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A STUDY ON THE STRUCTURE OF *RHUS TYPHINA* SECONDARY PHLOEM AND RESIN CANALS

MENG ZHAO*, LULU ZHAO, YUNXIA WANG AND SHUPENG WU

Life Science College, Shanxi Normal University, 041000 Linfen, Shanxi, P. R. China
**Corresponding author's email: zhaomeng@sxnu.edu.cn*

Abstract

Rhus typhina is a kind of indigenous species to North America, it has been accommodated in many mountain areas in north China because of this species can display luxuriant growth in different habitat even in unfertile soils. In this study, we observed the morphology and ultra structure of secondary phloem and resin canal. We focus on the detailed observation of the microstructure and ultrastructure of the secondary phloem and resin canal, and to figure out the characteristics on the structure and the secretory process of epithelial cells. Our results showed that the structure of the secondary phloem exhibits the characteristics of typical temperate trees. And the mature resin canal of *Rhus typhina* comprised epithelial cells delimiting a long, round lumen, where secretions accumulate in the lumen. In the active epithelial cell, Golgi apparatus shaped secretory granules with monofilm, then move to attach on the plasma membrane and fused with the internal flocculent material of secretory granule was then released to the lumen side of periplasmic space, and finally, the excretion spread to the lumen and formed the secretions. This study shows the details of information on the structure of the *Rhus typhina* secondary phloem and epithelial cells, and the secretion process.

Key words: *Rhus typhina*; Resin canal; Secondary phloem; Secretory.

Introduction

Rhus typhina is indigenous to North America, and has been used for a long time by indigenous people as food, and in non-food applications. In current years, there has been an increment interest in applying *Rhus typhina* because of its diverse chemical constituent and functional activities, and the use of *Rhus typhina* has been well documented (Olchowik *et al*., 2012). *Rhus typhina* has also been adapted in different parts of the world and planted as an ornamental plant and a potential source of functional food ingredients. And it was also introduced to Asia and used for medicinal and food purposes (Kossah *et al*., 2011; McCune & Johns, 2002; Rayne & Mazza, 2007).

The genus *Rhus* with over 250 species as the largest population in the family of Anacardiaceae, distributed mainly in the subtropics and warm temperate regions in the world. There are 6 species in China, found nearly all over the country in China (Yi *et al*., 2004). *Rhus typhina* is an ordinary cashew plant in North America, for now, this species also distribute in North and Northeast China and cultivated as an ornamental plant (Chen *et al*., 2006). *Rhus typhina* is always used for reclamation of degraded soil in most mountain areas of north China, particularly for its invasive characteristics of thriving even in unfertile land and rapid propagation (Wang *et al*., 2008). For now, the species is dispersed widely in all habitats ranging from city to mountainous and has been taken as a destructive aggressor to native species by many research. (Weber *et al*., 2008; Yuan *et al*., 2013; Zhang *et al*., 2009).

However, the microstructure and ultrastructure of *Rhus typhina* secondary phloem and resin canals stay largely obscure, which blocks the exploitation of *Rhus typhina* as a versatile and operational ingredient. Therefore, as part of this comprehensive anatomical study, we examined the microscopic structure and ultrastructure of the *Rhus typhina* secondary phloem and resin canals to locate the secondary metabolite biosynthesis site, and present details on the secretions.

Materials and Methods

The materials for this research were *Rhus typhina* bark blocks that consisted of the conducting phloem, nonconducting phloem, vascular cambium, and periderm. These blocks were knifed out from the trunks of living trees, at a height of 1-2 m from ground level. The bark blocks for our research were collected in July 2017 from Linfen, Shanxi Province, China (111.52 E, 36.08 N).

Optical microscopy: To obtain the permanent slides, the research materials were fixed in 0.1 mol L^{-1} phosphate buffer (pH 7.2) containing 2.0% glutaraldehyde for 4 h (4°C). After that, the research samples were washed with double distilled water 3 times, then let the materials post-fixed in 1.0% osmium tetroxide in the same phosphate buffer for 4 h at 4°C, after the post-fix, washed the osmium tetroxide with double distilled water for 3 times, dehydrated the samples through the graded acetone series, and finally the materials were embedded in Spi-812 resin at 60°C for 3 days. The resin blocks were trimmed with razor blade and semi-thin sections (1-2 μm) were cut with a Reichert–Jung ultramicrotome, sections were collected with glass slides and dyed with toluidine blue (Hayat, 1981; O'brien *et al*., 1964). Observation and documentation were carried out under microscope with a digital camera (Olympus FSX 100).

