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PAPAIN IN SEEDLINGS OF PAPAYA

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Abstract. **Two types of articulated anastomosing laticifers occur in stems and hypocotyls of seedling papayas (*Carica papaya* L.), which have attained a height of 4.0 to 22.5 cm above ground level. Small laticifers occur between the parenchyma and collenchyma cells of the cortex, parenchyma cells of the vascular rays and to a lesser extent parenchyma cells adjacent to the primary xylem. Large laticifers occur principally in the cortex but also occur in the vascular rays. Formation of large laticifers appears to be associated with tubular structures which extend through the transverse walls of series of cells. The tubes have indistinct pores in their lateral walls and frequently appear to be attached to the lateral walls of the enclosing series of cells by loops. It is suggested that following rupture of the tubes, the enclosing series of cells enlarge, their walls rupture and large articulated anastomosing laticifers develop. The abundance of laticifers in seedling papayas suggests the possibility of their utilization as a commercial source of papain.**

The papaya, *Carica papaya,* is the source of papain, a proteolytic enzyme used as a meat tenderizer, in the brewery industry, and for various medical purposes. Commercial papain is obtained from the skin of immature papaya fruit. The fruit is scratched with a sharp instrument and the latex collected, dried, and powdered. Balls (1) found that papain could be obtained from all parts of the papaya plant except the roots and suggested that extraction of papain from the whole plant is feasible.

Laticifers in mature papaya trees are articulated and anastomosing (4,5,6,7,8,10). A literature survey revealed no specific information pertaining to laticifers in seedling papayas. This investigation was undertaken to determine the stage in papaya seedling development in which lacticifers occur.

Materials and Methods

Seeds were planted approximately 2.5 cm apart in potting soil in shallow flats and grown in a greenhouse. Papaya seedlings are epigeal, therefore measurements of above ground height include hypocotyl as well as epicotyl or young stem. Preliminary studies were made with freehand sections of seedlings ranging from 4.2 to 5.5 cm in height and were found to contain papain in their hypocotyls. Seedlings measuring 4.0 to 4.5 cm, 10 to 10.5 cm, and 16.8 to 22.5 cm above ground level were used for this study.

Specimens were fixed in FAA, evacuated, dehydrated and embedded in paraffin-plastic. Transverse, tangential, and radial serial sections were stained with Foster's tannic-acid ferric chloride and counterstained with safranin and fast green.

The following observations are based on a study of transverse and longitudinal sections prepared from seedlings ranging from 4.0 to 22.5 cm in height and presented as composite data.

Results

Two types of articulated anastomosing laticifers occur in papaya seedlings. Small laticifers occur between the parenchyma and collenchyma cells of the cortex, parenchyma cells of the vascular rays and to a lesser extent the parenchyma adjacent to the primary xylem. Large laticif-

