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Development of Air Passages, and Crystal Distribution, in the Stem of *Bacopa caroliniana* (Scrophulariaceae)

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LERSTEN, NELS R., and JAMES L. GUNNING (Department of Botany and Plant Pathology, Iowa State University, Ames, Iowa 50010). Development of air passages, and crystal distribution, in the stem of *Bacopa caroliniana* (Scrophulariaceae). *Proc. Iowa Acad. Sci.* 82(2): 109-112, 1975.
About 20 vertical air passages occur in each internode, separated at each node by a solid diaphragm of parenchyma, in which leaf traces depart. Air passages are schizogenous and begin to form just below the shoot apex. This may be the first report of druse crystals in Scrophulariaceae. Druses become visible in the second nodal plate below the shoot apex. In the mature stem, druses are more common in nodes, but they also occur in internodes. The stele appears to be an ectophloic siphonostele, without separate vascular bundles at any stage of development. This appears to be a good species for further study by electron microscopy.
INDEX DESCRIPTORS: Scrophulariaceae, *Bacopa*, stem anatomy, druse crystals.

The Scrophulariaceae is among the larger dicot families, with 220 genera and 3,300 species (Willis, 1973). Considering its size, anatomical studies have been rather sparse. According to Metcalfe and Chalk (1950), representatives of 63 genera, only slightly more than a quarter of the total, have been examined for various aspects of vegetative anatomy.

One genus that has never been studied anatomically is *Bacopa*, which consists of about 100 species of herbs (Willis, 1973), mostly of warm or tropical regions, including many of aquatic and semi-aquatic habitats (Fernald, 1950). Metcalfe and Chalk (1950) did not mention any work on *Bacopa*, and no anatomical studies were uncovered during an examination of *Biological Abstracts* from the late 1940's through 1974.

We examined several aspects of the anatomy of mature and developing stems of *Bacopa caroliniana* (Walt.) Robins., a rhizomatous perennial herb native to the southeastern United States. Our most important observations were of the development of the characteristic air passages, and crystal formation, below the shoot apex. These are described, along with other anatomical observations.

MATERIALS AND METHODS

Mature stems and stem tips were collected from plants of *Bacopa caroliniana* growing in a greenhouse in the department of Botany and Plant Pathology, Iowa State University, and preserved in formalin-acetic acid-alcohol (Sass, 1958). The material was dehydrated in an ethanol-xylene series, embedded in 56° mp. paraffin wax (Fisher Tissuemat), and sectioned at 8-12 μm. The sections were stained with safranin and counterstained with either fast green or chlorazol black E. Sections were examined under bright field optics, or with polarized light to allow clearer observation of druse crystals. Photographs were taken with an Orthomat automatic camera fitted to a Leitz Ortholux microscope.

OBSERVATIONS

There is no true aerenchyma in the stem, but instead there are several conspicuous air passages that extend the length of each internode (Figures 2, 7). The air passages are separated by uniseriate vertical septa, so that the stem in cross-section appears similar to a wagon wheel. The number of passages per internode varies only slightly, from 19 to 22, based on an examination of 43 internodes. In the inner cortex, between the air passages and the stele, there are also conspicuous intercellular spaces. One is indicated by the lower arrow in Figure 7. The arrowhead in Figure 3 points to another, seen at higher magnification. The air passages are interrupted at each node by a solid diaphragm of parenchyma (Figures 1, 2). Within this diaphragm, a single trace per leaf departs abruptly from the vascular cylinder and separates into three bundles as it enters the base of each of the two leaves per node.

A study was made of the developing air passages from a series of cross-sections, cut 8 μm thick, from the shoot apex downward to the mature stem. The first sign of initiation of air passages was noted at about 56 μm below the apex, at which level there occurred a thickening of walls and a slight splitting between cortical cells (Figure 4). Slightly lower, at approximately 64 μm below the apex, splitting was more obvious (Figure 5). At about 144 μm the cells flanking the spaces had subdivided to form several vertically and periclinally flattened cortical cells (Figure 6, arrow). These cells expanded later to form the radially arranged vertical septa in the mature stem (Figure 7).

The levels in the stem at which the sections shown in Figures 4-6 were taken are indicated in longitudinal view in Figure 1 by the three horizontal black lines at the upper right. The increase in diameter of the developing air passages can also be seen in Figure 1, where the left arrowhead indicates the youngest visible passage, below which there are two larger air passages.

Druse crystals were observed throughout the mature stem, but they tended to be concentrated in the nodal diaphragms (Figures 1, 2) in both the developing and mature stem. Figure 3 shows some detail of individual druses. The first visible crystals were seen, under polarized light, at the level of the second node, approximately 100 μm below the apex (Figure 1, uppermost arrow).