Transmission electron microscopy (TEM): To analyze the subcellular features of the secondary phloem and resin canal of *Rhus typhina*, same fix and embed method was carried as semi-thin section in 2.2.1, ultrathin sections (50-70 nm) were acquired by a Leica EM UC6 ultramicrotome, sections were collected on the copper grids, and contrast stained with uranyl acetate and lead citrate (Reynolds, 1963), and observation were made with an FEI-20 transmission electron microscope.

Results

Micro-structure of *Rhus typhina* secondary phloem: The structure of *Rhus typhina* secondary phloem exhibits the characteristics of typical temperate trees, the *Rhus typhina* secondary phloem was formed during the initial phases of development, and was made up of phloem rays, sieve elements, phloem parenchyma, and resin canals. Vascular cambium was the innermost ring with 3-5(7) flat cells (Fig. 1-a), and the outermost was the periderm with phellem, phellogen and phelloderm (Fig. 1-b). Secondary phloem comprises conducting phloem that originated from the vascular cambium formed in the current year, and nonconducting phloem from earlier. The boundary lines between conducting phloem and nonconducting phloem was easy to find (Fig. 1-c). Sieve elements and phloem parenchyma were radially distributed, as seen in the full perspective view (Fig. 1-d).

Rhus typhina sieve elements were like squares or polygons in the conducting phloem, and parenchyma cells located at the sieve elements corners (Fig. 1-e). In contrast, the sieve elements in the nonconducting phloem were restricted to the phloem parenchyma, and the significant difference was observed in the size of sieve tube elements. While the bigger parenchyma cells were tangentially enlarged (Fig. 1-f). The nonconducting phloem had big, thin-walled, and oval to circular parenchyma cells. Sieve elements in the nonconducting phloem extruded as the volume of phloem parenchyma cells enlarged, which caused the cell walls of sieve elements to bend or even overlap. Sieve elements were shuttle-like cells, as observed in longitudinal sections (Fig. 1-g), which also clearly reveals the enlarged size of nonconducting phloem parenchyma cells (Fig. 1-h). *Rhus typhina* phloem rays were mostly uniseriate, and constitute a few roughly isodiametric cells; nonconducting phloem rays have been extruded (Fig. 1-i).