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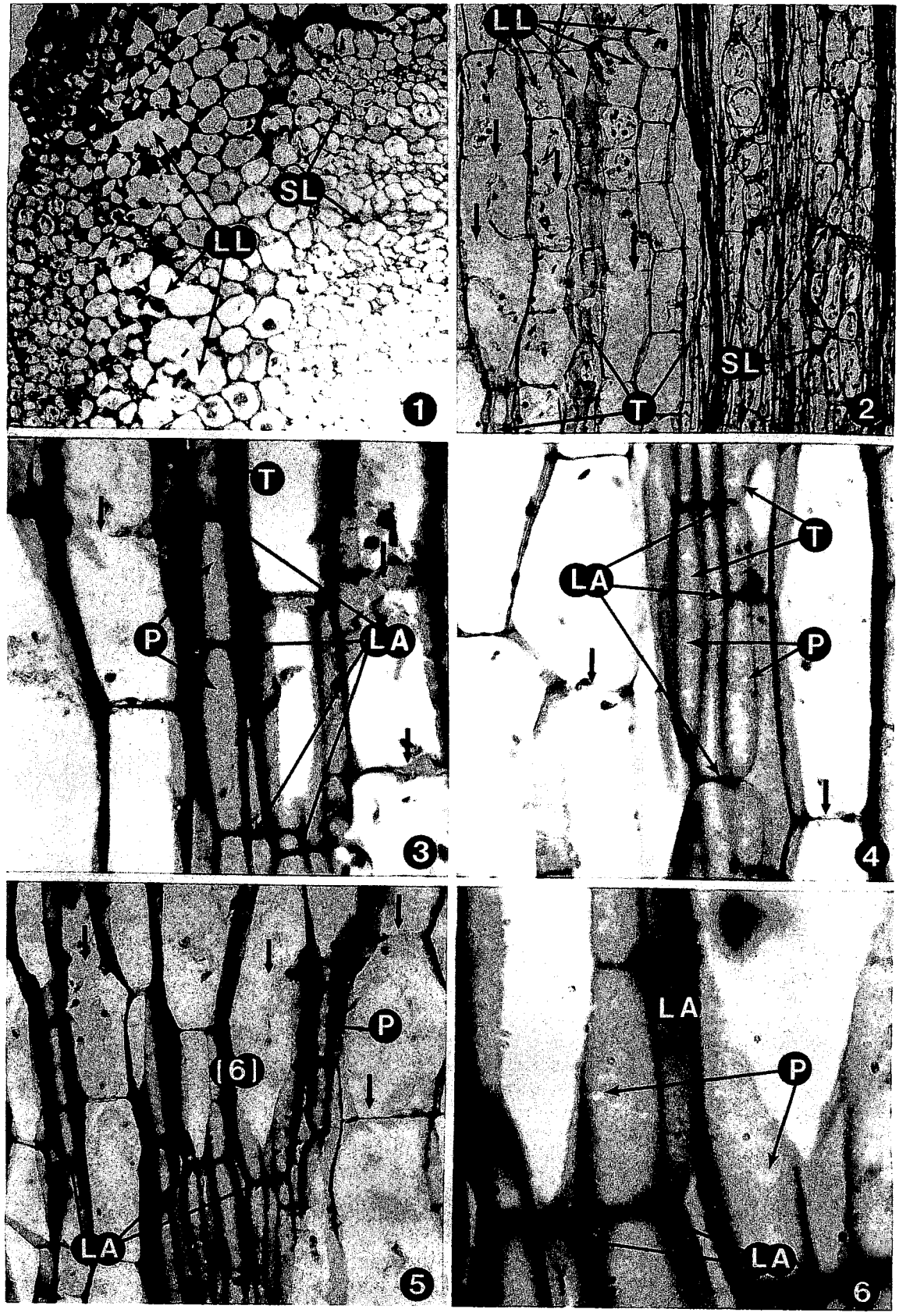

Key to Labeling: LA, loop attachment; LL, large laticifer; P, pit; SL, small laticifer; T, tube; vertical unlabeled arrows point to ruptured transverse walls in large laticifers.

Fig. 1-6. 1. Transverse section through stem of a 10 to 10.5-cm seedling papaya. × 125. 2. Tangential section through hypocotyl of a 4.2 to 4.5-cm seedling. Three large laticifers contain fragments of tubes and 3 show ruptured transverse walls (arrows). × 125. 3. Tangential section through cortex of hypocotyl of a 4.2 to 4.5-cm seedling. Note breakdown of transverse walls, loop attachment of tubes to walls of enclosing cells and pits in lateral walls of tubes. × 400. 4. Tangential section through outer cortex of hypocotyl of a 4.2 to 4.5-cm seedling. Note partial constriction of tubes by loop attachment and pits in lateral walls of tubes. × 400. 5. Tangential section through outer cortex of a 4.2 to 4.5-cm seedling. Note abundance of tubes within parenchyma cells, loop attachments, the enlargement of parenchyma cells following rupture of tubes and breakdown of transverse walls in the large laticifers. × 400. 6. Enlargement of central portion of Fig. 5. × 1000.