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In addition to what has been described about the developing air passages and druse crystals, there are other features worthy of mention. The stele does not develop as separate vascular bundles, but is initiated instead as a cylinder of procambium. Because of this pattern, any longitudinal section, such as in Figure 1, shows the two sides of the continuous stelar cylinder, which appears at various levels of maturation in Figures 5-7.

There is no sclerenchyma in the stem, which is common among hydrophytes. Small glands develop early on the stem and leaves (Figure 1). They consist of a large basal epidermal cell, a short stalk cell, and four cap cells. Two views of these glands are shown in Figures 8 and 9 and a young gland is seen at left in Figure 9.

Adventitious roots are common, some arising at nodes, others from internodes. Figure 7 shows an adventitious lateral root emerging from the stele in an internode. In mature roots, air passages were seen that appeared quite similar to those seen in the stem.

DISCUSSION

Kaul (1971) included in his introduction some general remarks about internal ventilation in aquatic plants. The various types of inner spaces store and transport oxygen, and such an architectural pattern may also give ". . . the greatest strength with the least tissue . . ." Compared with many other aquatic plants, the vertical internodal air passages of *Bacopa caroliniana* are rather simple internal spaces. They begin to develop quite early, with the first signs of splitting and wall modification detected in the first internode below the youngest pair of leaf primordia. Subsequent development is quite orderly and symmetrical, and mature internodes differ very little from each other in number of air passages. We did not subject the plants to varying growing conditions, so we do not know how closely this pattern is genetically controlled.

Crystal formation in the stem in relation to the shoot apex does not appear to have received much study. In two recent reviews of crystals in plants (Arnott and Pautard, 1970; Arnott, 1973), nothing is included about this. The only paper we uncovered that gives comparable information is that of Scott (1941) on *Ricinus communis* (castor bean), in which "The earliest identifiable druse crystals [under polarized light] occur in the vacuolating cells of the third internode . . ." We noted crystal initiation in *Bacopa caroliniana* at the second node, using polarized light.

Druse crystals were abundant in our material. Metcalfe and Chalk (1950) state that calcium oxalate crystals in the Scrophulariaceae are ". . . rather infrequent, when present nearly always small; prismatic, octahedral or acicular in shape." We have not been able to explore the subsequent literature for reports of crystals, but it is possible that this is the first report of druse crystals in this family.

The small external glands that we have described are common in the family, according to Metcalfe and Chalk (1950). Their secretions are probably responsible for the characteristic lemony odor of this species, although we did not verify it beyond doubt.

Our finding that the stele appears to be an ectophloic siphonostele rather than a eustele needs to be investigated more intensively, but there is no doubt that the procambial cells from their inception are so close together that individual vascular bundles appear to be lacking. Leaf gaps are also virtually lacking; the single trace per leaf departs abruptly in the nodal diaphragm at a 90° angle to the stele, and vascular tissue appears immediately above the place where the trace departs.

