esau, 1965), nectarthode (Lewis, 1968), squamellae (Woodson, 1935), squamellae or collectors (Ramayya and Bahadur, 1968) and collectors (Lersten, 1974 a, b; Lersten and Curtis, 1974; Metcalfe and Chalk, 1979), pectinate glands (Pichon, 1949), stipular glands (Patel and Zaveri, 1975), and extrafloral nectaries (Arekal and Ramakrishna, 1980; Inamdar *et al.*, 1985; Mohan and Inamdar, 1986). The term collector is used in the present study. The collectors of Apocynaceae have been studied by Ramaya and Bahadur (1968), Fjell 9(1983), Dave *et al.* (1987b), Thomas *et al.* (1988), and Thomas and Dave (1989a, b). Williams *et al.* (1982) have revealed the shoot apex organization of *Nerium* in detail, but the collectors are not described in sufficient details. Here the collectors of *Nerium indicum* are studied for their detailed structural, developmental and histochemical aspects.

### MATERIALS AND METHODS

Plant materials were collected from Sardar Patel University Botanical Garden and fixed in

F.A.A. They were then dehydrated through tertiary butyl alcohol-ethanol series infiltrated with and embedded in "Tissue Prep" (Sass, 1958). Sections of 6-8  $\mu\text{m}$  thickness were cut and stained with toluidine blue O, safranin O-fast green FCF, and tannic acid-ferric chloride (Johansen, 1940). Observations and drawings were made by using Nikon 305, Stereo and Projection microscopes. Photographs were taken using a Carl-zeiss photomicroscope, and the specimens were photographed using a Tessoovar microscope. For scanning Electron Microscopy, fresh samples of both young and mature leaf bases were taken and dehydrated through an alcohol series and fixed on the stub with double sided tape. Then it was coated with gold-palladium and observed using a Cambridge Stereoscan S4-1- electron microscope.

Histochemical tests for contents of the colletar tissue were conducted. Young colleters washed with double distilled water were used for detection of sugars. Dinitrosalicylic acid reagent (Miller, 1959) and thin layer chromatography were used to determine sugars; periodic acid-schiff's reagent (Jensen, 1962) for polysaccharides; mercuric bromophenol blue (Mazia *et al.*, 1953) for proteins, sudan black (Jensen, 1962) for lipids.

## RESULTS

*Nerium indicum*, a beautiful garden plant has colleters on the adaxial base of its petiole, bract, bracteole, calyx and corolla. Leaves are simple, lanceolate, ex-stipulate and whorled. Petiolar colleters are long finger shaped structures arranged in 3-4 alternate rows intermingled with petiolar unicellular hairs (Figs. 1-5). Young colleters are secretory and the secretion or exudation covers each colleter (Fig. 3). Rarely the colleters are lobed or branched (Fig. 4, at arrow). There are about 30-35 colleters at the base of a mature leaf covering about 6 sq.mm. area of the leaf (23 cm in length and 3.3 cm in breadth)-the lowermost colleters on the leaf base are translucent yellow and upper ones are dark brown differentiated into a long head on a short stalk (Fig. 6).

White, pink or red flowers with either single or double corolla are borne in racemose cymes. Each flower has 7-9 colleters present on the adaxial base of bract (Fig. 7) bracteole, calyx (Fig. 8) and corolla (Fig. 9). Calycine colleters are persistent at the base of the fruit (Fig. 10). There is an average of 70-90 colleters per single flower and 130-150 per double corolla flower.

**Origin, development and structure** Petiolar colleters were studied for their origin and development. At the site of colleter initiation (i.e. on the adaxial side of the petiole base) two to three protodermal cells and four to eight hypodermal cells become distinct due to denser staining than neighbouring cells (Fig. 11). These densely stained meristematic cells have abundant cytoplasm and prominent nuclei and undergo a number of periclinal divisions (Figs. 12, 13, 21-23). After a number of anticlinal and periclinal divisions a peg-like structure is formed and there is gradual increment in the size of the colleter primordium (Figs. 14, 15, 26). The outermost cells of the colleter primordium divide anticlinally producing a layer of epithelial cells, the epithelial cells measure 7.5  $\mu\text{m}$  in length during thin early stages of

development; later they elongate radially as much as 40-60  $\mu\text{m}$  and have a width of 10-15  $\mu\text{m}$ . The young collector grows more parallel to the ventral axis of the organ than at right angle to it. This is caused by the rapid proliferation and elongation of the central cells bounded by epithelium (Figs. 16-19, 24, 27). This also increases the length of the collector. Collecters are of "standard" type have a central core of parenchyma cells surrounded by radially elongated palisade like epithelial cells and a thin cuticle outside to it. A fully mature collector is differentiated into a tall head and a short stalk (Figs. 6, 20). The short parenchymatous stalk not bounded by the epithelium becomes distinct (Figs. 20, 29).

