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Histochemical and Anatomical Studies of Phloem and Xylem Cells of Jackfruit (*Artocarpus heterophyllus* Lam.) Tree

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ABSTRACT

The localization of starch, lipid and protein, nuclei in the phloem and xylem cells of stem of Jackfruit trees (*Artocarpus heterophyllus*) has been studied. The optical digital images of anatomical features and localization of nuclei and reserve materials were obtained by light microscopy and fluorescence microscopy. Starch granules in the ray parenchyma cells were more abundant in the outer xylem close to the cambium than in the inner xylem and phloem. Radial localization of starch granules provided more clear data than transverse and tangential observations. Lipid and protein appeared as droplets and were uniformly distributed in the outer xylem. The parenchyma cells of phloem have large amount of lipid bodies but those were almost absent in cambium and xylem ray parenchyma cells. The results on the localization of storage starch, lipid droplets and proteins in phloem, cambium and xylem cells indicating that reserve materials might be important for wood formation in jackfruit trees. This data would be helpful for further study in tree breeding program and clarification of the mechanism of utilization of such reserve materials and their distribution pattern within the cells.

Key words: Localization, starch, lipid droplets, protein, *Artocarpus heterophyllus,* autofluorescence, phloem and xylem cells.

INTRODUCTION

Jackfruit belongs to the genus *Artocarpus* (family Moraceae) which is one of the most important tropical fruit trees. It has 3 botanical names, which are *Artocarpus integra* Merr., *Artocarpus integrifolia* Linn. And *Artocarpus heterophyllus* Lam. of which *Artocarpus heterophyllus* is now widely accepted [1]. It is a fairly large sized tree, bearing largest fruit among edible known fruits and produced very high quality timber that has a great market value [2]. It is mostly cultivated in South and Southeast Asia.

Jackfruit is the national fruit of Bangladesh and Indonesia. It occupies an area of 29,306 hectares having annual production of 31,200 tons [3]. It is an evergreen tree growing height of 8-25 m (26-82 ft) tall at 5 years of age. The demand and market price of jackfruit wood is very high. Mainly the heartwood of jackfruit is used as quality wood. The heartwood is formed from the regular conversion of sapwood. The conversion of sapwood to heartwood is a natural phenomenon of trees. There are some biochemical and physiological activities involved during the transformation process. The cambial zone encircled through xylem and phloem. Conduction of food materials by phloem cells and water conduction by xylem cells occurs during tree growth and development. Besides those, there are some metabolites such as starch, lipid and protein also engaged in tree growth and development.

Localization of reserve materials in different types of cells may vary species to species. It was reported that stored materials are crucial to radial growth, wood density and formation of the current year's tree ring. There have been several reports of variations in levels and partitioning of storage starch, lipid droplets, proteins in trees such as in *Betula pendula* [4], *Pinus sylvestris* [5]; *Juglans nigra* and hybrid *Juglans major* × *J. regia* [6], *Pinus cembra* [7], *Quercus petraea* and *Fagus sylvatica* [8-9]. The data in the cited studies indicate that levels of different reserve materials from phloem to xylem provides one of the key to a full understanding of growth and development of trees.

However, the histochemical changes of reserve material in phloem and xylem cells of *Artocarpus heterophyllus* trees are not fully understood. Therefore, the present study has been undertaken to observe the localization of reserve materials in different types of cells of phloem and xylem. The main purpose of this study was to investigate the anatomical features and histochemical changes of reserve materials from phloem to xylem cells. We also showed the xylem and phloem cells regarding their autofluorescence in relation to deposition of different chemical contents.

MATERIALS AND METHODS

Plant materials: Two jackfruit (*Artocarpus heterophyllus*) trees, which were 50 years old that were growing in the field nursery of the Bangladesh Agricultural University, Mymensingh, were used in the present study.

Collection of samples: Blocks containing outer bark, phloem, cambium and xylem were removed from the main stem at breast height from jackfruit trees (*A. heterophyllus*). The small block was removed with a

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scalpel and chisel. Samples were removed in a zigzag pattern to eliminate any effects of wounding. All the samples were wrapped with aluminum foil to protect them from moisture and light.