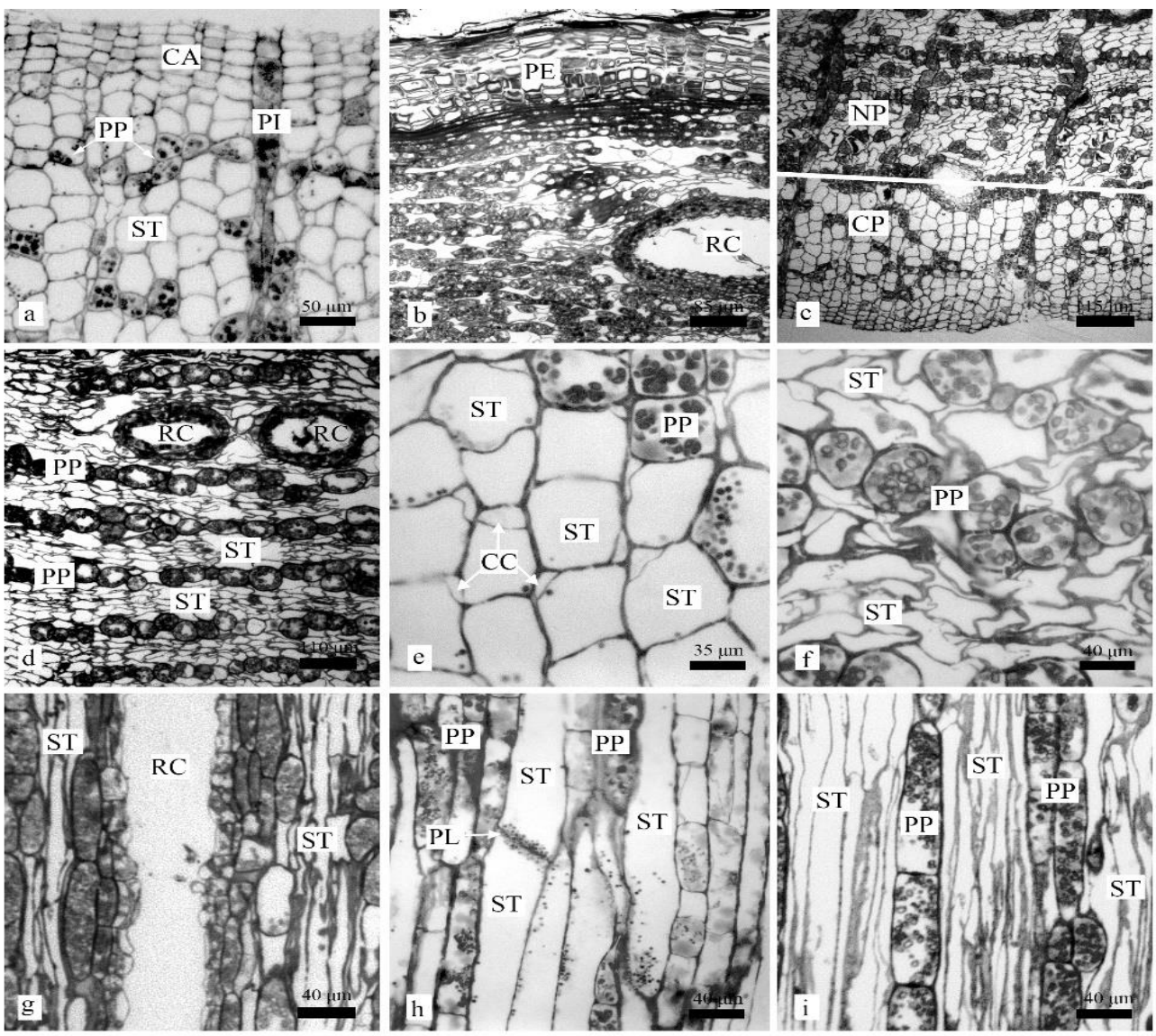

Fig. 1. Micro structure of *Rhus typhoid* secondary phloem.
Fig. 1-a. Cross section of cambium and conducting phloem; Fig 1-b. Cross section of non-conducting phloem and periderm; Fig. 1-c. Cross section of secondary phloem, the white line show the boundary; Fig. 1-d. Cross section of non-conducting phloem, show the "bark ring" of secondary phloem; Fig. 1-e. Cross section of conducting phloem, show the sieve elements; Fig. 1-f. Cross section of non-conducting phloem, show the sieve tubes were crushed; Fig. 1-g. Micro structure of resin canal; Fig. 1-h. Micro structure of conducting phloem, show the sieve tubes and P-protein; Fig. 1-i. Longitudinal section of non-conducting phloem.

Resin canals: Observation of cross sections revealed that mature resin canals comprised secretory epithelium delimiting a wide and round lumen, and the lumen was the place where the secretions accumulate, and the epithelium cells of resin canal appeared with dense cytoplasm and voluminous nuclei (Fig. 2-a). And longitudinal sections revealed the resin canals as long-tube shuttle-like cells (Fig. 2-b). During the activities in the vascular cambium, outside of the nonconducting phloem, the resin canal extruded by periderm and phloem cells assumed an elliptical shape, ultimately crushing the epithelial cells that lost their function as a result and atrophied (Fig. 2-c).

Ultrastructure of secondary phloem elements and resin canal of *Rhus Typhina*: TEM research on the *Rhus typhina* secondary phloem showed that it contains of sieve elements, parenchymal cells and phloem rays. Sieve tubes in the conducting phloem are possessed a large central vacuole, clear nucleus, (Fig. 3-a). Meanwhile, the sieve tubes in the nonconducting phloem were shranked and deformed after being squeezed by parenchymal cells into irregular shapes in narrow intracellular spaces, with intracellular P-protein, thick cell wall and no nuclei (Fig. 3-b).