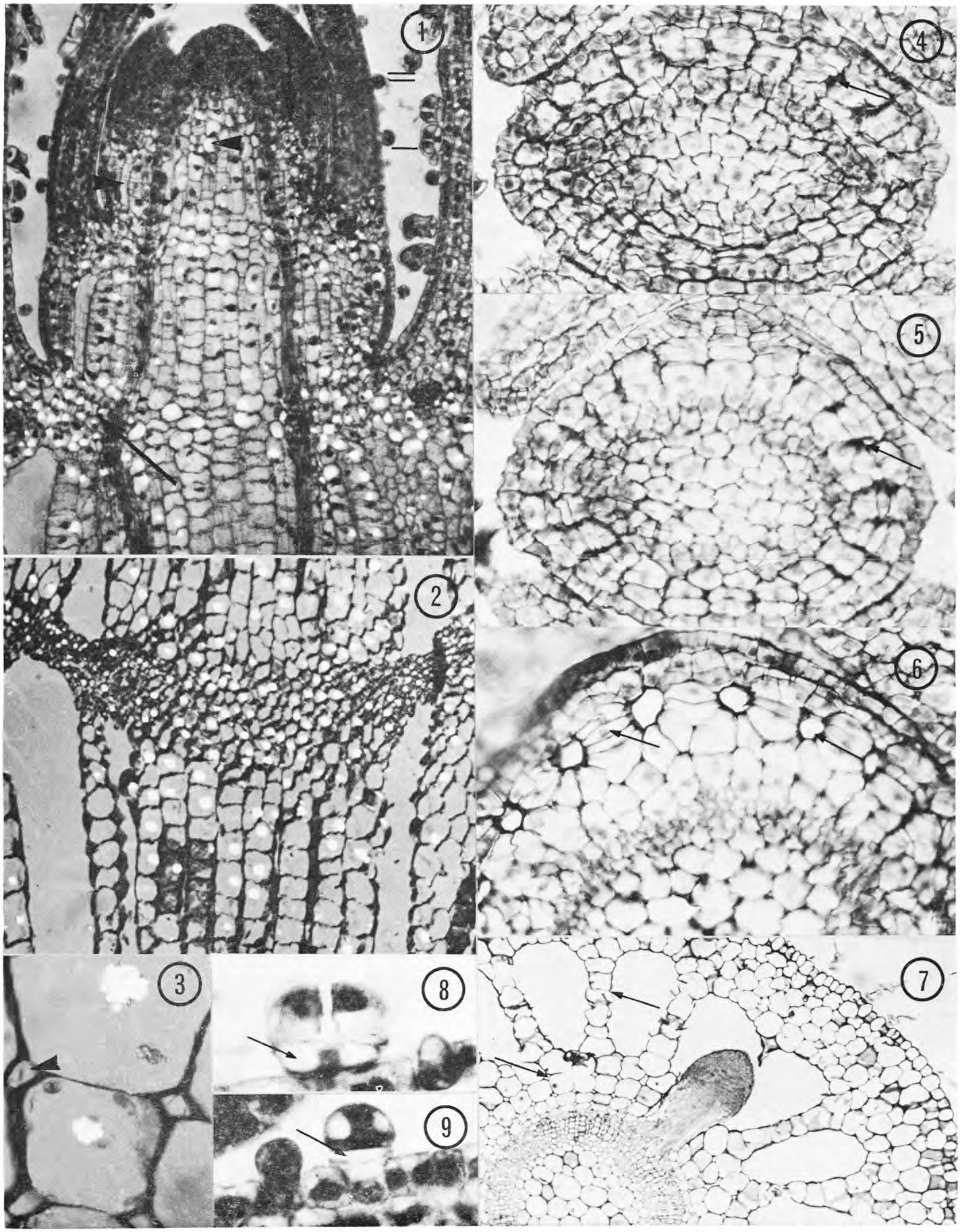
Bacopa caroliniana is a desirable species in which the development of at least three structures can be studied, separately or at the same time, at the fine structure level. Plants of this species are small and easy to grow in the greenhouse, and they rapidly proliferate new branches. Leaf arrangement is decussate, a favorite pattern to provide orientation for longitudinal sections of the shoot tip, and the three structures of interest are initiated just a short distance beneath the apex.

The schizogenous air passages are numerous and predictable in position. The changes in primary wall and middle lamella as cell separation occurs have never been described. Such information could be obtained here in an orderly way.

Crystal initiation and development remains a complex and little understood process (see Horner and Whitmoyer, 1972, for the most complete study and a review of other recent studies using electron microscopy). The peculiar crystal aggregate that makes up a druse has never been investigated. This species seems technically convenient for studying druse crystal development.

Finally, a question that still remains little explored is the series of changes by which procambial cells become distinct from other meristematic cells and gradually take on the characteristics of xylem or phloem. The procambium in *Bacopa*

Figures 1-9. Stem anatomy of *Bacopa caroliniana.* 1. Median longitudinal section of stem tip under partially polarized light. Upper vertical arrow indicates first visible crystal. Upper arrowhead indicates uppermost mature crystal. Left arrowhead is on earliest visible stage of developing air passage. Lower arrow points to a nodal diaphragm with numerous crystals. Three horizontal lines at upper right indicate levels of section in Figures 4-6. X68. 2. Enlarged longitudinal portion of extra-stelar part of stem showing node under partially polarized light. Air passages, nodal diaphragm, and crystals are apparent. X66. 3. Two enlarged cortical cells under partially polarized light. Two druse crystals are seen. Arrowhead indicates a conspicuous intercellular space. X368. 4. Stem cross-section 56 μm below shoot apex (level of upper line in Figure 1). Arrow indicates first visible sign of splitting to form air passage. X368. 5. Stem cross-section at 64 μm (level of middle line in Figure 1). Splitting is more conspicuous (arrow). X357. 6. Stem cross-section at 144 μm (level of lower line in Figure 1). Definite air passages now seen (right arrow). Cortical cells separating passages are dividing periclinally (left arrow). X357. 7. Cross-section of mature stem. Area indicated by left arrow in Figure 6 has developed into a vertical septum like that indicated by upper arrow. Inner cortex has regularly arranged parenchyma cells with prominent intercellular spaces (lower arrow). A young adventitious root is seen extending into two air passages. X51. 8. Enlarged view of a gland. Arrow indicates the stalk cell. X756. 9. Longitudinal view of young (left) and mature (right) gland. Arrow indicates stalk cell, below which the basal cell is located. X756.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caroliniana is initiated as a cylinder, so the possibility of missing individual procambial strands is avoided. This is important when observing the earliest stage of procambial differentiation, where differences among meristematic cells must be very slight.

This rather brief study has contributed some new information to systematic and developmental anatomy, but it is perhaps more important as a preliminary report which points out where further research using electron microscopy could be conveniently undertaken on some questions of current interest.

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