Preparation of samples for light microscopy: The samples were fixed in 4% glutaraldehyde in 0.1M phosphate buffer (pH 7.3) under vacuum for 1 h at room temperature. Fixed samples were washed in 0.1 M phosphate buffer and trimmed to 3 mm in length for subsequent fixation in 1% osmium tetraoxide in 0.1 M phosphate buffer for 2 h at room temperature. After washing in phosphate buffer, specimens were dehydrated in a graded ethanol series and embedded in epoxy resin. Transverse sections were cut at a thickness of approx. 2 µm with a glass knife on an ultramicrotome (Ultracut N; Reichert, Vienna, Austria) for sequential observations of cambial reactivation and xylem differentiation. Sections were stained with a solution of 1% safranin in water for observations of anatomical features of tissues of phloem, xylem and cambium and then examined under a light microscope (Axioscop; Carl Zeiss, Oberkochen, Germany) [10-13].

Preparation of samples for observation of starch, lipid droplets and proteins: The radial, transverse and tangential sections were cut at a thickness of approximately 40 µm with a stainless steel microtome blade on the freezing stage of a sliding microtome (MA-101; Komatsu Electronics, Tokyo, Japan). For light microscopic observations of storage starch, sections were stained with iodine-potassium iodide (I_2-KI) for 2-3 min [10, 14]. After staining, sections were washed with distilled water. For visualization of lipid droplets, sections were washed in a series of ethanol, namely, from 30% to 70% followed by stained with Sudan Black B in 70% ethanol for 5 minutes. After staining with Sudan Black B in 70% ethanol, sections were washed with 70% ethanol for 2-3 min. For visualization of proteins, sections were stained with Amido Black 10B for 5 minutes followed by washed with distilled water. For observation of anatomical structures of phloem and xylem, thick sections about 40 µm, were observed without staining and then examined under a light microscope (as described above).

Preparation of samples for observation of nuclei: The 40 µm sections were stained with acetocarmine for 2 hours. The stained sections were firstly washed with distilled water two times followed by series of ethanol, namely, from 30% to 100% ethanol. Permanent slides were prepared by using xylene and resin for future microscopic observations. The slides were kept as it is for overnight to prepare good quality sections. The localization of nuclei was observed under a light microscope with acetocarmine stained sections and fluorescence microscopy was used to visualize nucleus without any staining by using green light (wave length 561 nm; Axioscop; Carl Zeiss, Oberkochen, Germany).

Preparation of samples for observation of autofluorescence: About 40 µm sections were washed with distilled water and examined under fluorescence microscope. UV rays (wave length 405nm), blue light (wave length 488nm) and green light (wave length 561nm), were used separately to visualize the light intensity and absorbance of different types of cells of phloem and xylem. In this study, a comparison of fluorescence microscopy with different lights and filters was observed to understand the difference of light intensity and absorbance capacity of different types of cells.

RESULTS AND DISCUSSION

Anatomical observations of *Artocarpus heterophyllus* stem: Different types of cells were observed from outer bark to inner xylem without any staining. The anatomical observations focused on cell arrangement and chronological alignment from outer bark to inner part of *A. heterophyllus* stem. There were many variations among the cells visualized as well as their natural color (Fig. 1). The outer bark of the stem was composed of dead parenchyma cells which have black colored. The dark colored observations appeared in phloem ray parenchyma cells and longitudinal phloem parenchyma cells in the inner bark (Fig. 1a). Inner part of phloem was composed of live parenchyma cell and those are dark colored due to containing more food materials (Fig. 1a-c). Cambial zone was located between phloem and xylem portion of the stems. The lighter color of cambial zone was very narrow, which have meristematic activity and produced many new phloem and xylem cells during active seasons of cambium (Fig. 1d). Secondary xylem cells were mostly composed of xylem ray parenchyma cells, axial parenchyma cells, xylem vessel (not shown) and wood fibers that were visualized in figure 1 (d-f).

Cellular structural variation observed from phloem to xylem of jackfruit stem under light microscope (Fig. 2). The transverse sections showed clearly the cell size, shape and arrangement from phloem to xylem. Phloem was consisted with phloem ray parenchyma, phloem parenchyma and phloem fibers. New cell plate was clearly observed in phloem ray parenchyma and phloem parenchyma cells. Cambial zone was consisted with 10-12 radial layers of fusiform cambial cells during active seasons of cambium in July. Many differentiating wood fibers and xylem vessel elements were observed. During differentiation, cell walls were thin and gradually increased thicknesses of cell walls of wood fibers. Xylem ray parenchyma was multiseriate consisted with 2-4 layers of rays (Fig. 2a). The differentiation of vessel elements was observed from active cambial derivatives (Fig. 2b).