With the comparison of phloem elements in the surrounding tissues, there were more Golgi apparatus and plastids in epithelial cells. Plastids were evenly distributed in the cytoplasm. Epithelial cells have conspicuous cell nucleus and many small vacuoles. As the resin canals developed, the surrounding epithelial cells is getting mature, and this invasive growth causes an increase in the lumen diameter (Fig. 3-c), number of plastids and types of mitochondria; Golgi apparatus and ER also increased (Fig. 3-d), with a large amount of flocculent stuffs in the latter (Fig. 3-e). The epithelial cells contained comparatively more ER (Fig. 3-f).

Secretion process from the secretory cells to the lumen: When the resin canal gets mature, the epithelial cell nucleus was still very distinct, different with other kinds of cells in secondary phloem, the epithelial cells has no central vacuole exists, epithelial cells have strong secretion ability, copious flocculent materials in the small vesica which was covered by monofilm appear in the cytoplasm. The Golgi apparatus produces monofilm-covered secretion granules that were transported to the lumen side of plasma membrane (Fig. 4-a); and subsequently, the vacuolar membrane and epithelial cells membrane fuse together to steer these granules primarily toward the cell surface adjacent to the lumen. Numerous vesicles are observed in epithelial cells, especially where they were close to the plasma membrane and face the lumen. The flocculent materials which in the vehicles will be released into the lumen side periplasmic space (Fig. 4-b), and electron-dense inclusions spread into the lumen by permeating through cell walls. Flocculent materials then fuse and integrate to generate an abundance of secretion in the lumen (Fig. 4-c).

Aging and disintegration of epithelial cells: When the epithelial cells gradually lose their synthesis and secretion functions. Senescent epithelial cells were thus hollowed, lack cellular components such as the nucleus, ER, and Golgi apparatus, and have lesser monofilm-covered flocculent materials in the epithelial cells cytoplasm. Meanwhile, central vacuoles are formed, and the number of organelles significantly reduces, with only a few plastids left in the cells (Fig. 5-a). Aged secretory cells become vacuolated, shrink, and have a small amount of starch; a disappearance of endoplasmic reticulum and Golgi apparatus as well releases fewer inclusions into the lumen through senescent epithelial cell walls (Fig. 5-b). And finally, their cell walls become fibrous and disintegrated (Fig. 5-c).

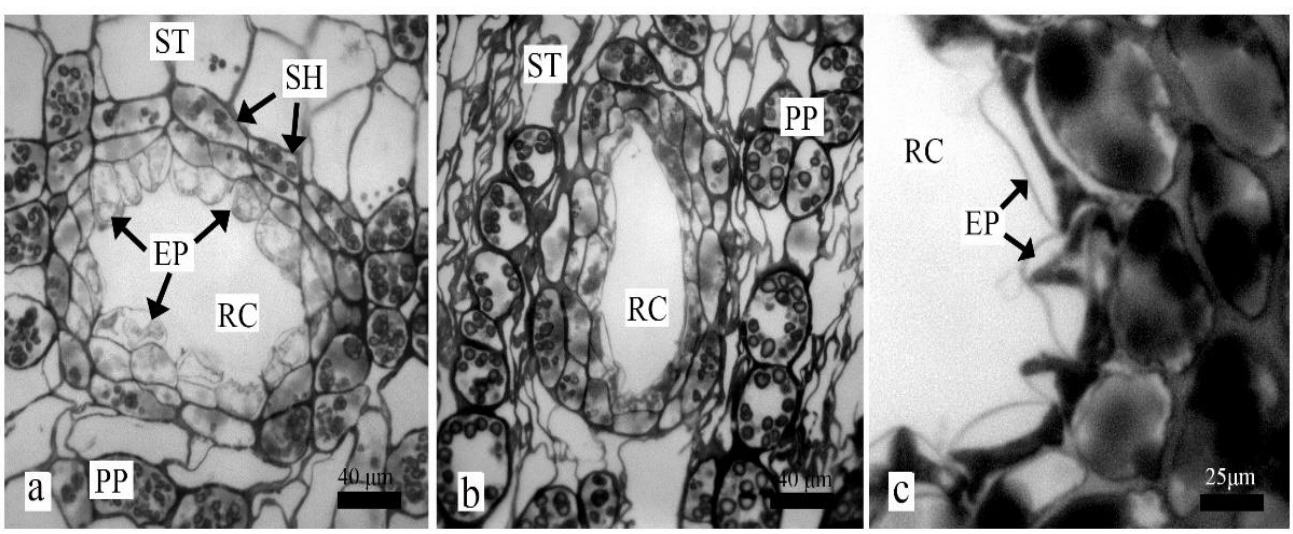

Fig. 2. Micro structure of *Rhus typhoid* resin canal.
Fig. 2-a. Cross section of conducting phloem, show the active epithelial cells of resin canal; Fig. 2-b. Cross section of nonconducting phloem, show the micro structure of resin canal; Fig. 2-c. Cross section of nonconducting phloem, show the aging resin canal epithelial cells.