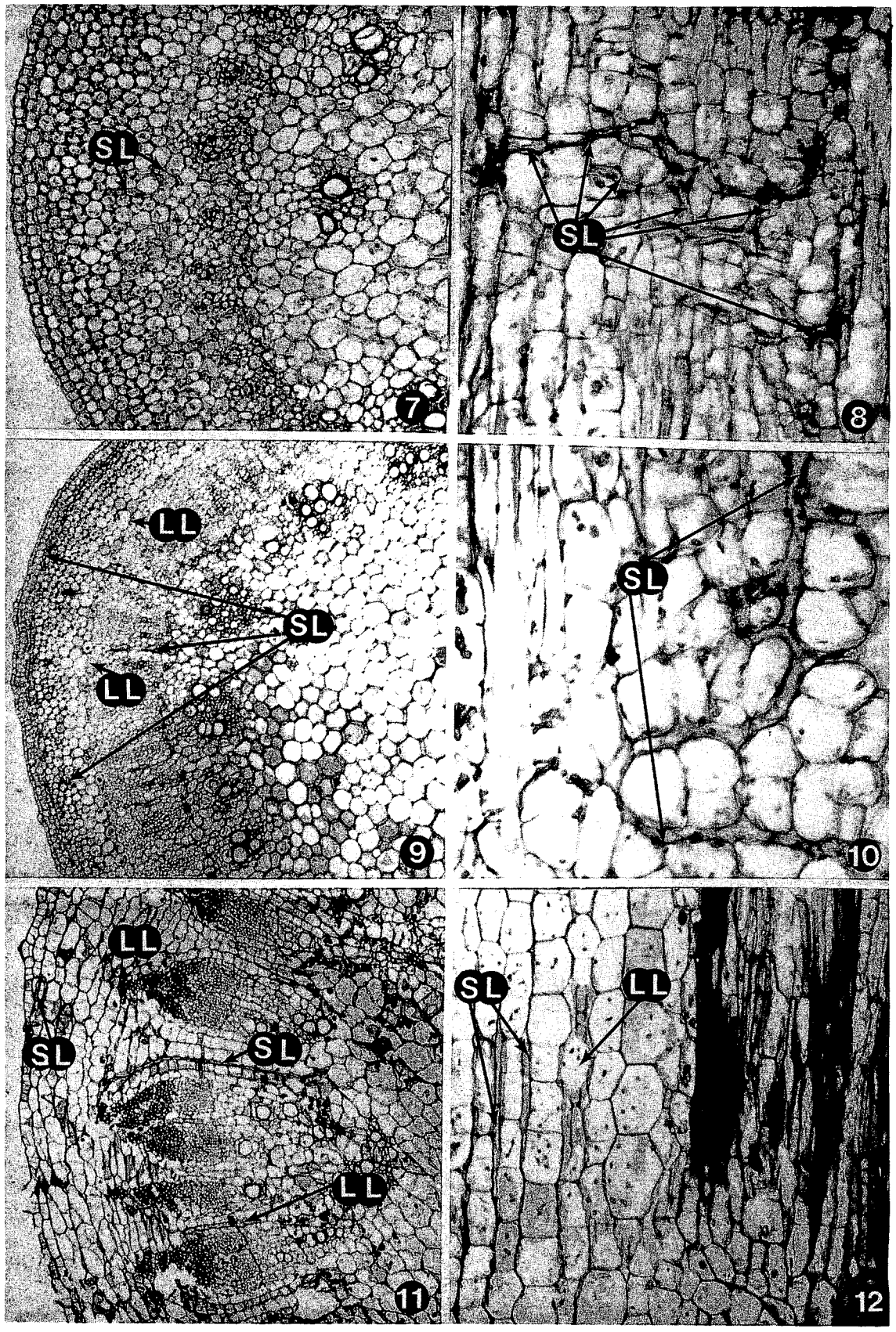

Fig. 7-12. 7. Transverse section near apex of stem of a 10 to 10.5-cm seedling papaya. × 125. 8. Radial section near apex of stem of a 10 to 10.5-cm seedling. Note extensive anastomosing of small laticifers between parenchyma cells of vascular ray. × 250. 9. Transverse section of epicotyl between cotyledons and leaves of a 10 × 10.5-cm seedling. × 100. 10. Radial section of epicotyl of a 10 to 10.4-cm seedling showing extensive anastomosing of small laticifers between parenchyma cells of vascular ray. Note the presence of some transverse walls in these laticifers. × 430. 11. Transverse section of hypocotyl of a 10 to 10.5-cm seedling. × 130. 12. Radial section of hypocotyl of a 10 to 10.5-cm seedling. Note the presence of both small and large laticifers in the cortex external to the fibers of a vascular bundle. × 250.

ers are located principally in parenchyma of the cortex and to a lesser extent within collenchyma of the cortex and parenchyma of vascular rays (Fig. 1,2).

Of particular interest are the tubes, of unknown composition, which appear to penetrate the transverse walls of series of cells (Fig. 2,3,4,5). The walls of the tubes are flexible and appear to contain a fluid under pressure as evidenced by their enlargement above and below areas of partial constriction (Fig. 3,4,5,6). The nature of the structure causing the partial constrictions is not known. In many longitudinal sections, the constrictions appear to be associated with loops which encircle the tube and attach it to the wall of the enclosing series of cells (Fig. 3,4,5,6). In other longitudinal sections, the areas of partial constriction seem to occur where the tubes pass through the transverse walls of the series of enclosing cells (Fig. 2, 3, 5). Indistinct pits occur in the lateral walls of the tubes (Fig. 3, 4, 5, 6). Following rupture of the tubes, the enclosing series of cells enlarge, their transverse and less frequently their lateral walls break down and large articulated anastomosing laticifers are formed (Fig. 2, 3, 4, 5).