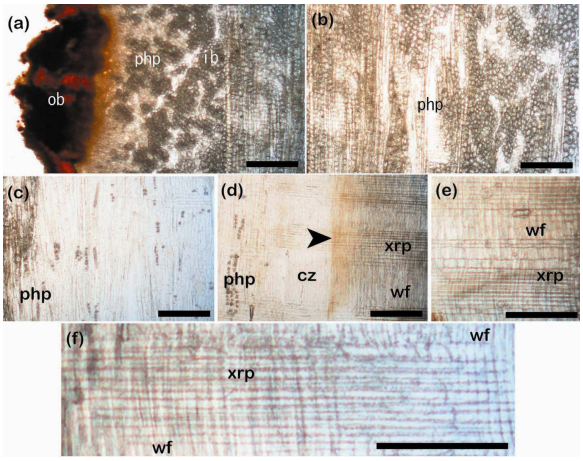

Figure 1: Light micrographs of radial sections (without staining), showing cell arrangement from phloem to outer xylem chronologically of the stem of *Artocarpus heterphyllus*. a) Outer phloem (outer bark), b) inner phloem (inner bark), c) innermost phloem, d) cambial zone, e-f) ray parenchyma cells in outer xylem. The left side of the micrographs corresponds to the outer side of the tree. ob, outer bark; php, phloem parenchyma; ib, inner bark; cz, cambial zone; wf, wood fiber; xrp, xylem ray parenchyma; scale bars = 50 µm.

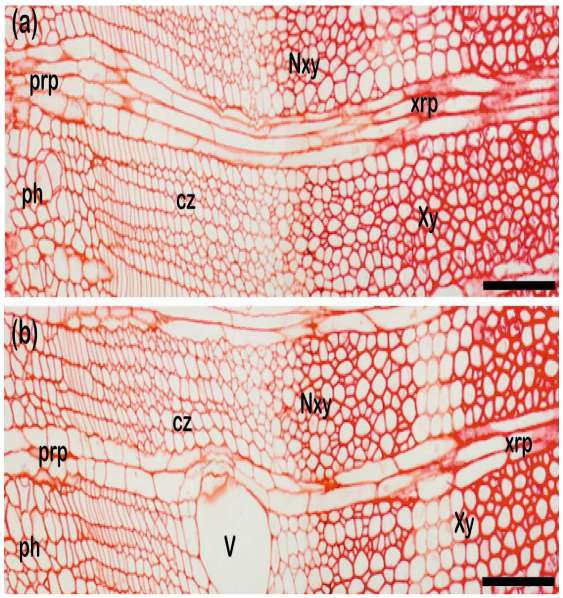

Figure 2: Light micrographs of transverse images of phloem, cambium and xylem of *Artocarpus heterphyllus*. Sections were stained with safranin, (a) different cells from phloem to xylem tissue, (b) differentiating vessel element along with different types of cells of phloem and xylem. The left side of the micrographs corresponds to the outer side of the tree. prp, phloem ray parenchyma; ph, phloem part; cz, cambial zone; Nxy, new xylem; xrp, xylem ray parenchyma; Xy, xylem part; V, vessel; scale bars = 25 µm.

Localization of starch in phloem and xylem cells: Starch grains in the ray parenchyma cells were more abundant than other cells. Both axial and ray parenchyma cells of outer xylem contained starch granules (Fig. 3). In phloem, the occurrence of starch was relatively smaller. Since, the outer bark contained dead cell, no starch granules were present.