The head has a mean length of 1100  $\mu\text{m}$  and a mean diameter of 150  $\mu\text{m}$  at its base, 130  $\mu\text{m}$  at its middle and 40  $\mu\text{m}$  at the terminal region. The stalk of a collector is 25  $\mu\text{m}$  in length. The epithelial and central cells are reduced in number and size towards the terminal end of the collector (Figs. 20, 25a-c). Some of the collectors on the calyx show a layer of radially elongated cells centripetal to the epithelial cells (Fig. 31). Collecters borne of the calyx or bract have well differentiated vascular elements supplied from the organ on which they are borne (Figs. 30,32,33). Collecters are also found on the margin of the bract, bracteole and calyx (Figs. 28,34).

It is found that collectors are more active during winter. They secrete a highly viscous fluid coming out on rupture of the cuticle. This exudate is found to contain sugars such as rhamnose, glucose and arabinose. Histochemical studies of young or active collectors show the presence of starch, protein and lipids.

**Senescence of collectors** The senescence of collector occurs basipetally by the vacuolation of the cytoplasm and the thickening of the epithelial cell walls (Figs. 19,20). The nuclei in the epithelial cells appear disorganized, whereas the cytoplasm is still present although withdrawn from the lignified cell walls (Fig. 20). The remains of the cytoplasm are recognizable even in the dead collectors of *Nerium*. Both epithelial and central cells accumulate densely stained globular or irregularly shaped materials. Later the columnar epithelial cells and central cells are filled with dark brown contents that make the appearance of collector dark brown. The visible sign of a collector senescence is the change from its translucent yellow colour into dark brown; which happens approximately at the petioles of fifth or sixth node below terminal shoot.

The spherical nuclei of the central cells become spindle shape or linear, then gradually disappear along with the cytoplasm of the cells (Figs. 19,20). Solitary sphaerocrystals are seen on the peripheral region of the central cells. The stalk cells lack tannin deposit, but later become lignified (Figs. 29,35). Several such dead collectors with tall, hard heads borne on short, narrow stalks are seen attached to the petioles of old leaves borne at lower nodes of the shoot and on the calyx persisting with the mature fruit.

## DISCUSSION

*Nerium indicum* is a genus having collectors on the adaxial base of petiole, bract, bracteole,

calyx and corolla. But most of the taxonomist (Cooke, 1958; Duthie, 1960; Gamble, 1957; Haines, 1961 and Saldanha and Nicolson, 1976) reported its presence only on the petiole and calyx. In *Roupeia* (Thomas *et al.* 1988) and *Aganosoma* (Dave *et al.*, 1987b) colleters are present on the petiole, bract and calyx. Ramayya and Bahadur (1968) reported only petiolar and calycine colleters in *Tabernaemontana* and *Allamanda*, but Thomas and Dave (1989) reobserved *Allamanda* and found the occurrence of colleters on the bract and bracteole too. In an elaborated study of Apocynaceae flowers, Woodson and Moore (1938) have emphasized the taxonomic importance of colleters according to the number and mode of distribution. We thoroughly observed 14 Indian genera and agreed to the taxonomic significance of colleters on the basis of position, number, size and whether the colleters are intermingled with hairs or not. Besides the morphological characters, anatomically also colleters differ considerably, such as the presence of epithelial hair (*Aganosoma*), subepithelial layer (*Nerium* and *Roupeia*), vasculature (*Aganosoma* and *Nerium*) and branched laticifer (*Allamanda*).

Anatomically colleters of *Nerium* are of 'standard' type as Lersten (1974) observed in many Rubiaceae members with a central core of elongated cells surrounded by epithelial cells. Colleters are usually non-vascularized, but vascularized colleters are present in *Holarrhena*, *Wrightia* and *Vallis* (Rao and Ganguli, 1963). Vascular traces are bicollateral in colleters of *Strophanthus gratus* and *Funtumia elastica*, which receive the supply from the organ to which they are attached (Woodson and Moore, 1938). Rao and Ganguli (1963) considered the calycine colleters of *Nerium indicum* to be non-vascularized. Many of the colleters of *Nerium* observed in this study however, are vascularized. According to Williams *et al.* (1982) colleters are organs of limited growth and have no vascular tissue. They appear to obstruct the early development of the leaves and may effectively inhibit the growth of axillary buds thus their presence is a factor governing apical dominance in vegetative shoots of *Nerium* (Williams *et al.*, 1982).