Stem near apex. Transverse sections of the stem near the apex show a uniseriate epidermis. The cortex is divided into 3 layers, an outer cylinder of parenchyma, a middle cylinder of developing collenchyma and an inner cylinder of parenchyma. Primary phloem appears as isolated bundles separated by phloem parenchyma rays. Internal to the cylinder of phloem bundles and separated from them by parenchyma cells is a cylinder of isolated bundles of primary xylem. The number of phloem bundles is markedly greater than the number of xylem bundles. The pith is composed of parenchyma (Fig. 7).

Transverse and longitudinal sections show small laticifers between the parenchyma of the cortex, the parenchyma of vascular rays and the parenchyma located between the primary xylem and phloem (Fig. 7,8). Large laticifers are present within the parenchyma of the cortex, within the parenchyma of vascular rays and in the parenchyma located between the xylem and phloem.

Epicotyl. Transverse sections of stems just above the cotyledons show additional differentiation compared to those near the apex. The primary phloem has completed its differentiation, the vascular cambium has become active and there is marked thickening of cell walls in the cortical collenchyma (Fig. 9).

Transverse and longitudinal sections show small laticifers between the cells of the outer cylinder of cortical parenchyma, the inner cylinder of cortical parenchyma, the parenchyma of vascular rays and in the parenchyma located between the primary xylem and primary phloem. Large laticifers are present within the outer cylinder of cortical parenchyma, the cylinder of cortical collenchyma, the inner cylinder of cortical parenchyma and the parenchyma of vascular rays (Fig. 9, 10).

Hypocotyl. There is a complete absence of cortical collenchyma in hypocotyls. Transverse sections show vascular bundles separated by parenchyma rays. The parenchyma rays are dilated at their outer ends. Associated with each vascular bundle is a bundle cap of fibers (Fig. 11).

Transverse and longitudinal sections show small laticifers between the cells of the parenchyma of the cortex, the parenchyma of vascular rays and the parenchyma just internal to the xylem. Large laticifers are present within the aprenchyma of the cortex and within the parenchyma of the vascular rays (Fig. 11,12).

Discussion

This investigation shows that 2 types of articulated anastomosing laticifers occur in the apical portion of the stem, in the epicotyl and in the above ground portion of the hypocotyl of seedling papayas ranging in height from 4.0 to 22.5-cm. Small laticifers are found principally between the parenchyma cells of the cortex, parenchyma cells of the vascular rays, and parenchyma cells adjacent to the xylem. Large laticifers, formed by the breakdown of transverse walls of series of cells, occur most frequently within cortical parenchyma but are also present in cortical collenchyma and in parenchyma of vascular rays (Fig. 1,2).

Distribution of laticifers in the papaya is widespread (2,7,8,10). It is quite possible that large and small articulated laticifers were observed but no distinction was made between them.

A literature survey provided little information which would explain the association of tubes observed in this investigation and the development of the larger laticifers. De Bary (3) refers to the presence of pits in the lateral walls of laticiferous tubes in some species of plants. He also mentions that certain representatives of the family Papayaceae, now known as Caricaceae which includes as a representative the papaya, contain laticiferous tubes in which the cross-walls partially persist and are "usually somewhat constricted at the cross-walls". Moyer (9) refers to the presence of a protoplasmic sheath lining the latex duct of *Carica.* It seems entirely possible that the tubes observed in this study might appear as such a sheath during the differentiation of large laticifers.

Based upon this study it is suggested that the tubes enclosed in series of cells may represent an early stage in the development of large laticifers in papayas. Further it seems probable that the tubes contain latex under pressure. Following rupture of the tubes, the enclosing cells enlarge, their transverse and lateral walls break down and large laticifers are formed.

Laticifers found in young papaya plants may be used as a commercial source of papain. It might prove feasible to grow extensive plantings of closely spaced young papayas, mechanically harvest them, and extract the papain. Such a procedure would reduce production costs compared to the current practice of scratching the skin of the individual immature fruit and collecting the exuded latex.

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