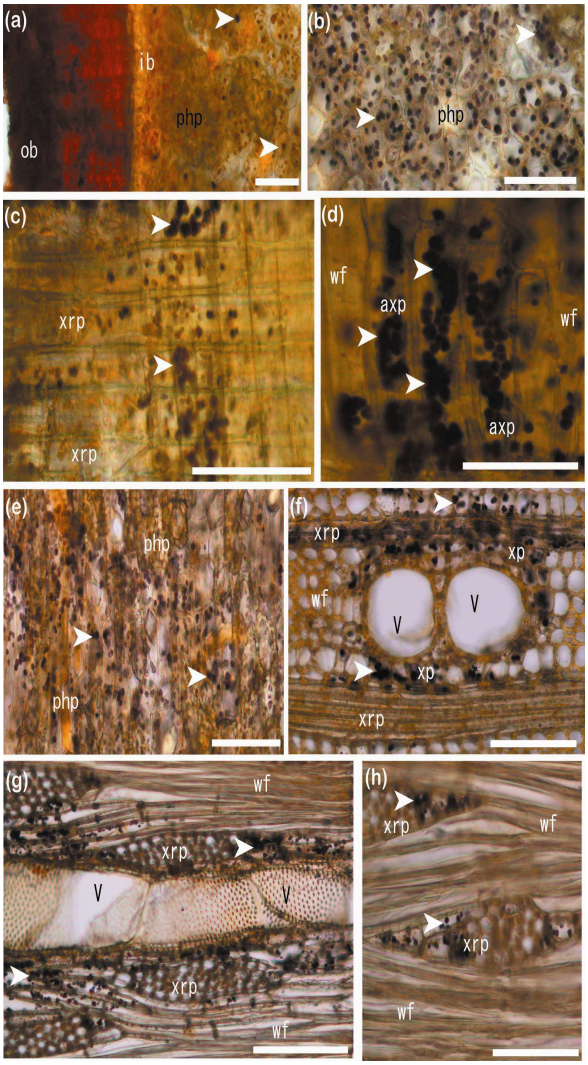

Figure 3: Light micrographs showing radial (a-d), transverse (e-f) and tangential (g-h) views of localization of storage starch (arrows) around phloem and outer sapwood of samples from *Artocarpus heterphyllus* stem. a) no storage starch was evident in outer phloem, b) abundant storage starch was evident in middle portion of phloem parenchyma cells, c) abundant starch appeared in xylem ray parenchyma cells, d) axial xylem parenchyma cells contained abundant starch granules, e) middle portion of phloem parenchyma cells contained abundant storage starch, f) abundant storage starch around the vessels and xylem ray parenchyma cells, g) abundant storage starch visualized around the vessels, h) ray parenchyma showed abundant starch content. ob, outer bark; ib, inner bark; php, phloem parenchyma; wf, wood fiber; xrp, xylem ray parenchyma; axp, axial parenchyma; V, vessel. Scale bars = 50 µm.

In inner bark, limited amount of starch granules were visualized in some phloem parenchyma cells (Fig. 3a). The innermost phloem parenchyma cells contained large amount of starch granules (Fig. 3b). Radial, transverse and tangential sections showed the clear visualization of starch content inside the phloem and xylem cells. Among them, radial observation (Fig. 3a-d) provided more clear localization than transverse sections (Fig. 3e-f) and tangential sections (Fig. 3g-h). The axial parenchyma

cells around the vessel contained much more starch granules that was observed in transverse (Fig. 3f) and tangential (Fig. 3g) sections. Almost all of the living cells contains starch granules, on the other hand no starch available in wood fibers in xylem.
The changes on distribution and localization of storage starch in the phloem and outer xylem was observed. In the outer part of phloem, no starch particles were localized and increased gradually inside of the phloem. In xylem cells, almost all of the ray parenchyma and axial parenchyma tissue contained much storage starch than phloem parenchyma cells. There was no considerable starch localization occurred in wood fibers than ray and axial parenchyma cells. These results supports the results reported by Begum [12], starch grains must be needed as a source of energy for the initiation of cambial cell division and xylem differentiation in *Cryptomeria japonica*.

The present results showed clearly that, the level of starch in phloem parenchyma cells falls significantly at the start of xylem differentiation during active seasons of cambium. The abundance of starch granules in phloem and cambium cells decreased from cambial reactivation to xylem differentiation in stems of *A. heterphyllus*. A marked change of starch granules was also observed in phloem ray parenchyma cells and longitudinal phloem parenchyma cells during xylem differentiation (data not shown). The decreases of starch granules were closely related to the change in their levels. Therefore, the reduced starch granules in phloem and cambium might provide energy during cambial reactivation and xylem differentiation [12].