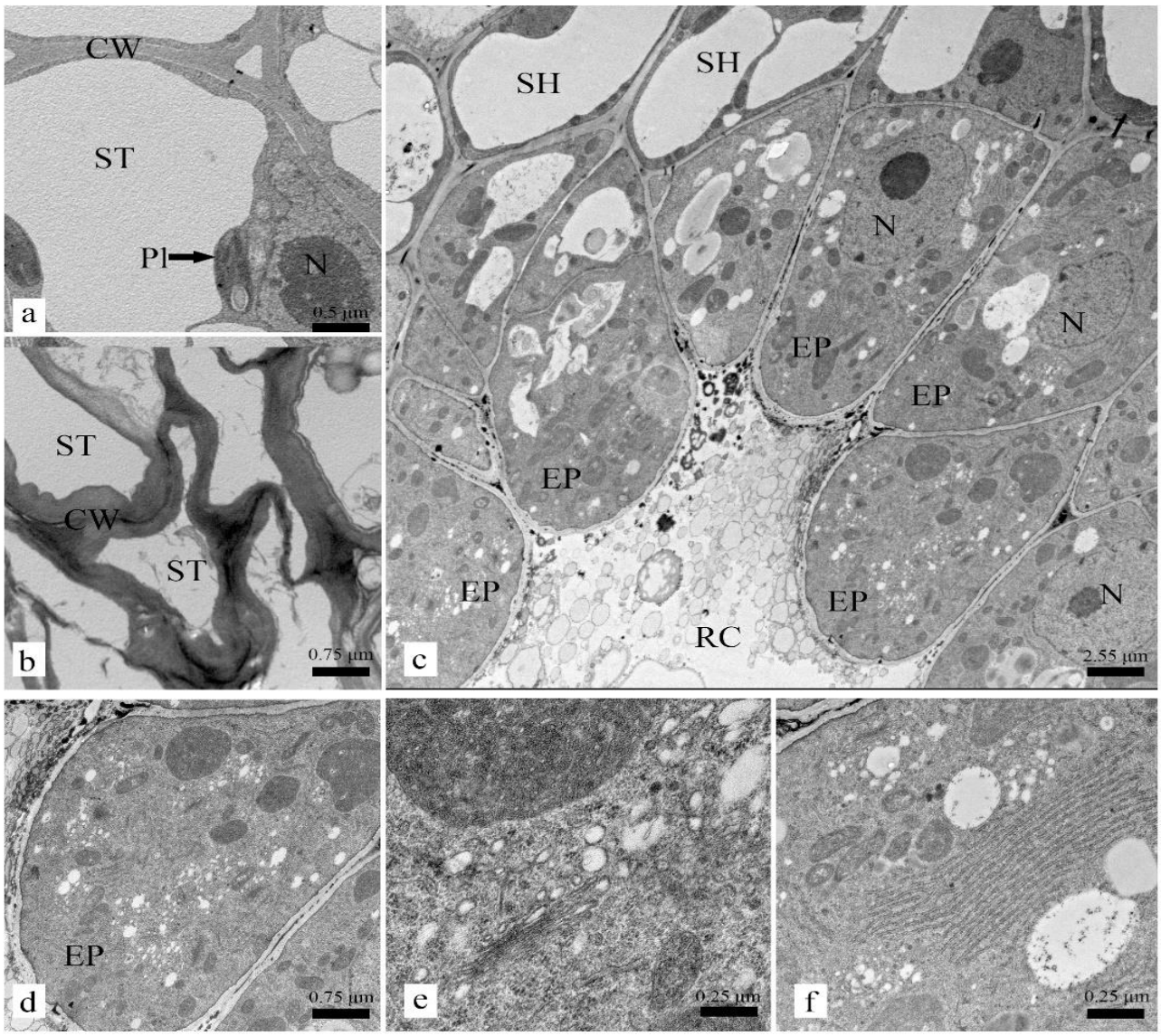

Fig. 3. Ultrastructure of secondary phloem and resin canal of *Rhus typhoid*.
Fig. 3-a. Ultrastructure of sieve elements in conducting phloem; Fig. 3-b. Ultrastructure of sieve elements in non-conducting phloem, show the bent cell wall of sieve tubes; Fig. 3-c. Ultrastructure of resin canal, the white arrows show the invasive growth of epithelial cells; Fig. 3-d. Ultrastructure of epithelial cells, show the epithelial cell