Williams *et al.* (1982), Mueller (1985) and Thomas *et al.* (1988) emphasized the protective function of colleters in *Nerium*, *Allstonia* and *Roupeia* respectively. The young colleters secrete a highly viscous fluid which comes out on rupture of the cuticle (Dave *et al.*, 1987a) or through the gap present in between the epithelial cells (Thomas and Dave, 1989b) and covers the entire meristem. Fjell (1983) observed colleter secretion in *Allamanda* and *Thevetia*, but not in *Vinca*. Thin layer chromatography of the secretory material in *Nerium* revealed the presence of rhamnose, glucose and arabinose sugars whereas in *Aganosoma* and *Roupeia* only rhamnose has been detected. Glucose and rhamnose are present in the exudate of *Allamanda* (Thomas and Dave, 1989b).

Senescence of the colleter in *Nerium* initiates from the tip of the head and proceeds basipetally by vacuolation of the central cells and thickening of the epithelial cell wall. Necrosis in colleters of *Roupeia* (Thomas *et al.*, 1988) and *Allamanda* (Thomas and Dave, 1989b) show identity with *Nerium* colleters in the appearance of deeply stained globular bodies in the epithelial cells. Appearance of crystals during necrosis is noticed in *Alstonia*, *Allamanda*, *Gardenia* and *Roupeia*. But the significance of persisting petiolar and calycine colleters are unknown.

Woodson (1930) described the collectors as staminodes while studying the flowers of Apocynaceae, but later Woodson and Moore (1938) discarded that view in favour of a stipular theory when they found that in collectors of some genera of Apocynaceae there is vascular supply. Gluck (1919) has also interpreted that the collectors on the petiole are stipular in nature in spite of the fact that the leaves of Apocynaceae are exstipulate. From our point of view it is difficult to agree with the stipular theory of collectors because of its occurrence both in the vegetative and floral parts; structural specializations such as epithelial hair, laticifer, vasculature and subepithelial cells. Moreover the term "shaggy hairs" is inappropriate for collectors. In fact many members of Apocynaceae have petiolar collectors intermingled with unicellular unbranched hairs. So in accordance with Metcalfe and Chalk (1979) it is better to consider collectors as multicellular, glandular structures secreting various mucilages, gums or resins and are to be found on the leaves, stipules and bud scales in many families of dicotyledons.