Localization of lipid droplets in phloem and xylem cells: Lipids appeared as droplets in phloem portion (Fig. 4a). In contrast, no lipid droplets were found in outer xylem portion (Fig. 4b). The ray parenchyma cells of xylem have no remarkable lipid droplets whereas it was observed in phloem. Lipid droplets were fully absent in outer bark (Fig. 4a). Most of the cases their appearance observed in mass of droplets or in a cluster (Fig. 4a).
In *Robinia pseudoacacia*, lipid droplets accumulated mainly in the cambium and the level of lipids (as droplets) fell steadily as the distance from the cambium to the phloem and xylem increased during cambial dormancy [15]. Similarly, in the present study of *A. heterophyllus*, the relative area occupied by lipid droplets was higher in the cambium than in the phloem and this area remained unchanged in phloem ray parenchyma cells and longitudinal phloem parenchyma cells. Previous research works [5, 16] proposed that the storage lipids in the secondary xylem ray parenchyma cells might be generated from sugars or from starch and that the small-scale conversion of starch to lipids might occur in the autumn in Scots pine and poplar trees, respectively. In addition, a research group [16] reported that, in the secondary xylem ray parenchyma cells that were located close to the cambium of poplar trees (*Populus* × *Canadensis*), there were clear reductions in levels of lipids (as droplets) from cambial reactivation to the start of xylem differentiation. Therefore, these authors postulated that lipid droplets might be mobilized for the production of new cells in this variety of poplar in spring time.

Localization of proteins in phloem and xylem cells: The stained protein droplets were visualized as blue colored and appeared mostly in phloem and xylem parenchyma cells (Fig. 4c-e). The outer bark showed no protein content in their cells as those cells were died (Fig. 4c). There was no detectable protein visualized in the cells of the outer xylem while inner xylem contains little bit. Innermost portion of phloem, have limited powdery protein droplets that was visualized inside the phloem parenchyma cells (Fig. 4c-d).

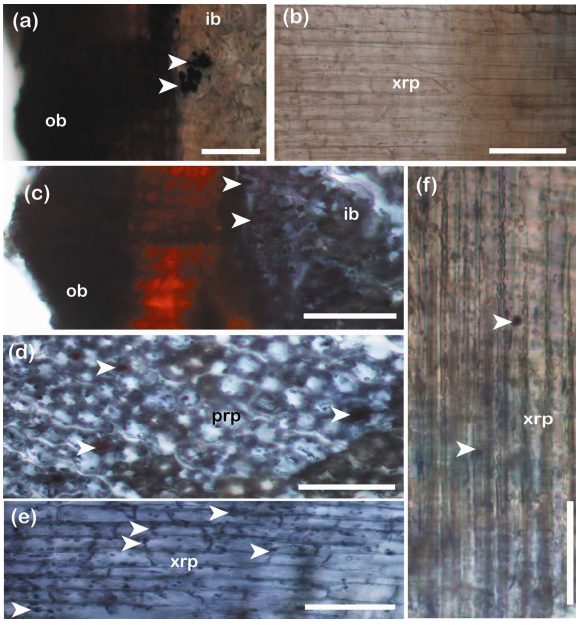

Figure 4: Light micrographs showing visualization of lipid droplets (a-b) and protein (c-f) in *Artocarpus heterphyllus* stem. (a) phloem with outer and inner bark and their lipid contents, (b) no considerable lipid droplets in outer xylem, (c) limited protein droplets in phloem parenchyma cells, (d) abundant protein droplets in mid phloem parenchyma cells, (e) xylem ray parenchyma cells of outer xylem contained protein droplets, (f) inner part of xylem ray parenchyma cells contained limited protein droplets. Arrowheads indicated lipid and protein drop/droplets. ob, outer bark; php, phloem parenchyma; ib, inner bark; xrp, xylem ray parenchyma; prp, phloem ray parenchyma; scale bars = 50 µm.

The xylem ray parenchyma cells contained more protein than the wood fibers and phloem parenchyma cells. There was relatively more protein droplets were visualized in outer part of xylem (Fig. 4e) than innermost part of xylem parenchyma cells (Fig. 4f).
A number of studies have provided information on distribution of protein in both deciduous and coniferous trees. The information of protein

localization among phloem and outer xylem tissue of *A. heterophyllus* supported the previous investigations [5-7]. Abundant presence of protein droplets within xylem ray parenchyma cells indicated that the ray tissue in the secondary xylem of woody plants plays an important role in the translocation, storage, and mobilization of nutrients [17].