rich in organelles; Fig. 3-e. Show the Golgi apparatus in epithelial cell; Fig. 3-f. Show the ER in epithelial cell.

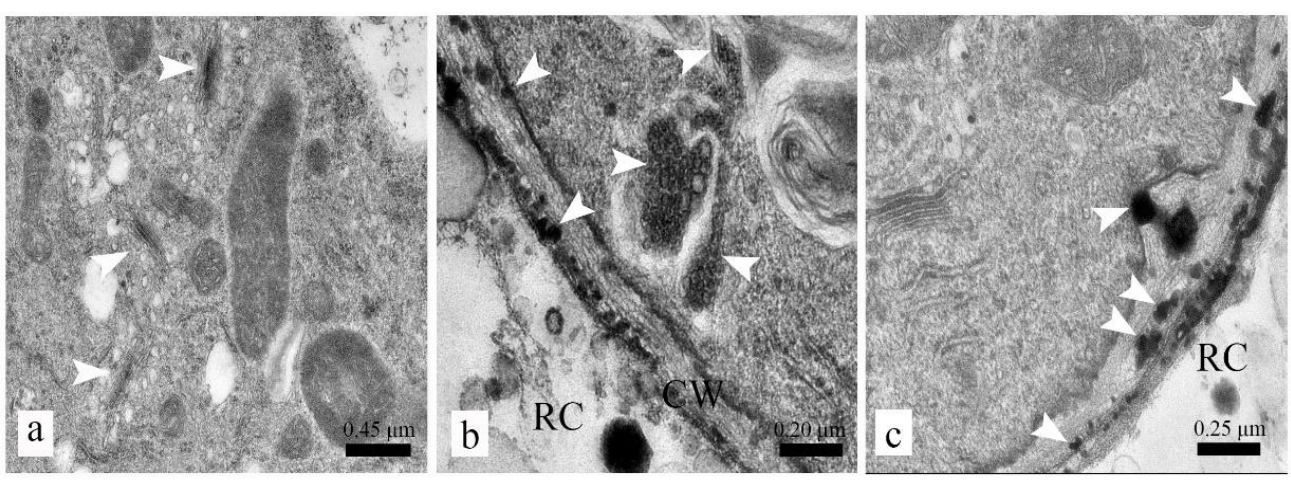

Fig. 4. Ultrastructure of secondary phloem and resin canal of *Rhus typhoid*.
Fig. 4-a. The Golgi apparatus produces monofilm-covered secretion granules; Fig. 4-b. The vesicles filled with flocculent materials in the epithelial cell; Fig. 4-c. Inclusions spread to lumen by permeating through the cell wall.