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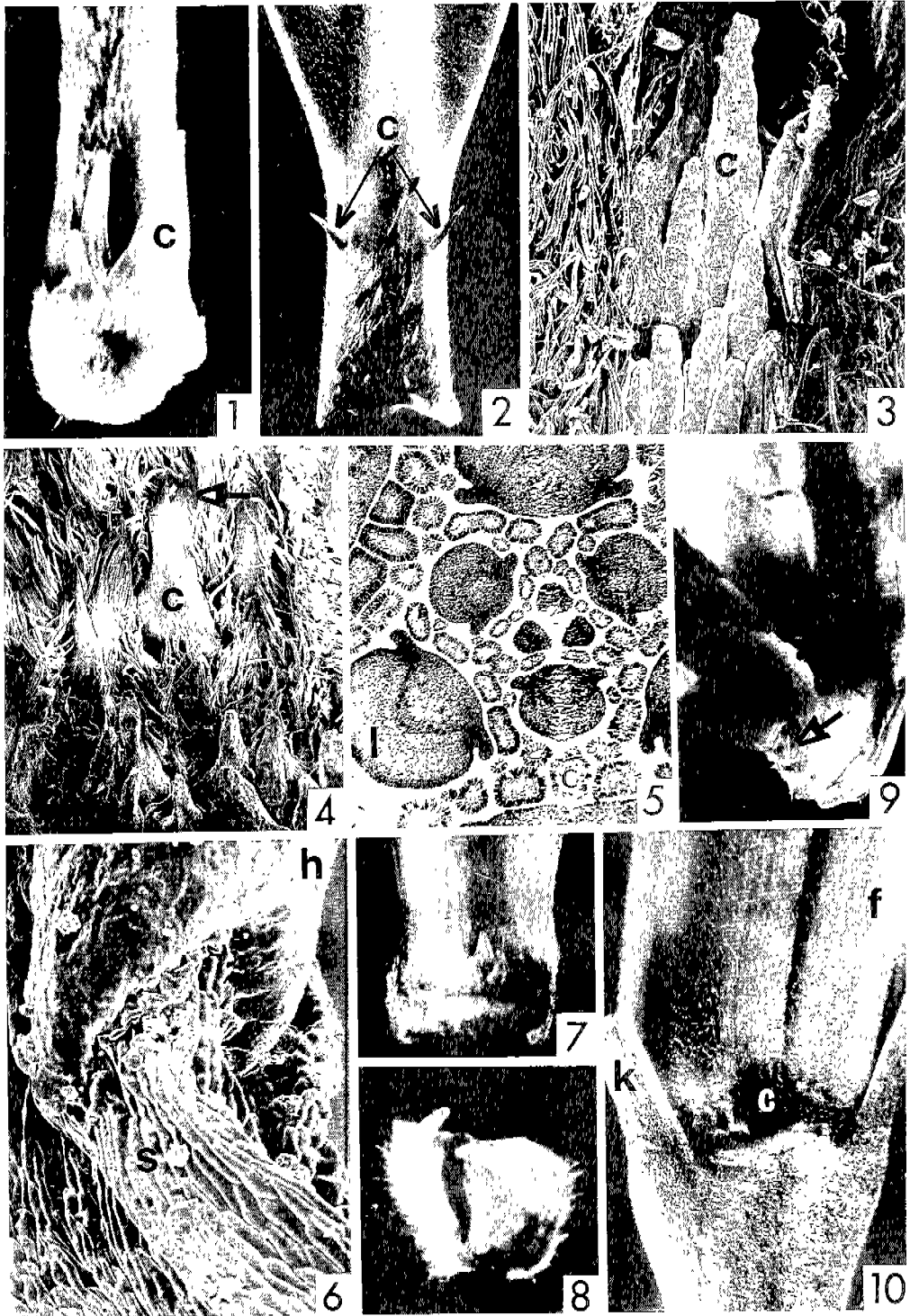
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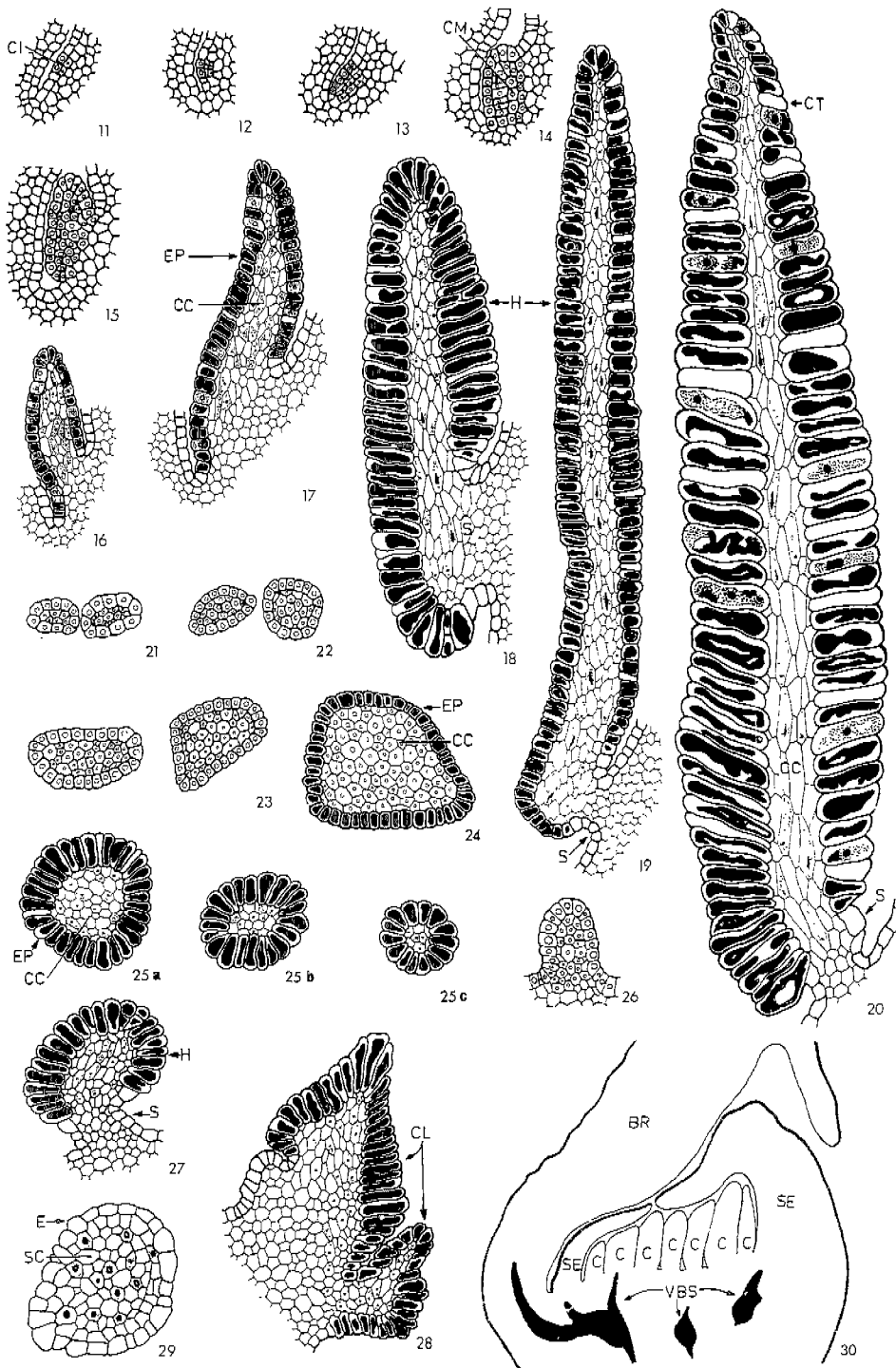
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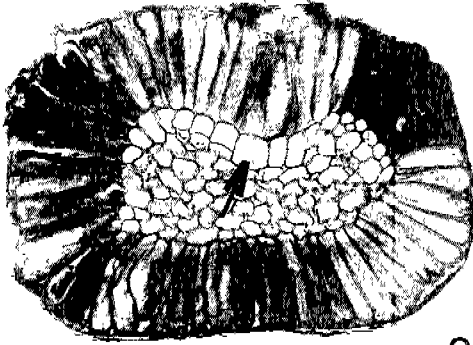
### Explanation of Figures