Such types of accumulation and localization of reserve materials (e.g. starch, lipid and protein) between phloem and xylem cells is well known from extended studies on different trees [5, 6, 12, 18], rather than jackfruit. The results showed clearly the information of localization of reserve materials of *A. heterophyllus* but their intracellular localization and specific function, are still obscure. Although, some dark substances within the vacuoles of woody species have been referred to as tannins or phenolic substances [19-20], for several reasons, we cannot exclude the presence of electron-dense intra-vacuolar bodies of storage proteins in our present study. Firstly, Reports [19-20] loosely resembled the structure of protein bodies in oat starchy endosperm cells, described in detail by Saigo [21]. Strikingly, similar results have been obtained during the accumulation of storage proteins in vacuoles of cotyledonar and endosperm cells [22].

Localization of nuclei in phloem and xylem cells: The presence or absence of nuclei was observed to distinguish dead and live cells. Using acetocarmine staining, nuclei of live cells were observed as small reddish droplets (Fig. 5a-e). Outer bark has no nuclei since those were dead cells (Fig. 5a). Inner bark of investigated sample was almost consisted with phloem parenchyma cells that contained nuclei (Fig. 5a-b). The presence of nucleus showed in phloem ray parenchyma cells (Fig. 5c). Xylem ray parenchyma also showed the presence of nuclei in the cells (Fig. 5d). The axial xylem parenchyma cells also contained nuclei (Fig. 5e). Presence of nuclei was observed using green light (wavelength 561 nm) without any staining, which showed in Figure 5 (f-g). Cell death plays an important role in the functions of secondary xylem cells in wood. Research on the death and live cells of phloem, cambial zone and xylem parts, for the most part, cells are live rather than outer bark of the phloem. Although short-lived tracheary elements of xylem part observed but this portion was not our present focus, similar reports investigated [23]. Every cell death in ray parenchyma cells that were in contact with ray tracheids in conifers, such as *Pseudotsuga menziesii* and *Pinus strobus*, was observed [24-25]. Similarly our results revealed that cell death earlier in outer phloem parenchyma cells, ray tracheids of outer xylem cells.

Autofluorescence of different cells of phloem and xylem: To compare the difference of light intensity in a same section, green light (wavelength 561 nm), blue light (wavelength 488 nm) and UV (wavelength 405 nm) lights was used (Fig. 6). The outer and inner bark showed distinguished light intensity depending on different light absorbance capacity. Phloem and xylem cells contained different combination of chemicals which varied cell to cell investigated under different light sources (Fig. 6a-c).

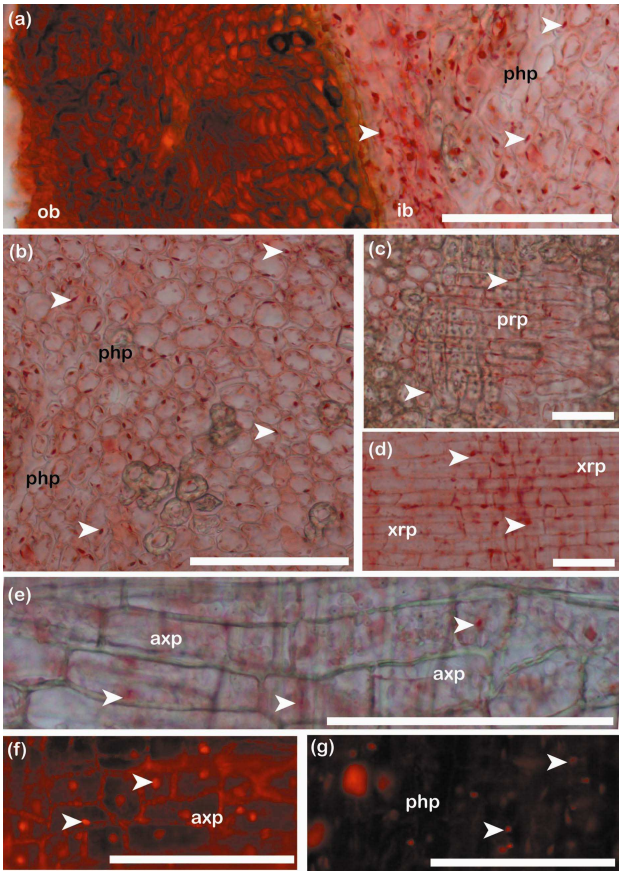

Figure 5: Light micrographs showing visualization of nuclei in *Artocarpus heterphyllus* stem (a-d, radial; e, tangential; f-g, transverse), stained with acetocarmine (a-e) and fluorescence microscopy with green light (f-g). (a) outer and inner bark, (b) phloem parenchyma cells in middle of phloem part, (c) phloem ray parenchyma cells in middle of phloem part, (d) xylem ray parenchyma cells of outer xylem portion, (e) axial xylem parenchyma cells, (f) axial cells in xylem part and (g) parenchyma cells in phloem part. Arrowheads indicated nuclei. ob, outer bark; php, phloem parenchyma; ib, inner bark; xrp, xylem ray parenchyma; prp, phloem ray parenchyma; axp, axial parenchyma; scale bars = 50 µm.