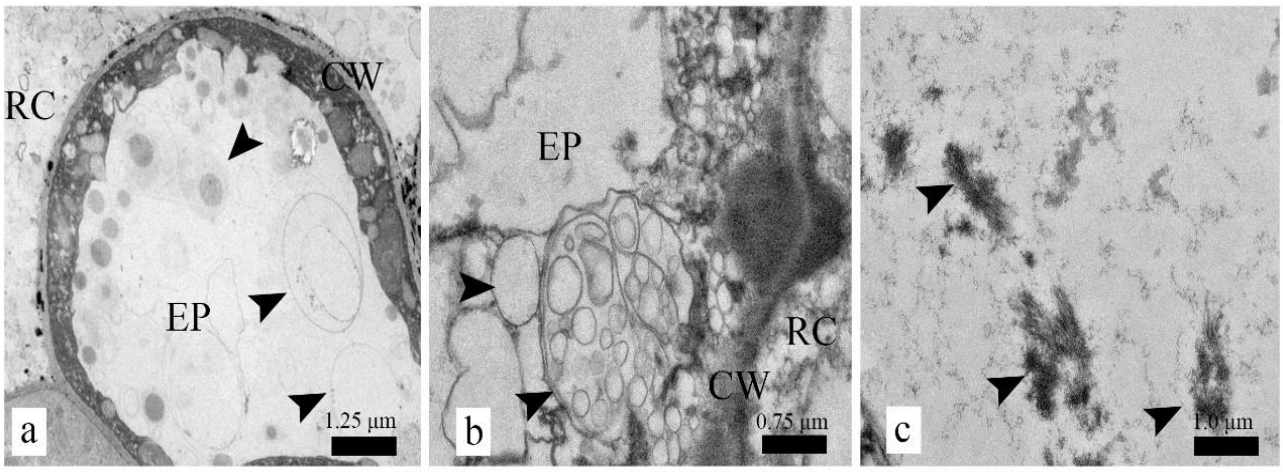

Fig. 5. Aging and disintegration of epithelial cells.
Fig. 5-a. Senescent epithelial cells, black arrows show the hollowed vesicles; Fig. 5-b. Aged epithelial cells become vacuolized and shrink; Fig. 5-c. Cell walls of epithelial cell became fibrous and disintegrated, black arrows show the fragments of cell wall.

Discussion

The structure of *Rhus typhina* secondary phloem exhibits the characteristics of typical temperate trees, sieve elements and phloem parenchyma were radially distributed, as seen in the full perspective view. Trees in temperate regions have the partial layer of secondary phloem, the secondary phloem produced each year can be divided into different layers, similar to the rings formed in the secondary xylem, that is, the "bark ring" (Esau *et al*., 1969; Trockenbrodt, 1990). A full description about the resin canal of the Anacardiaceae plant was first given in 1918 (McNair, 1918), and later anatomical studies of resin canals are generally similar to previous reports of many other species of Anacardiaceae (Aguilar-Ortigoza & Sosa, 2004; Bachelier & Endress, 2009). Resin canals consist of epidermis with one or two layers of cells, and sheath cells below (Evert, 2006; Fahn, 2000). Our results will help to understand the structure of phloem and secretory canals in *Rhus typhina,* even though anatomical facts are already known (Diao, 2016). Our observations with optical and transmission electron microscopy have substantiated the structure of the *Rhus typhina* resin canal. The structure pattern achieved by this study is likely to be the most common pattern in Anacardiaceae, and similar organization patterns have also been found in resin canals of *Toxicodendron vernicifluum*, *Anacardium humile*, *Lannea coromandelica*, *Pachycormus discolor* and other species of Anacardioideae (Gibson, 1981; Zhao & Hu, 2018; Royo *et al*., 2015; Venkaiah, 1992; Venning, 1948).

Epithelial cells of resin canal in *Rhus typhina* share lots of common characters with other secretory and metabolically active cells, in the present study, *Rhus typhina* epithelial cells have organelles including extensive RER (rough endoplasmic reticulum), abundant ribosomes, Golgi apparatus, vacuoles that can fuse with the lumen side plasma membrane, and plentiful vesicles situated in the lumen side space between the epithelial cell wall and plasma membrane. The occurrence of dictyosomes and plentiful vesicles in these cells is the symptom of the formation of secretions (Machado *et al*., 2017; Milani *et al*., 2012; Palermo *et al*., 2018; Tolera *et al*., 2013). These organelles are consistent with the requisite syntheses in epithelial cells, and they help to transport secretions into the lumen or cavity (Evert, 2006; Fahn, 1988; Fahn, 2000).

Three different secretion release mechanisms in plants secretory structures have been described, after the secretions are synthesized in the epithelial cells, exudates commonly accumulate and then cross the plasma membrane with the help of transporters (eccrine mechanism), through endoplasmic reticulum profiles (granulocrine mechanism), or by fusing with membranous vesicles (Evert, 2006; Fahn, 1979; Machado *et al*., 2017), the secretions then cross the cell walls into the lumen of the secretory (Bosabalidis & Tsekos, 1982). Our observations suggest membranous vesicles fusing with the lumen side plasma membrane and extrusion of secretion at the site facing the cell wall, which are all features typical of the eccrine process of secretion release into the lumen of secretory canals.

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