- Fig. 1-10.** Collecters on different organs of the plant.
- 1,2. Collecters (C) on the adaxial side of the young and mature leaf petiole.
  3. Young collecters (c) are covered with secretory material. X 375
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- Fig. 21-25c.** Developmental stages of collector in transverse sections.
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- Fig. 33.** Collector showing vascular strand (vs) in connection with the sepal trace. X 245
- Fig. 34.** Marginal collector present on the calyx (k). X 145
- Fig. 35.** Lignified stalk (s) of the collector in longitudinal section (portion of head (h) is obliquely cut). X 115

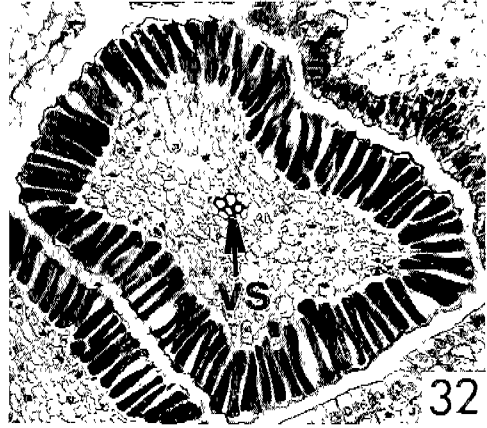




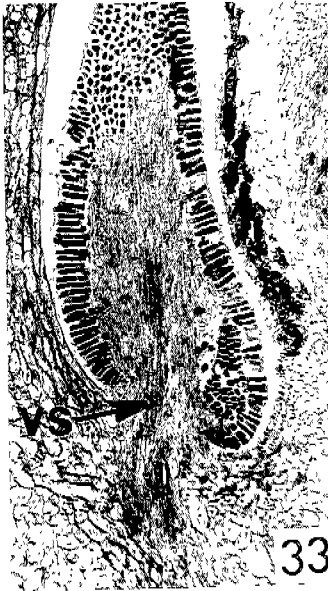




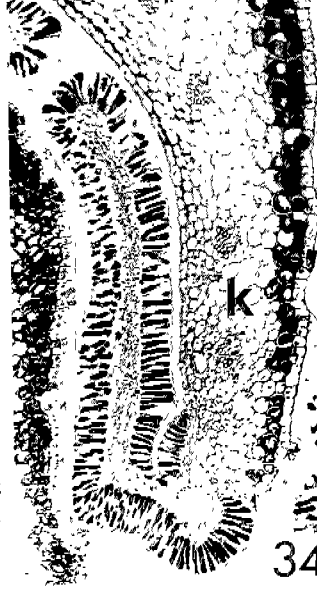
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