There were enormous difference observed in outer and inner bark due to their composition and light absorbance capacity. Similar results also observed in xylem portion, where green and blue light sources illuminated some part of the sections that was absent in case of UV light source (Fig. 6d-f). This observation proved the difference of autofluorescence among cells in plant tissues (Fig. 6a-f).

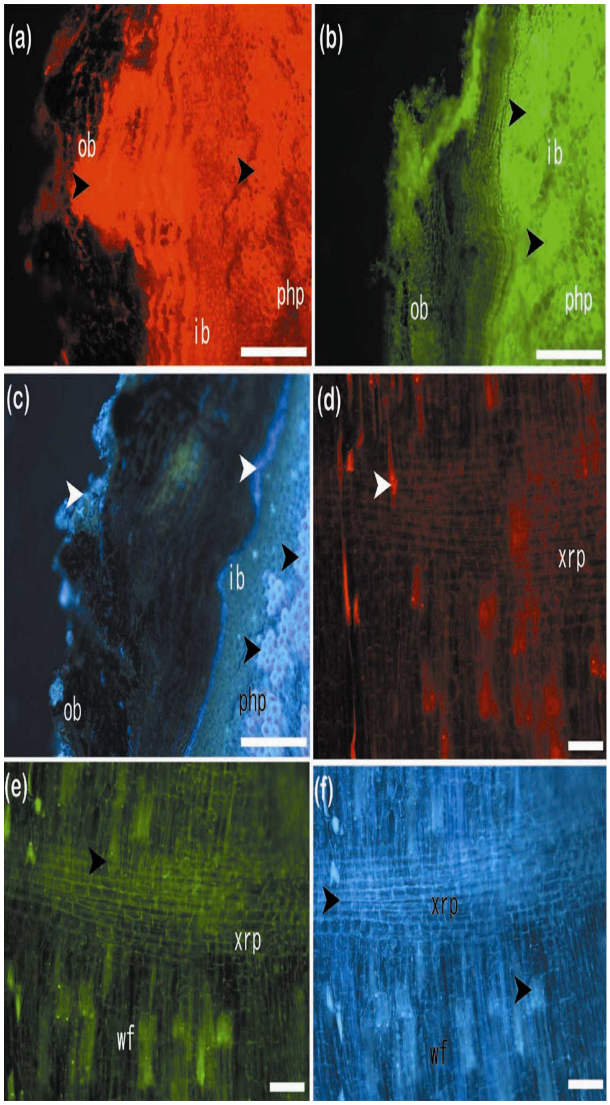

Figure 6: Fluorescence microscopy showing radial images of cell contents and cell walls of phloem and xylem cells in *Artocarpus heterphyllus* stem. Outer and inner phloem (a. green, b. blue, and c. UV lights) and outer xylem consisted with ray parenchyma and fiber cells (d. green, e. blue and f. UV lights). ob, outer bark; php, phloem parenchyma; ib, inner bark; xrp, xylem ray parenchyma; prp, phloem ray parenchyma; wf, wood fibers; scale bars = 50 µm.

The color of wood depends on chemical components interacting with light, as well as the presence or absence of extractives [26]. Therefore, our observation showed difference of color intensity and color absorbance under different light sources. However, the information about autofluorescence of different cell organelles showed variation of their composition between phloem and xylem cells. The occurrence of unknown different compounds in the cells might also indicate their roles as functions and importance in growth and development in plants. This result may be helpful for further query on chemical content related to their light absorbance of cells in plant.

CONCLUSION

Considering the prominent changes in storage starch, lipid and protein droplets between phloem and xylem and their use in active cambial zone, it appeared interesting to search for a relationship between reserve materials and biochemical phenomena in ray parenchyma cells. In addition, an attempt is made to follow the changes of amount of storage materials followed by cell death in phloem and xylem cells. This distribution and pattern of such storage materials within the cells of tree has significant effect on growth and development. It would be helpful for further research on tree breeding program as well as improving the quality of wood.

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